Synucleinopathies

Brás, Inês Caldeira; Dominguez-Meijide, Antonio; Gerhardt, Ellen; Koss, David; Lázaro, Diana F.; Santos, Patrícia I.

Published in:
Journal of Neurochemistry

DOI:
10.1111/jnc.14965

Publication date:
2020

Licence:
CC BY

Document Version
Publisher's PDF, also known as Version of record

Received: 7 November 2019 | Revised: 7 January 2020 | Accepted: 8 January 2020

DOI: 10.1111/jnc.14965

REVIEW

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

Synucleinopathies: Where we are and where we need to go

Inês Caldeira Brás[1] | Antonio Dominguez-Meijide[1,2] | Ellen Gerhardt[1] | David Koss[3] | Diana F. Lázaro[1] | Patrícia I. Santos[1] | Eftychia Vasili[1] | Mary Xylaki[1] | Tiago Fleming Outeiro[1,3,4]

[1]Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany

[2]Laboratory of Neuroanatomy and Experimental Neurology, Department of Morphological Sciences, CIMUS, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

[3]Institute of Neuroscience, The Medical School, Newcastle University, Newcastle upon Tyne, UK

[4]Max Planck Institute for Experimental Medicine, Göttingen, Germany

Correspondence
Tiago Fleming Outeiro, Department of Experimental Neurodegeneration, University Medical Center Göttingen, 37073 Göttingen, Germany.
Email: touteir@gwdg.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB1286; European Union's Horizon 2020, Grant/Award Number: 721802

Abstract

Synucleinopathies are a group of disorders characterized by the accumulation of inclusions rich in the a-synuclein (aSyn) protein. This group of disorders includes Parkinson's disease, dementia with Lewy bodies (DLB), multiple systems atrophy, and pure autonomic failure (PAF). In addition, genetic alterations (point mutations and multiplications) in the gene encoding for aSyn (*SNCA*) are associated with familial forms of Parkinson's disease, the most common synucleinopathy. The Synuclein Meetings are a series that has been taking place every 2 years for about 12 years. The Synuclein Meetings bring together leading experts in the field of Synuclein and related human conditions with the goal of discussing and advancing the research. In 2019, the Synuclein meeting took place in Ofir, a city in the outskirts of Porto, Portugal. The meeting, entitled "Synuclein Meeting 2019: Where we are and where we need to go", brought together >300 scientists studying both clinical and molecular aspects of synucleinopathies. The meeting covered a many of the open questions in the field, in a format that prompted open discussions between the participants, and underscored the need for additional research that, hopefully, will lead to future therapies for a group of as of yet incurable disorders. Here, we provide a summary of the topics discussed in each session and highlight what we know, what we do not know, and what progress needs to be made in order to enable the field to continue to advance. We are confident this systematic assessment of where we stand will be useful to steer the field and contribute to filling knowledge gaps that may form the foundations for future therapeutic strategies, which is where we need to go.

Abbreviations: A30P, aSyn substitution of an alanine for a proline at position 30; A53E, aSyn substitution of an alanine for a glutamic acid at position 53; A53T, aSyn substitution of an alanine for a tyrosine at position 53; ALP, autophagy-lysosomal pathway; APLP1, Aβ precursor-like protein 1; aSyn, alpha-synuclein; BiFC, bimolecular fluorescence complementation; CMA, Chaperone-mediated autophagy; CNS, central nervous system; co-IP, co-immunoprecipitation; Cryo-EM, cryo-electron microscopy; CSF, cerebrospinal fluid; Cu, copper; DLB, dementia with Lewy bodies; E46K, aSyn substitution of a glutamic acid for a lysine at position 46; ENS, enteric nervous system; ER, endoplasmic reticulum; Fe, iron; FRET, fluorescence resonance energy transfer; G51D, aSyn substitution of a glycine for a aspartic acid at position 51; GBA1, gene encoding for glucoceribrosidase; GCase, glucoceribosidase; GCIs, glial cytoplasmic inclusions; GWAS, genome wide association studies; HSP10, heat shock protein 10; HSPG, heparan sulfate proteoglycan; IDP, intrinsically disordered protein; iPS, Induce pluripotent stem cells; LAG3, lymphocyte Activating 3; LBDs, Lewy bodies diseases; LBs, Lewy bodies; LNs, Lewy neurites; LRKK2, leucine-rich repeat kinase 2; MAM, mitochondria-associated membranes; Mn, manganese; MPP^+, 1-methyl-4-phenylpyridinium; MSA, multiple systems atrophy; NAC, amyloid component; Ni, nickel; NMR, nuclear Magnetic Resonance; PAF, pure autonomic failure; PBMC, peripheral blood mononuclear; PD, Parkinson's disease; PET, positron-emission tomography; PFFs, pre-formed fibrils; PLA, proximity ligation assay; PMCA, protein misfolding cyclic amplification; PRKN, Parkin RBR E3 ubiquitin protein ligase; PrP^c, prion protein; PTM, posttranslational modifications; RBC, red blood cells; RBD, REM sleep behavior disorder; RT-QuiC, real time quaking induced complementation; S129, aSyn phosphorylation on serine 129; S87, aSyn phosphorylation on serine 87; SN, *Substantia nigra*; SNCA, gene enconding aSyn; VMAT2, vesicular monoamine transporter 2; WT, wild type; Y125, aSyn phosphorylation on tyrosine 125; Zn, zinc.

All authors contributed equally to this manuscript.

This article is related to the Special Issue "Synuclein"

KEYWORDS
alpha-synuclein, dementia Lewy bodies, multiple system atrophy, neurodegeneration, Parkinson's disease, synucleinopathies, toxicity

1 | INTRODUCTION

1.1 | Synucleinopathies: from clinics to pathology

Synucleinopathies are a group of disorders characterized by the deposition of aggregated forms of alpha-synuclein (aSyn) in neuronal and non-neuronal cells in the brain. In Lewy body diseases, including Parkinson's disease (PD) and dementia with Lewy bodies (DLB), aSyn typically accumulates in Lewy bodies (LBs) (Figure 1) and Lewy neurites (LNs) in neuronal cells. In multiple systems atrophy (MSA), aSyn typically accumulates in oligodendrocytes, in glial cytoplasmic inclusions (GCIs). aSyn deposits are also present in other diseases, such as Alzheimer's disease, pure autonomic failure, and in REM sleep behavior disorder.

aSyn is genetically associated with PD (Polymeropoulos et al., 1997) and, since this discovery, it has been the subject of intense research in synucleinopathies.

In September 2019, the Synuclein Meeting brought together over 300 experts in the field in order to discuss current challenges and future goals in the field of synucleinopathies, in the hope of bringing new ideas that may pave the way for the development of future therapeutic strategies (Outeiro & Mestre, 2019).

We still face many challenges and open questions, but perhaps the hardest one to overcome is the need for maintaining an open mind, leaving biases aside and keeping hypothesis and theories as such, and not as dogmas that hinder progress. After all, a major question we still cannot completely discard in the field of synucleinopathies is: are aSyn inclusions (LBs, LNs, or GCIs) relevant or simply an epiphenomenon? Time will tell.

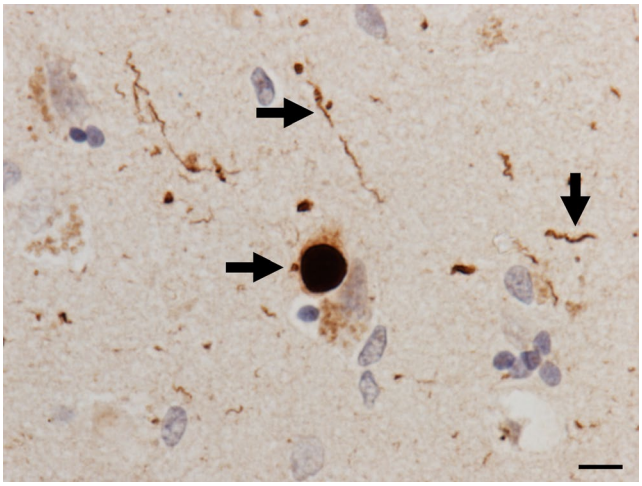

FIGURE 1 Cortical Lewy Body and Lewy neurites. Visualization of cortical Lewy pathology in the entorhinal cortex of a dementia with Lewy bodies case. Fixed tissue section stained with pSer129 aSyn antibody (EP1536Y, 1:500, Abcam) and Universal Polymer HRP kit (Menarini Diagnostics) with DAB chromogen and hematoxylin counterstain. Arrows point to LB and LN. Scale bar represents 10 μm

2 | WHAT DEFINES A LB AND WHAT IS ITS SIGNIFICANCE?

2.1 | What we know

LBs and LNs are the main histopathological hallmarks of PD and DLB. LBs were first identified by Fritz Jakob Heinrich Lewy as eosinophilic circular structures (Lewy, 1912). These circular lesions were initially found to be comprised of dense proteinaceous central cores surrounded by radially oriented filaments (Duffy & Tennyson, 1965; Roy & Wolman, 1969). Later, LBs were found to be highly enriched with ubiquitin (Kuzuhara, Mori, Izumiyama, Yoshimura, & Ihara, 1988; Lowe et al., 1988), although this is not a strict requirement, and with aSyn, which is said to be the main protein component of LBs (Figure 1) and LNs (Spillantini et al., 1997). LBs were then found to be enriched in aSyn phosphorylated on serine 129 (S129) (Anderson et al., 2006), but other posttranslational modifications (PTMs) are known to occur in LBs (discussed below).

Additional studies on the composition of LBs resulted in the identification of an array of more than 90 different molecules (Wakabayashi et al., 2013), including synphilin-1 (Wakabayashi et al., 2000), Parkin (Schlossmacher et al., 2002), Tau (Ishizawa, Mattila, Davies, Wang, & Dickson, 2003), chaperone proteins (Outeiro et al., 2006), or leucine-rich repeat kinase 2 (LRRK2) (Zhu et al., 2006), among many others. Very recently, the architecture of LBs was brought to the limelight through the use of powerful imaging techniques that suggest the presence of membranous components, including mitochondria and lysosomes, but with little aSyn fibrils (Shahmoradian et al., 2019).

Different neuropathological criteria have been proposed to correlate the brain regions affected by LB pathology with PD and DLB symptomology (Beach et al., 2009; Braak et al., 2003; Leverenz et al., 2008; McKeith et al., 2017). Intriguingly, the deposition of LBs is not widespread throughout the brain, but seems restricted to particular subsets of neurons. In the brainstem and midbrain, monoaminergic neurons, such as the dopaminergic neurons of the *substantia nigra* (SN), seem particularly prone to the formation of LBs (Braak, Ghebremedhin, Rub, Bratzke, & Tredici, 2004; Halliday et al., 1990). In contrast, in the limbic system and cortex, particularly in layers 3 and 5 of the neocortex, the greatest frequency of LBs is observed in the glutamatergic pyramidal neurons (Bernstein et al., 2011; Marui, Iseki, Kato, & Kosaka, 2003; Wakabayashi, Hansen, & Masliah, 1995), alongside the cholinergic neurons of the basal forebrain (Dugger & Dickson, 2010).

2.2 | What we do not know

Although Lewy pathology is used as a central pathological hallmark of PD and DLB, the precise molecular composition and architecture remains obscure. Despite much speculation, we still do not know whether LBs and LNs are toxic, inert, or even protective. Numerous studies have reported conflicting evidence associating LB burden with the severity of symptoms (Beach et al., 2009; Harding, Stimson, Henderson, & Halliday, 2002; Mattila et al., 2000), neuronal dysfunction (Bergeron, Petrunka, Weyer, & Pollanen, 1996; Katsuse, Iseki, Marui, & Kosaka, 2003; Kramer & Schulz-Schaeffer, 2007) and neuronal cell death (Bernstein et al., 2011; Dijkstra et al., 2014; Fukuda, Takahashi, & Tanaka, 1999; Halliday et al., 1990; Marui et al., 2003; Milber et al., 2012). The lack of such agreement, may suggest that: (a) cells most vulnerable to LBs accumulation may die rapidly and disappear without a trace, and that only those few LB-resistant neurons persist; (b) there may be subpopulations of LBs which are distinct in their composition and on the types of post-translationally modified aSyn they accumulate, rendering some more toxic than others; or (c) the active process of LBs formation is toxic rather than the final product, which would be consistent with the hypothesis that smaller aggregated forms of aSyn would actually be the culprits. This idea is supported by observations suggesting the absence of LB pathology in the brains of patients with certain familial forms of PD (Kalia et al., 2015).

Why some neuronal populations form LBs and others not, likely depends on a variety of as of yet unidentified functional and biochemical parameters. A high energy demand, a disproportional state of Ca^{2+} transients versus buffering capacity, and a high dependence on protein clearance systems are generalized features of neuronal vulnerability to cell death across neurodegenerative diseases. However, the contribution of these pathways to the formation of LBs is not well established. We also do not understand whether and how different types of pathology in the brain influence each other, for example, how tau pathology affects LB pathology.

Although aSyn aggregation has been observed in the gut and other peripheral tissues, we also still do not know in detail whether Lewy pathology can exist in other tissues besides the brain.

2.3 | What key experiments/tools are necessary?

We need to continue to investigate both the biochemical composition of LBs and their molecular architecture. This will require the use of highly selective and sensitive tools (such as antibodies and mass spectrometry), and of powerful imaging techniques, respectively.

In addition, we will need a greater understanding of factors modulating the formation of LBs (inhibitors and enablers), as these may prove useful for then testing the relevance of Lewy pathology in Lewy body diseases.

All these studies will require access to high-quality post-mortem human tissue, which in turn, requires high-quality brain banks.

A technical advance that should prove extremely important would be the development of aSyn ligands for positron-emission tomography (PET) imaging (Kotzbauer, Tu, & Mach, 2017). Such PET ligands would be extremely relevant for following Lewy pathology during disease progression and for establishing the its actual relevance for disease.

From the therapeutic perspective, developing cell-based models capable of recapitulating the accumulation of LBs and LNs would be extremely important. Patient-derived induced pluripotent stem (iPS) cells and 3D culturing techniques offer hope, but it is still early to tell whether they will prove to be the ideal systems for studying Lewy pathology to the extent we still need.

3 | WHAT IS THE FUNCTION AND SUBCELLULAR LOCALIZATION OF ASYN?

3.1 | What we know

Synuclein was the name used to describe a protein that was found to occur in the synapse and nucleus (Maroteaux, Campanelli, & Scheller, 1988). However, because of the connection with LBs and neurodegeneration (Spillantini et al., 1997) and also as a result of a predominant occurrence in the pre-synaptic compartment, the synaptic role of aSyn has been extensively investigated, in contrast to other roles it may play in the nucleus and in other subcellular compartments. aSyn is found in a fine equilibrium between a soluble cytosolic form, and in an insoluble membrane bound form (Fortin et al., 2004; Iwai et al., 1995). aSyn shows a high preference for binding lipid rafts with high content in unsaturated and polyunsaturated fatty acids (Fortin et al., 2004; Kubo et al., 2005) and displays a high specificity for membranes with high curvature. aSyn interacts with synaptic vesicles and is involved in synaptic vesicle regulation (Chandra, Chen, Rizo, Jahn, & Sudhof, 2003). In addition, the identification of synaptobrevin as an interacting partner implicated aSyn in SNARE complex formation (Burre et al., 2010).

However, despite some controversy, aSyn is present in other organelles. Studies with post-mortem brain tissue from PD patients suggest aSyn is enriched in mitochondria in different brain regions such as striatum, SN, and cortex (Devi & Anandatheerthavarada, 2010; Li et al., 2007). In particular, aSyn appears to associate with the inner mitochondrial membrane, because of the interaction with a specific mitochondrial lipid component called cardiolipin (Ghio, Kamp, Cauchi, Giese, & Vassallo, 2016; Zigoneanu, Yang, Krois, Haque, & Pielak, 2012). It also interacts with the mitochondrial outer membrane via direct binding to TOM20a, a subunit of the outer import machinery (Di Maio et al., 2016).

aSyn is also associated with endoplasmic reticulum (ER) and Golgi membranes (Colla et al., 2012) and, over-expression of aSyn impairs the ER-Golgi trafficking and induces ER stress and Golgi fragmentation (Cooper et al., 2006; Mazzulli, Zunke, Isacson, Studer, & Krainc, 2016; Oaks, Marsh-Armstrong, Jones, Credle, & Sidhu, 2013). It was recently demonstrated that a fraction of aSyn is localized in

mitochondria-associated membranes, a region mainly composed of intracellular lipid rafts, which interconnects the ER with mitochondria (Grassi et al., 2018).

Lastly, since the initial description of aSyn in the nucleus, and despite ongoing controversy, several studies have confirmed this finding (Goncalves & Outeiro, 2013; McLean, Kawamata, Ribich, & Hyman, 2000; Pinho et al., 2019). In the nucleus, aSyn may interact with histones and affect histone acetylation, thereby modulating gene expression (Goers et al., 2003; Kontopoulos, Parvin, & Feany, 2006). In addition, aSyn can bind DNA, induce DNA fragmentation, and modulate repair processes (Pinho et al., 2019; Schaser et al., 2019).

3.2 | What we do not know

Despite the existing evidence on the role aSyn plays in the synapse, the function aSyn plays in other cells/tissues (e.g., heart, blood, kidney, gastro-intestinal tract, etc) remains unclear. Intriguingly, studies with knock out animals suggest aSyn, along with the other members of the synuclein family (beta-synuclein and gamma-synuclein), are not essential proteins.

An important aspect that is often overlooked is the fact that aSyn is present in blood cells, but what the protein does there is unclear. The major source of blood aSyn are red blood cells (>99%), and only minor amounts are founded in peripheral blood mononuclear cell (0.05%), platelets (0.2%), and in the plasma (0.1%). However, platelets seem to contain the highest levels of aSyn (Barbour et al., 2008).

The dual nature of aSyn, as a cytosolic and membrane-bound protein, has been correlated with both physiological and pathological conditions. The disruption of this equilibrium, by unclear mechanisms, might affect aSyn homeostasis, and promote pathology.

In mitochondria, it is unclear whether certain lipids, such as cardiolipin, drive the binding of aSyn to mitochondrial membranes, and affect its import into the lumen.

Another important issue, that further complicates our understanding of the function, is that aSyn may assemble into different oligomeric forms, some of which might be relevant in terms of its physiology.

Several studies have linked certain PD-associated mutations in aSyn (aSyn substitution of an alanine for a proline at position 30, aSyn substitution of a glycine for a aspartic acid at position 51, and aSyn substitution of an alanine for a tyrosine at position 53), and phosphorylation on S129, with increased nuclear localization (Fares et al., 2014; Kontopoulos et al., 2006; Lazaro et al., 2014; Pinho et al., 2019). How these aSyn variants are linked with toxicity or physiological function in the nucleus remains unclear.

3.3 | What key experiments/tools are necessary?

Additional studies of the interactomes of aSyn in different compartments may provide additional insight into its normal function. In addition, novel, super-resolution imaging techniques may provide important information on the subcellular distribution of aSyn, and on the accumulation of specific types of aggregated species.

Studying the role of aSyn in mitochondria, nucleus, and other subcellular compartments, is very important, but we also need to investigate what the protein does in tissues besides the brain. We posit that studying aSyn in the blood may provide insight into its normal biology and, at the same time, as a biomarker.

4 | WHAT DO WE KNOW ABOUT THE STRUCTURE OF ASYN?

4.1 | What we know

aSyn is composed of three distinct domains: the N-terminal region contains four imperfect KTKEGV motif repeats that fold into amphipathic helices; the non-amyloid component domain is hydrophobic and is crucial for aSyn aggregation, and the C-terminal region, enriched in highly acidic and charged amino acids (Uversky, 2003). aSyn is classified as an intrinsically disordered protein that may exist, perhaps depending on the surrounding environment, as an unstructured monomer. Upon membrane/lipid binding, the N-terminal region adopts alpha-helical structure, to promote membrane curvature, and the protein may adopt other quaternary arrangements, forming tetramers (Bartels, Choi, & Selkoe, 2011; Chandra et al., 2003; Eliezer, Kutluay, Bussell, & Browne, 2001). Furthermore, the conformation of aSyn may change from alpha-helical-rich to beta-sheet-rich during the process of aggregation to oligomers, protofibrils, and amyloid fibrils (Caughey & Lansbury, 2003).

4.2 | What we do not know

Despite tremendous efforts over the years, the precise native structure(s) of aSyn is(are) still a matter of debate. In addition, the triggers that initiate the misfolding of aSyn into insoluble fibrillar aggregates, and the specific protein conformations that might be related to toxicity in different synucleinopathies are still unknown.

Very little is also known about the effects of PTMs on the structure and function of aSyn, or their role during disease progression and spreading of pathology.

4.3 | What key experiments/tools are necessary?

Investigating the conformation of aSyn in the context of different environments, such as lipids, membranes or pH, should provide insight into the role it may play in different subcellular compartments. More detailed studies on the effects of mutations associated with familial forms of PD, and on the effects of different PTMs, would also inform on the role different regions of the protein play in biology and pathobiology.

Elucidating the native conformation of aSyn and that of pathogenic species formed during disease would provide important insight into rationale drug development. To achieve this, we will need to continue to employ and develop techniques to study protein structure in situ, such as in cell nuclear magnetic resonance, cryo-electron microscopy (cryo-EM), or super-resolution microscopy techniques. These approaches would also provide important insight into the issue of pathological strains, shedding light into the molecular basis of different synucleinopathies.

5 | POSTTRANSLATIONAL MODIFICATIONS, METALS AND ASYN: CAUSE OR CONSEQUENCE OF PATHOLOGY?

5.1 | What we know

The primary amino acid sequence of aSyn reveals sites with distinct binding affinities to certain metal ions. This type of interactions can ultimately change the folding, aggregation, and membrane-binding propensity of aSyn. One of the most physiologically relevant metal interactions seems to be the binding of copper (Cu(I/II)) to the N-terminal region (^{1}MDVFMK^{6}) of aSyn, and/or to histidine 50 (H50). These Cu interactions can accelerate fibrilization in vitro at micromolar concentrations (Binolfi et al., 2006; Rasia et al., 2005).

Interactions with calcium (Ca(I/II)) became of interest because of recent data suggesting a role for aSyn in Ca-dependent vesicular transport (Lautenschlager et al., 2018). Ca(II) ions, as many other ions ((Fe(II), Fe(III), Ni(II), Zn(II), Mn(II), Cu(II)), bind preferentially to the C-terminus (^{119}DPNEA^{125}) of aSyn, leading to the destabilization of mono/dimeric aSyn, and the formation of various types of aSyn aggregates.

In addition, aSyn undergoes several PTMs that affect its structure, membrane binding, localization and, likely, its function. Phosphorylation on S129 is, by far, the most studied PTM of aSyn, but other phosphorylation sites are being identified and studied. The phosphorylation on S87 was reported to reduce aSyn fibrillization (Paleologou et al., 2010; Xiong & Yu, 2018).

Some PTMs may also affect the aggregation state of aSyn by modulating the binding of metal ions. For example, phosphorylation on tyrosine 125 (Y125) and on S129 may regulate ion metal binding, thereby influencing the structure and aggregation of aSyn.

Physiologically, aSyn is acetylated at the N-terminus, and this PTM stabilizes the binding of Cu(I) ions, and increases the alpha-helical content and, therefore, the binding of aSyn to membranes (Abeyawardhane et al., 2018). Interestingly, acetylation was recently reported on lysines 6 and 10, and found to reduce aSyn aggregation and toxicity (de Oliveira et al., 2017).

Other PTMs, such as ubiquitination, nitration, and sumoylation, have also been reported, and appear to modulate the aggregation and biology of aSyn in ways that need to be further investigated.

5.2 | What we do not know

Most studies conducted thus far have used recombinant aSyn produced in bacteria. Without specific manipulation, the aSyn produced in bacteria lacks N-terminal acetylation, as well as other eukaryotic-specific PTMs and, therefore, it is still unclear how the presence of this specific PTM affects the overall biology and aggregation of aSyn. In other words, some of the findings reported using non-acetylated aSyn need to further validated.

Another key question is how different PTMs talk to each other, and to metal binding, and how this affects the biology of aSyn. Do the interactions of aSyn with metals, such as Cu or Fe affect the oxidative stress status of the cell? Although this is highly likely, we also do not know whether different PTMs and different metal interactions occur in different cell types, and how these would affect the aggregation and spreading of aSyn pathology.

A recent study showed that aSyn purified from blood or human brain tissue does not exhibit classical properties of a metalloprotein (Lothian et al., 2019), suggesting that additional studies, and more accurate model systems are necessary.

The levels and relevance of different PTMs, in the context of synucleinopathies and disease progression, are still unclear. Furthermore, whether measuring PTMs of aSyn in blood or cerebrospinal fluid (CSF) holds value as putative biomarkers is also unclear, and demands further attention.

5.3 | What key experiments/tools are necessary?

We are currently limited by the sensitivity and specificity of existing methods for detecting aSyn PTMs, such as mass spectrometry-based, or antibody-based. Despite recent advances in both types of methods, they have limitations, and these will need to be overcome to enable us to tackle outstanding questions in the field. In addition, the generation of purified forms of aSyn carrying specific PTMs is essential, so that each of them can be studied in detail, and also in the presence of metals of interest. Another limitation pertains to the existing model systems available, which do not always enable the full modulation of PTMs and metal interactions.

6 | GENES, PHENOTYPES, AND TERMINOLOGY

6.1 | What we know

The three most common synucleinopathies are PD, DLB, and MSA. Despite some controversy, the clinical differences between the different diseases are documented. However, the factors leading to the various clinical phenotypes remain elusive.

Although the vast majority of synucleinopathies appear to be sporadic, genetic studies have been instrumental for our understanding of the underlying molecular mechanisms. Classical human

genetic studies have identified mutations in several genes that have been linked to either dominant or recessive forms of PD. Among these, mutations in the *SNCA* gene were the first to be identified. More recently, genome wide association studies have identified additional genetic variants in PD (Blauwendraat, Nalls, & Singleton, 2019), DLB (Orme, Guerreiro, & Bras, 2018), and MSA (Katzeff, Phan, Purushothuman, Halliday, & Kim, 2019). These findings suggest a complex interplay between genetics, aging, and environmental factors in the various synucleinopathies.

6.2 | What we do not know

SNCA mutations and multiplications are very rare, and it is not clear why they would cause one synucleinopathy and not another.

Genetic forms of PD associated with mutations in *LRRK2* and in Parkin RBR E3 ubiquitin protein ligase, seem to complicate the spectrum of synucleinopathies even further. *LRRK2* variants are the most common cause of parkinsonism, but post-mortem analyses of some brains from carriers of those mutations showed pure nigral degeneration and no Lewy pathology (Bardien, Lesage, Brice, & Carr, 2011). Similarly, Parkin RBR E3 ubiquitin protein ligase variants are linked with early onset parkinsonism that is often evident without LBs (Poulopoulos, Levy and Alcalay, 2012).

The identification of *GBA1* mutations as a risk factor for PD, DLB, and possibly MSA also shed light into a possible interplay between glucoceribrosidase and aSyn, suggesting autophagy impairments as a mechanism that needs further investigation (Lwin, Orvisky, Goker-Alpan, LaMarca, & Sidransky, 2004; Sidransky *et al*., 2009).

6.3 | What key experiments/tools are necessary?

Overall, genetic studies in synucleinopathies should help define similarities and differences between them, and may point to underlying molecular mechanisms that may identify targets for therapeutic intervention. Therefore, we need to couple genetic studies with neuropathological and mechanistic studies, using patient-derived material and improved models.

Genetics may also enable the definition of subtypes of each synucleinopathy, which may enable personalized therapies in the future.

Finally, the upcoming field of epigenetics, can provide valuable insights into disease-related players that are currently essentially unknown, and should also be further investigated.

7 | THE INTERACTOME OF ASYN

7.1 | What we know

Interacting partners of aSyn have been extensively investigated in cells and brain extracts. Some examples of known aSyn interactors include, among many others, Synphilin-1 (Engelender et al., 1999), LRRK2 (Guerreiro et al., 2013; Qing, Zhang, Deng, McGeer, & McGeer, 2009), p25alpha (Ejlerskov et al., 2013), DJ-1 (Zondler et al., 2014), ATP13A2 (Gitler et al., 2009; Lopes da Fonseca, Pinho, & Outeiro, 2016), chaperones (Nakhjavani et al., 2010; Outeiro et al., 2006), Rab proteins (Breda et al., 2015; Chutna et al., 2014; Masaracchia et al., 2018; Yin et al., 2014), heparan sulfate proteoglycans (Ihse et al., 2017), and even histones and DNA (Figure 2) (Pinho et al., 2019).

This vast panoply of putative interactors complicates our understanding of the normal function of aSyn, and suggests a lot still needs to be done, as this knowledge may impact on different aspects of synucleinopathies. For example, the mechanisms involved in the spreading of aSyn pathology, but the hypothesis of internalization based on a receptor-mediated mechanism has gained traction with the identification of several putative receptors, including neurexin 1b, and Aβ precursor-like protein 1, lymphocyte activating 3 (Ferreira et al., 2017; Mao et al., 2016) 26,323,479. All these receptors might contribute to aSyn pathology, possibly by binding and guiding the internalizing aSyn. For example, lymphocyte activating 3 binds preferentially to aSyn, with only minimal to monomers. In another study, the prion protein (PrP^C) was found to act as a sensor of aSyn oligomeric species, leading to synaptic dysfunction (Ferreira et al., 2017).

aSyn associates with lipid membranes via the N-terminal region (Jo, McLaurin, Yip, St George-Hyslop, & Fraser, 2000), a process thought to be important in the context of aggregation, although it is still unclear how. In fact, aSyn associates with different membranes types, including synaptic vesicles (Maroteaux et al., 1988), axonal transport vesicles (Jensen, Nielsen, Jakes, Dotti, & Goedert, 1998), lipid droplets (Cole et al., 2002), yeast membranes (Outeiro & Lindquist, 2003), and lipid rafts (Fortin et al., 2004). Interestingly, several studies suggest that the PD-associated mutation aSyn substitution of an alanine for a proline at position 30 impairs membrane interactions, suggesting

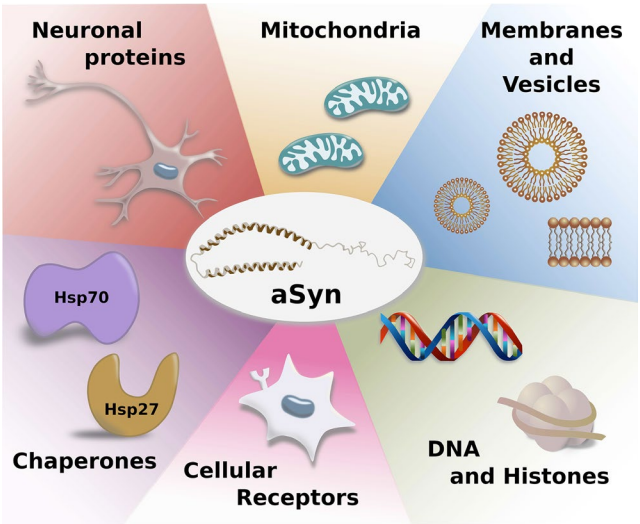

FIGURE 2 aSyn interactors. Selected examples of known aSyn interactors, including proteins, organelles, and DNA

this may be related to the pathogenesis of this particular mutant form of aSyn.

At the presynaptic terminal, aSyn can interact with a lot of proteins, such as as Rab3 (Chen et al., 2013), SNARE proteins (Burre, Sharma, & Sudhof, 2012; Burre et al., 2010), synapsin III (Zaltieri et al., 2015), Vesicular monoamine transporter 2 (Guo et al., 2008), dopamine and serotonin transporters (Butler et al., 2015; Swant et al., 2011; Wersinger, Rusnak, & Sidhu, 2006), piccolo (Scott et al., 2010), and tyrosine hydroxylase (Baptista et al., 2003; Perez et al., 2002; Yu et al., 2004).

Several observations have implicated mitochondria in the pathogenesis of PD. Therefore, the possible connection between aSyn and mitochondria has been a topic of interest for several years, with several studies suggesting aSyn may occur within mitochondria (Devi, Raghavendran, Prabhu, Avadhani, & Anandatheerthavarada, 2008; Martin et al., 2006; Shavali, Brown-Borg, Ebadi, & Porter, 2008). aSyn has also been shown to interact with mitochondrial proteins, such as heat shock protein 10 (Hsp10) (Szego et al., 2019).

7.2 | What we do not know

aSyn is an intrinsically disordered protein, enabling a panoply of conformations that may explain the various interacting partners, localizations, and functions. However, this also complicates our study of aSyn biology and pathobiology. Since our understanding of the localization and function of aSyn are limited, we may not have a complete understanding of all interacting partners, and of the consequences of such interactions, for example, as in the context of aSyn aggregation and of the role membrane interactions play in this process.

7.3 | What key experiments/tools are necessary?

The identification and characterization of aSyn interactors is important for the identification of putative targets for therapeutic intervention, and for our understanding of putative side-effects associated with the disturbance of the normal function(s) of aSyn. Currently, the toolbox for assessing protein interactions is large, and includes classical assays such as co-immunoprecipitation or crosslinking, or more recent assays such as mass spectrometry, nuclear magnetic resonance, bimolecular fluorescence complementation, fluorescence resonance energy transfer, or proximity ligation assay, to name just a few. When selecting a particular approach, one needs to carefully consider their strengths and limitations, especially with respect to sensitivity and specificity. Ideally, an assay should (a) allow a screening approach to identify and validate interacting partners; (b) rely only on direct interactions between two proteins; (c) enable testing at endogenous levels of expression of the proteins, to avoid false results. It is also important to consider that all available models are only approximations to the real situation in the human brain.

Computational approaches also promise to assist us in this quest, as new information about protein structure and computational power become available, although they will still require experimental validation.

8 | ASYN AGGREGATION AND STRAINS: HOW DOES IT HAPPEN?

8.1 | What we know

By definition, synucleinopathies are characterized by changes in the typical pattern of aSyn distribution in the cell, resulting in its accumulation in aggregated species (Masuda-Suzukake et al., 2013; Peng et al., 2018; Prusiner et al., 2015). However, the type of aSyn aggregate varies depending on the synucleinopathy. Recently, aSyn was found to form different types of aggregated structures in vitro, coined as ribbons and fibrils, and these were hypothesized to represent different 'strains', in an analogy with the prion field (Figure 3) (Bousset et al., 2013; Guo et al., 2013). Accumulating evidence suggests that the different conformational variants of aSyn differ in size, structure, toxicity, and lipid-binding efficacy, and they can be distinguished also based on their seeding capacity. Thus far, the factors triggering the formation of different types of aSyn aggregates (LBs, LNs, GCIs, etc) are unknown but, in vitro, salt concentration and pH determine the type of aggregate formed. In addition, the presence of natural pathogens, as bacterial endotoxins can also induce structural changes in aSyn fibrils (Kim et al., 2016).

In vivo studies in mice have shown that different aSyn strains lead to different biological effects. For instance, oligomers and ribbons have greater spreading potential than fibrils, but only ribbons lead to the accumulation of phosphorylated aSyn deposits.

Taken together, these data suggest that different strains of aSyn may explain the different clinical manifestations in different synucleinopathies.

8.2 | What we do not know

The studies described above demonstrate that recombinant aSyn is able to form different strains with distinct conformations and biological effects. Yet, the strain concept has not been validated in humans and, although it is appealing to think of this as the explanation for different pathologies and clinical outcomes, recent reports have even questioned the contribution of Lewy pathology to LBD (Moors et al., 2018; Shahmoradian et al., 2019). So, what could be the driving force for the heterogeneity of synucleinopathies? Could this be linked to PTMs, and not necessarily due to the occurrence of this type of strains? Phosphorylation, for instance, could be seen as a signal, to regulate aSyn aggregation. Truncations on the C-terminal of aSyn are present in the LBs, and are known to promote aSyn aggregation. Another important aspect is the binding to membranes and lipids, since this seems to also modulate aSyn aggregation.

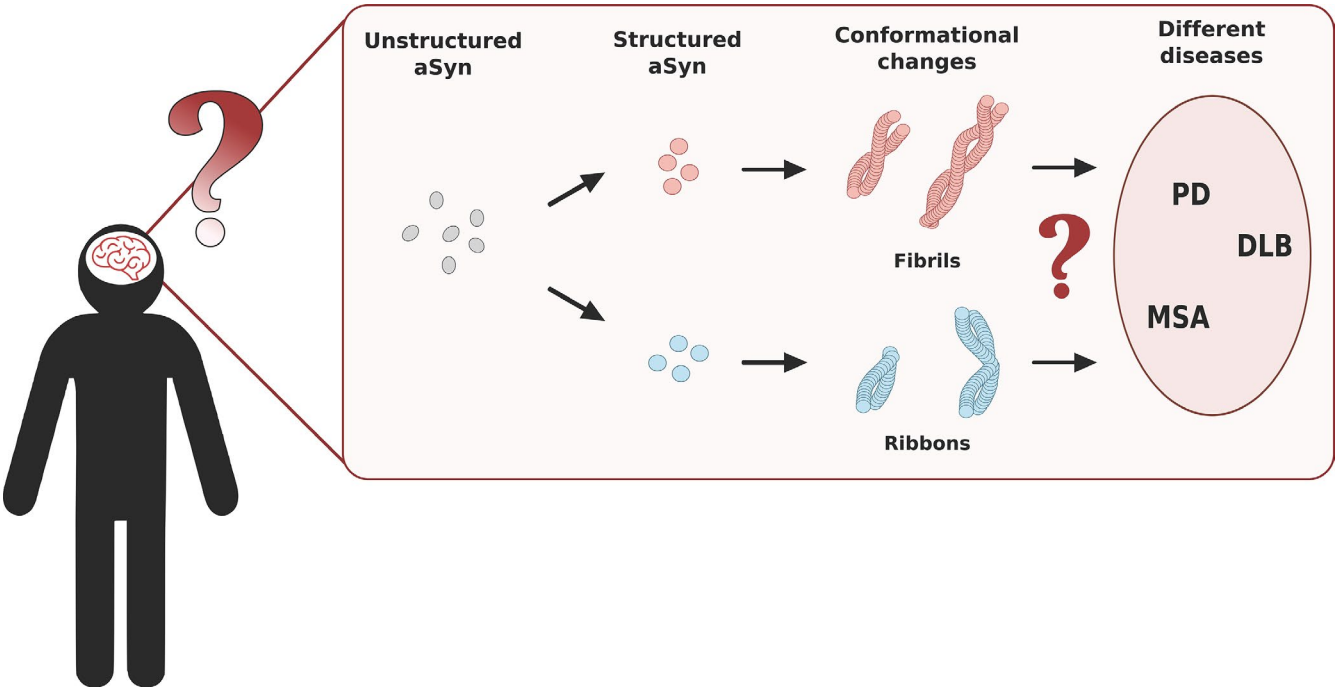

FIGURE 3 aSyn strains/aggregates. It still unclear if/or which type of aSyn conformation (e.g., ribbons or fibrils) influence the clinical phenotype in each synucleinopathy

Nevertheless, the concept of aSyn strains is important for the understanding of the molecular pathogenesis of synucleinopathies, and further studies are essential.

8.3 | What key experiments/tools are necessary?

An important aspect that needs to be considered when assessing strains formed by recombinant aSyn concerns the origin of the protein produced. Another aspect is whether the strains (aggregated forms) produced represent the same types of species formed in the human brain. Finally, another important aspect to consider is the variability in terms of aggregated forms of aSyn produced in different laboratories, using slightly different protocols, which may compromise direct comparisons. Therefore, specific guidelines are necessary to ensure the production and characterization of aggregated aSyn strains is consistent and physiologically relevant. Cryo-electron microscopy, and solid-state nuclear magnetic resonance are currently not only fashionable but very powerful techniques used to characterize protein aggregated species, and may prove instrumental for comparing human-derived with recombinantly produced aSyn aggregates. This requires the continued development of protocols for the extraction of aggregated aSyn from human brain tissue, to ensure minimal changes are induced during the extraction.

If distinct aSyn strains truly exist, the development of therapeutic strategies targeting conformation-specific forms of aSyn might prove beneficial. However, one will face issues of cases with mixed pathology, where multiple types of proteins deposits accumulate in the brain, and may need to be targeted independently.

9 | ANIMAL MODELS BASED ON ASYN EXPRESSION

9.1 | What we know

Numerous animal models have been developed in order to model specific aspects of synucleinopathies, such as aSyn aggregation and/or neuronal cell loss. These consist of the expression of wild-type or mutant forms of aSyn. Naively, we had thought that our understanding of PD genetics would enable us to generate good animal models of PD based on genetic manipulation of animals. However, we now understand that, at best, the models developed only model specific aspects related to the gene being manipulated. In some cases, it is likely that those models are not even relevant in the context of any human disease yet, they provide important information about the protein and basic molecular mechanisms and pathways where the protein might be involved.

Several transgenic animals (worms, flies, mice, rats, etc) have been generated over the years. In these models, aSyn has been over-expressed under the regulation of different types of promoters, enabling its expression in different tissues (Feany & Bender, 2000; Kahle et al., 2000; Masliah et al., 2000; Olsen & Feany, 2019). Invertebrate models, including worms and flies, have enabled powerful genetic and pharmacological screens, and are often used to bridge between cell-based and organism-based studies.

Another common approach for generating aSyn-based models is through the use of viral vectors (such as lentiviruses or adeno-associated viruses), which can be used to transduce, for example, populations of neurons in the brain, upon stereotactical injections.

More recently, models based on the injection of aggregated, possibly pathological forms of aSyn (known as pre-formed fibrils, PFFs), are used to study the spreading of pathology, as this is believe to correlate to the progression of PD and other synucleinopathies (Luk et al., 2012, 2009).

Importantly, all models offer advantages, but suffer also from disadvantages. It is essential to be aware of all of them, so that the correct animal is used to address the questions one is interested in studying.

9.2 | What we do not know

Although aSyn aggregation has been detected in several animal models, it is still unclear why the inclusions observed do not more closely resemble LBs. Several hypothesis have been put forward, but this will require additional investigation.

Another issue is that neurodegeneration is not often present in most of the transgenic mouse models, so the issue of selective vulnerability of the dopaminergic neurons from the SN is also unclear.

9.3 | What key experiments/tools are necessary?

Given the limitations of the current models, we still need to continue our efforts to refine and improve animal models, so we can mimic relevant aspects of synucleinopathies more closely. With CRISPR/Cas9 technology, it is likely that we will be able to achieve this in the near future.

Given the recent findings with tau-based models, it will be essential to characterize existing mouse models in much greater detail, in order to avoid artifacts, such as those recently reported for a mouse model of tauopathy (Gamache et al., 2019), and to 'humanize' the models, using promoters and regulatory elements that will ensure we can maximize the chances of obtaining relevant phenotypes (Walsh et al., 2017).

Additionally, we will need to incorporate other biological aspects into animal models, such as expression profiles, PTMs, and interacting partners, and we will need to develop novel knock-in models in non-human primates, as these may prove highly valuable for the study of specific brain circuits and physiological aspects.

10 | CELL MODELS OF ASYN TOXICITY AND AGGREGATION

10.1 | What we know

Over the years, many cellular models have been developed for studying different aspects of aSyn pathology, such as aggregation and toxicity. Such models, despite the minimalistic approach, have been very useful for our understanding of specific aspects of aSyn biology and pathogenicity.

The available cellular models range from powerful yeast cells, to neuronal and non-neuronal mammalian cell lines (human and non-human), primary neuronal cultures and, more recently, patient-derived iPS cells, to name just a few (Delenclos et al., 2019; Marvian, Koss, Aliakbari, Morshedi, & Outeiro, 2019). Different aSyn expression systems have also been employed, enabling transient or stable expression of either wild-type or PD-associated mutant forms of aSyn (Delenclos et al., 2019; Lazaro, Pavlou, & Outeiro, 2017; Vasili, Dominguez-Meijide, & Outeiro, 2019). The use of cellular models affords numerous advantages, such as the ease of use and manipulation, both genetically and pharmacologically, the low maintenance costs, and the reduced ethical constraints.

However, the use of single-cell model systems also faces important limitations that complicates extrapolations to more complex, biological systems.

Unlike other proteins prone to aggregate, aSyn over-expression does not necessarily result in the formation of inclusions. Therefore, additional insults have been used to 'force' the system. For example, 1-methyl-4-phenylpyridinium, rotenone, paraquat, or proteasome inhibitor are used to increase the aggregation of aSyn (Lee, Shin, Choi, Lee, & Lee, 2002; McLean, Kawamata, & Hyman, 2001). Furthermore, the addition of extracellular PFFs in primary neuronal cultures showed that these are able to recruit endogenous aSyn into ubiquitinated, and hyperphosphorylation aggregates (Volpicelli-Daley et al., 2014). Fluorescently labeled PFFs have been useful for the study of aSyn internalization and transfer between cells (Freundt et al., 2012; Jiang, Gan, Yen, McLean, & Dickson, 2017; Karpowicz et al., 2017).

Cell models have enabled mechanistic studies of the relationship between aSyn and mitochondrial dysfunction, oxidative stress, impairment of protein degradation pathways, etc (Klucken et al., 2012; Lazaro et al., 2014; Outeiro et al., 2008).

Cell models have also been useful for identifying surface proteins interacting with aSyn (Mao *et al.* 2016; Ferreira et al., 2017).

Expression of aSyn in oligodendroglial cell lines has been useful to study molecular mechanisms involved in MSA. For example, glial degeneration has been studied in cells co-expressing of aSyn and p25alpha (Kragh et al., 2009).

Overall, cellular models are powerful tools that mimic certain aspects of PD, and enable detailed mechanistic studies, but they also suffer from inherent limitations that need to be acknowledged.

10.2 | What we do not know

We still do not understand in detail what are the cellular factors that affect aSyn aggregation and cytotoxicity. The current hypothesis suggests that, during the process of aSyn assembly, soluble and cytotoxic oligomers may form and cause cell dysfunction and death (Conway et al., 2000; Outeiro et al., 2008; Villar-Pique et al., 2016).

In MSA, it is unclear how aSyn ends up accumulating in oligodendrocytes, as these cells are thought to produce only low levels of aSyn. So how aSyn enters and accumulates in oligodendrocytes is still unclear (Abati, Fonzo, & Corti, 2018).

We also do not understand how certain proteins, such as synphilin-1 (Engelender et al., 1999; O'Farrell et al., 2001; Tanaka et al.,

2004), and p25alpha (Ejlerskov et al., 2013; Lindersson et al., 2004) modulate the aggregation of aSyn in cell models. This suggests that, perhaps, other proteins/factors may also influence aSyn aggregation.

10.3 | What key experiments/tools are necessary?

Experiments in cell models rely a lot on the use of antibodies or fluorescent protein tags for probing the biology and aggregation of aSyn. These methods offer several advantages, but also important limitations that need to be considered. Therefore, we need to continue to develop antibodies that are more specific and capable of detecting relevant species of aSyn, and also epitope/fluorescent protein tags that do not affect the normal biology of aSyn.

It would also be important to develop cell models where typical LB inclusions accumulate. Thus far, these do not exist and, at best, we can talk about LB-like inclusions (Danzer et al., 2012; Outeiro et al., 2008).

We also need models that fully mimic the genetic environment of the disease that is being studied. iPS cells promise to help on this front, but reprogramming issues seem to affect the epigenetic landscape of the original cells, suggesting they are also not perfect models. Therefore, continued efforts and new tools will be necessary to improve the cell models we have currently available.

11 | SPREADING OF ASYN: RELEVANT OR EPIPHENOMENON?

11.1 | What we know

Recent studies suggest that aSyn pathology may spread between different brain regions, possibly converting normal aSyn into aggregated and/or pathological forms. Some authors believe this process occurs in a 'prion-like' manner, but this is still controversial.

Transmission of aSyn between cells and throughout the different body tissues comprises different mechanisms, including aSyn diffusion through the cell membrane, secretion *via* extracellular vesicles or alternatively transfer *via* tunneling nanotubes (Figure 4) (Grozdanov & Danzer, 2018). aSyn may be internalized by micropinocytosis, with aSyn fibrils binding to heparan sulfate proteoglycan chains in the plasma membrane (Holmes et al., 2013). This interaction between aSyn fibrils and the sulfated glycosaminoglycan chains seems to be dependent on the aggregation state of aSyn and on the cell type. This uptake occurs, for example, in oligodendrocytes and in neurons (Ihse et al., 2017; Rodriguez, Marano, & Tandon, 2018).

Even though our knowledge of these processes is increasing, there are still a lot of uncertainties and questions to answer. One of the main points in which there seems to be full consensus is that spreading of aSyn is relevant and it is not an epiphenomenon, nevertheless. Additional advances are being made in our knowledge of the aforementioned cell-to-cell transmission mechanisms.

11.2 | What we do not know

A major point of current debate in the field is whether aSyn pathology spreads in a 'prion-like' manner, and whether it should, therefore, be considered 'prion'. A defining characteristic of prions is that they can be transmitted among individuals of the same, or even different species (Prusiner, 1991). Currently, we do not know whether aSyn can be transmitted between different individuals. The occurrence of aSyn pathology in cells grafted into the brains of PD patients suggests aSyn pathology may spread in the brain (Kordower, Chu, Hauser, Freeman, & Olanow, 2008; Li

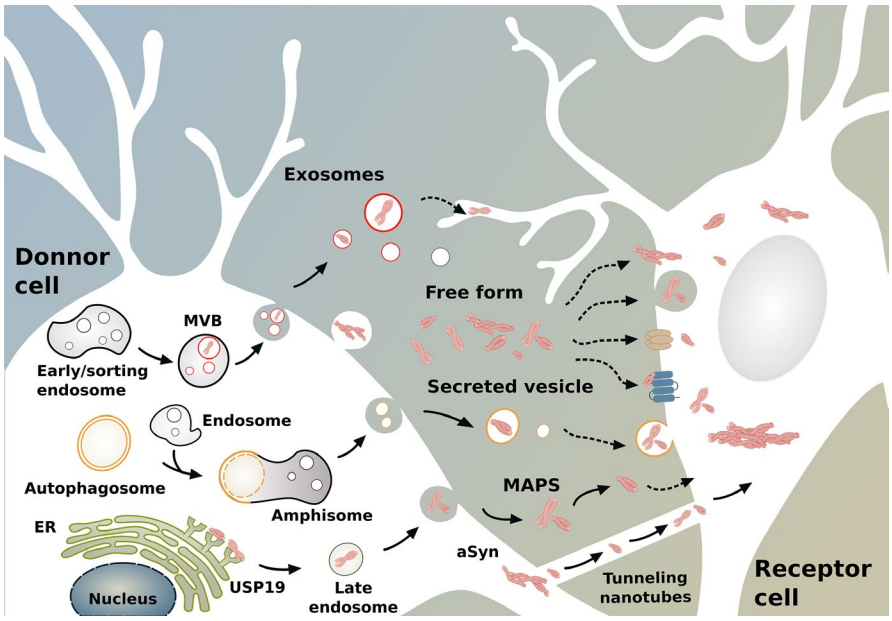

FIGURE 4 Possible mechanisms involved in the cell-to-cell transfer of aSyn. aSyn can be released through tunnelling nanotubes, extracellular vesicles or can directly cross the plasma membrane, leading to protein release through passive diffusion. Afterward, uptake of aSyn can happen via endocytosis of the extracellular vesicles, receptor-mediated endocytosis, interaction with heparan sulfate proteoglycans, pores in the plasma membrane or passive uptake from the extracellular space

et al., 2008). Several studies in animal models support this idea, but we still lack evidence confirming that aSyn pathology can spread when expressed at normal, endogenous levels (Killinger & Kordower, 2019).

We also have limited understanding of whether aSyn pathology can spread between different cell types in the human brain (e.g., neuron to glia, between different neuronal types, or between different glial types). This impacts on our understanding of the Braak staging, which might be because of selective vulnerability of specific neuronal populations (Killinger & Kordower, 2019).

Importantly, whether aSyn pathology spreads from the periphery to the brain, or vice-versa, has only been demonstrated in animal models, so we do not know whether this really happens in synucleinopathies.

Factors modulating the spreading of pathology, such as PTMs, are also under-studied at the moment, and we also do not know whether spreading of aSyn is a physiological phenomenon or, rather, an epiphenomenon that may bear little impact on the progression of PD and other synucleinopathies.

11.3 | What key experiments/tools are necessary?

We will need to refine our understanding of the biological processes and mechanisms associated with the possible spreading of aSyn pathology, as this will also enable us to clarify whether synucleinopathies are 'prion' disorders or, instead, simply disorders of protein aggregation. Therefore, we need to continue to improve and develop cell and animal models.

Furthermore, we will need to establish the mechanisms by which aSyn is released and taken up by cells, and whether PTMs or other factors affect these processes, as these may prove useful targets for therapeutic intervention.

12 | HOW IS ASYN CLEARED FROM THE CELL?

12.1 | What we know

Different protein degradation pathways seem to degrade different species of aSyn. These include chaperone-mediated autophagy (CMA), macroautophagy, and the ubiquitin–proteasome pathway. Impairments in the function/activity of these pathways may lead to the accumulation of aSyn, causing it to aggregate and accumulate in toxic species.

Macroautophagy is thought to be the most important pathway for the lysosomal degradation of higher order oligomeric and aggregated species of aSyn, since CMA is unable to handle such large protein species (Martinez-Vicente et al., 2008; Salvador, Aguado, Horst, & Knecht, 2000). In turn, monomeric aSyn can be degraded by the ubiquitin–proteasome pathway (Liu, Corboy, DeMartino, & Thomas, 2003). Mutant versions of aSyn associated with familial forms of PD, for example, H50Q, aSyn substitution of a glycine for a aspartic acid at position 51, aSyn substitution of a glutamic acid for a lysine at position 46, and aSyn substitution of an alanine for a glutamic acid at position 53, are more effectively degraded by macroautophagy than by CMA (Dahmene, Berard, & Oueslati, 2017; Fares et al., 2014; Khalaf et al., 2014; Vogiatzi, Xilouri, Vekrellis, & Stefanis, 2008; Yan, Yuan, Chu, Li, & Chen, 2018). Surprisingly, it was shown that intermediate, soluble, oligomeric aSyn can be partially degraded by the ubiquitin–proteasome pathway (Emmanouilidou et al., 2010).

Another mechanism by which cells can reduce the levels of aSyn is by secreting/releasing the protein (Ejlerskov et al., 2013).

The turnover of aSyn also seems to be modulated by PTMs, such as ubiquitination (Lee, Wheeler, Li, & Chin, 2008b; Nathan, Kim, Ting, Gygi, & Goldberg, 2013; Rott et al., 2008), sumoylation (Rott et al., 2017), glycation (Vicente Miranda, Szego, et al., 2017b), or phosphorylation (Oueslati, 2016; Tenreiro et al., 2014).

Only a few studies addressed the clearance of aSyn in vivo as animal models. Those suggested that the autophagy-lysosomal pathway was important to regulate the accumulation of aSyn oligomers and aggregates (Cullen et al., 2009; Ebrahimi-Fakhari et al., 2011; Friedman et al., 2012; Mak, McCormack, Manning-Bog, Cuervo, & Monte, 2010; Qiao et al., 2008; Xilouri et al., 2016).

The degradation of aSyn can also affect its transmission. In primary neurons, lysosomes seem to be the primary destination of internalized aSyn (Karpowicz et al., 2017). Interestingly, internalized seeds may compromise the endosomal/lysosomal machinery and the templating of endogenous aSyn (Karpowicz et al., 2017; Sacino et al., 2017). However, the degradation capacity of aSyn in neurons is lower than that in astrocytes, suggesting a possible cooperation between different cell types in the clearance of aSyn species (Loria et al., 2017).

Proteases, such as calpains and metalloproteinases have also been proposed to play a role in both intracellular and extracellular clearance of aSyn.

12.2 | What we do not know

Despite the various possible mechanisms for degrading aSyn in the cell, we still do not know which is more relevant for different aSyn species, and whether some of them fail, or at least have reduced activity, during aging and disease. We also do not know how PTMs affect the clearance of aSyn, so a lot needs to be done on this front.

It is thought that increasing the activation of clearance pathways might be a promising strategy for therapeutic intervention in synucleinopathies, but we need additional studies to rule out possible detrimental effects.

We also do not know whether and how certain types of cells in the brain, including microglia and astroglia, clear aSyn, which might bear important consequences not only during disease but also as targets for therapies.

12.3 | What key experiments/tools are necessary?

Investigating the contribution of different pathways for the clearance of aSyn will be essential. Live cell imaging methods might help us on this issue. We will also need to determine the role of different glial cell types on the clearance of aSyn. Better reporters and readouts for the activities of different clearance pathways would also be very important, and could then be used as readouts for the effects of possible therapeutic strategies.

13 | ANTIBODIES AGAINST ASYN: TOOLS AND THERAPIES

13.1 | What we know

Antibodies are powerful tools, produced by the immune system, with multiple uses in basic research, such as in immunostainings, immunoblotting, or immunoprecipitation. Antibodies are also useful in clinical research, as diagnostic tools and, more recently, as biological therapies (Figure 5). Given their specificity, there has been a great interest, and hope, in developing and applying immunotherapy for the treatment of neurodegenerative diseases, such as Alzheimer's disease, PD, and other synucleinopathies (Vaikath et al., 2019). Antibodies have several key features such as strong binding to the antigen, a long half life because of the constant part of the immunoglobulin, and the fact that they are occur naturally in the organism.

Recently, conformation-specific antibodies against aSyn have been developed, able to bind to aggregated forms of aSyn (Covell et al., 2017; Vaikath et al., 2015). These antibodies were tested in cell and animal models, yielding promising results that prompted ongoing clinical trials (Figure 5) (El-Agnaf et al., 2017; Spencer et al., 2017, 2016).

13.2 | What we do not know

One of the major handicaps for use of antibodies as therapeutic tools is that we still require more knowledge regarding their mechanism of action, pharmacokinetics, and pharmacodynamics. The routes of administration and the biodistribution of antibodies in the organism need to be considered, because of their large size they do not easily cross membranes or barriers and, consequently, may not easily reach intracellular targets. If the antibodies differ too much from our own, they may be recognized by the immune system and elicit an undesired reaction that can lead to their neutralization. We are still trying to understand how can we overcome these problems. These issues should be addressed before their use

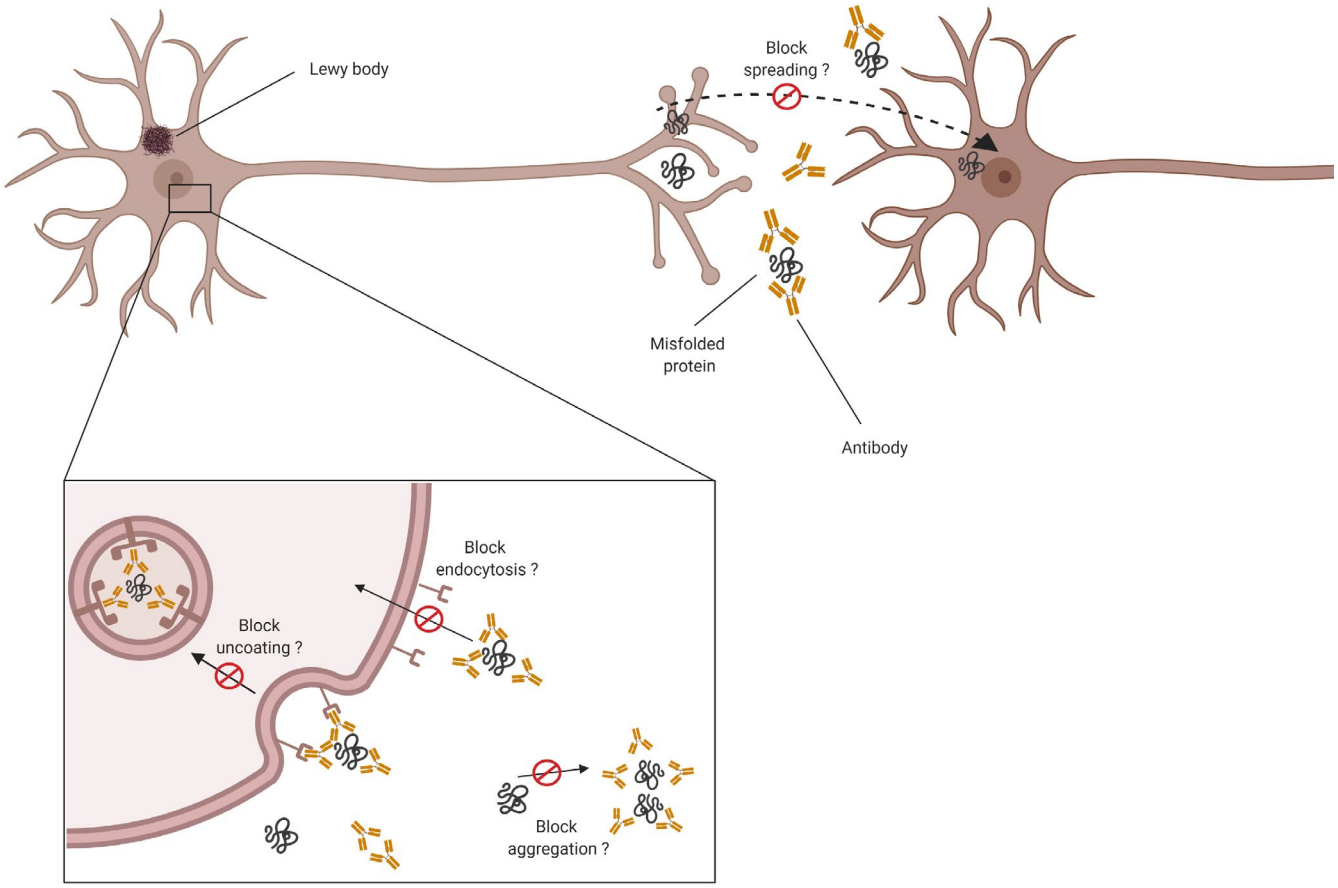

FIGURE 5 Antibodies against aSyn. The formation of misfolded aSyn antibody–antigen complexes may block aSyn aggregation and endocytosis. Additionally, formation of the complexes may block the possible effects of aSyn on synaptic vesicle trafficking by blocking the uncoating processes. Finally, may block the spreading of misfolded aSyn

become widespread. Additionally, most antibodies undergoing clinical trials for PD bind to the C-terminus of the protein (Table 1). As mentioned before, truncation of aSyn at the C-terminus seems to be a common event that affects aggregation (Sahin et al., 2017). Additional PTMs will also need to be considered, as they may enable the specific targeting of certain forms of aSyn. Another issue that needs to be addressed is the fact that we can only be sure that conformation-specific antibodies do not bind to monomeric aSyn, but we still do not know exactly to which conformation (oligomers or fibrillary structures) they are binding. Importantly, we still do not fully understand the pathological implications of aSyn aggregation and whether we should target oligomeric or fibrillar forms.

13.3 | What key experiments/tools are necessary?

An important issue is to establish the exact pharmacodynamics and pharmacokinetics of the different aSyn antibodies. Likewise, questions like whether these antibodies are able to cross the blood-brain barrier and their ability to be internalized by the cells still need to be addressed. Determining the affinity and avidity of the antibodies, and further advances in developing smaller antibodies will also be important (Vaikath et al., 2019).

It will also be important to determine what forms of aSyn should be targeted by the antibodies (El-Agnaf et al., 2017; Spencer et al., 2017).

Humanizing the antibodies is also important, and progress is being done on this front as well (Table 1).

14 | IMMUNE RESPONSES AND MICROBIOME IN PD

14.1 | What we know

Post-mortem studies in PD brains revealed extensive microgliosis in brain regions affected by synuclein pathology, such as the SN, hippocampus, or cortex (Doorn et al., 2014; Iannaccone et al., 2013; Imamura et al., 2003; Ouchi et al., 2005). This challenges the view that extensive microgliosis is simply a consequence of neuronal loss.

aSyn released by neurons triggers the initiate immune responses (Emmanouilidou et al., 2010) that involve microglia and macrophages in order to clear extracellular aSyn (Lee, Suk, Bae, & Lee, 2008a).

The aggregation state of aSyn impacts on both the uptake/internalization and clearance of aSyn (Hoffmann et al., 2016). As the disease progresses, it is thought that the aggregated forms of aSyn may become immunoreactive and trigger different immune responses. A sustained, long-term activation of microglia leads to a significant up-regulation of various pro-inflammatory components, thereby contributing to neurodegeneration (Wang, Liu, & Zhou, 2015). In summary, it is currently thought that microglial cells may play a dual role in PD: participating in the phagocytosis of aggregated and toxic species of aSyn, in order to prevent damage to neurons, and also as pro-inflammatory agents, contributing to neuroinflammation and, eventually, neuronal loss.

Recent studies demonstrated that PD patients exhibit an altered gut microbiome, accompanied by gastrointestinal inflammation, and that disease progression leads to a significant reduction in gut bacterial diversity (Heintz-Buschart et al., 2018; Hill-Burns et al., 2017; Keshavarzian et al., 2015; Li et al., 2017). In addition, intestinal aSyn is associated with increased intestinal permeability (Forsyth et al., 2011). Thus, the interaction between the gut microbiota and aSyn aggregation is of great interest, and is receiving increased attention as a putative risk factor and/or disease modifier in PD and, possibly, in other synucleinopathies.

14.2 | What we do not know

Current hypotheses for the initiation site of aSyn pathology consider it may occur outside the central nervous system (CNS),

TABLE 1 Anti-aSyn antibodies in clinical trials for PD

	Prasinezumab PRX 002	BIIB054	MEDI1341	Lu AFB2422	ABBV–0805
Company	Prothena/Roche	Biogen/Neurimmune	AstraZeneca/Takeda	Lundbeck/Genmab	Abbvie/Bioartic
Stage	Phase II	Phase II	Phase I	Phase I	Phase I
Isotype	Humanized IgG1	Full human IgG1	Human IgG agly (Fc Null)	Human IgG	Humanized (IgG1?)
Epitope	115–126	1–10	102–130	112–117	121–127
Affinity	3 nM (Biacore)	100 nM (ITC)	7 nM (Biacore) 74 pM (Kinexa)	16 nM (Biacore)	50–120 nM (Biacore)
Aggregate binding	Affinity/avidity to aggregated aSyn is 48 pM (Biacore)	Affinity/avidity to aggregated aSyn is 120 pM (ELISA). IHC shows no binding to control brain sections	High affinity, high selectivity (claimed); demonstrate target engagement in the CNS of the antibody in preclinical species	100 fold selective for aggregated aSyn. Reduce or prevent spreading	200 fold selective for aggregated aSyn (claimed)
Posted Trial Completion Date	Feb 2021	Apr 2022	Nov 2019	Mar 2020	

perhaps in the peripheral nervous system (gut or nasal epithelia) (Braak et al., 2003), implying that, probably, the peripheral immune cells might be the first ones affected by aggregated forms of aSyn forms, with microglia activation occurring as a secondary event. This is supported by several lines of evidence such us the fact that phosphorylated aSyn aggregates are detected in the enteric nervous system of PD patients (Barrenschee et al., 2017) and that alterations in the gut microbiome can induce the accumulation of aSyn aggregates in the enteric nervous system (Chen et al., 2016). In addition, apendectomy (Killinger et al., 2018; Mendes et al., 2015), and vagotomy are associated with a decreased risk for PD in humans (Liu et al., 2017; Svensson et al., 2015) and experiments in rats show that exogenous aSyn fibrils induce the formation of LB-like inclusions in the brain in a process that appears to involve propagation via the vagus nerve (Holmqvist et al., 2014; Kim et al., 2019). These findings raise the question of how brain and periphery cross-talk, and whether this is mediated by soluble components. Furthermore, we also do not know what are the stimuli that mark the initiation of these events.

14.3 | What key experiments/tools are necessary?

Recent studies showed the appearance of LB-like structures, some positive for phosphorylation of S129, in microglial cells, suggesting a link between pathological alterations taking place in neurons and microglia. Since the development of pathology in neurons and microglia occurs in parallel, we cannot exclude the existence of transfer of aSyn species between neurons and microglia, so it will be important to investigate this. Also, it will be necessary to assess the fate of the microglial cells that bear aSyn inclusions: do they die or they clear the inclusions and stay alive and functional? Do microglia share a common mechanism for inclusion formation with neurons? If this is the case, why do inclusions look different in microglia, and also in oligodendrocytes in MSA? The tools currently available may not enable us to address all these questions in detail, but we also need better approaches to characterize the full spectrum of immune responses in model organisms and in patients. Ultimately, such advances may enable the development diagnostic approaches, and also the identification of targets for intervention.

15 | PD BIOMARKERS BASED ON ASYN

15.1 | What we know

The diagnosis of PD is mostly based on clinical criteria that assess motor features. This means diagnosis is achieved once the disease is already established. A major limitation is that the typical motor features of PD overall with those present in other related synucleinopathies. Genetic testing is not a solution since the vast majority of synucleinopathies are sporadic. Therefore, there is a great need to identify markers that can aid in the diagnosis and in the monitoring of disease progression. In this context, and since aSyn was found in the CSF, suggesting it is released from brain cells, hope has been built on the possibility that measuring the levels of aSyn might be used as a biomarker in synucleinopathies.

Several different assays to measure aSyn have been developed, and these try to measure different forms of aSyn present in body humors, such as the CSF, plasma, blood, saliva, or tear fluid (Maass, Schulz, Lingor, Mollenhauer, & Bahr, 2019). Currently, the most advanced studies are based on measurements in CSF samples (Eusebi et al., 2017; Fayyad et al., 2019; Mollenhauer, Bowman, et al., 2019a).

Other studies suggest that aSyn PTMs may also hold value as possible disease biomarkers. These measurements might be done in CSF, but also in blood (Porro, Panaro, Lofrumento, Hasalla, & Trotta, 2019; Schmid, Fauvet, Moniatte, & Lashuel, 2013; Vicente Miranda, Cássio, et al., 2017a).

The ratio between different aSyn isoforms has also been proposed as a sensitive and specific diagnostic tool for PD (Hansson et al., 2014; Parnetti et al., 2014; Tokuda et al., 2006).

15.2 | What we do not know

Although aSyn is regarded as a possible biomarker for PD, we do not know at what stage of the disease we should measure aSyn, on which biological fluid, and which form (monomeric, oligomeric or fibrillar). We also need more standardized assays to ensure that different laboratories in the world can measure aSyn and obtain comparable results (Mollenhauer, Caspell-Garcia, et al., 2019b).

We also need to establish whether certain aSyn PTMs can indeed be used as biomarkers of disease, but this requires additional knowledge of their effects (Magdalinou et al., 2017).

Finally, we also do not know whether Lewy pathology load correlates well with disease progression, as we lack adequate tools to study this in vivo, with minimally invasive procedures, such as PET or other imaging-based techniques.

15.3 | What key experiments/tools are necessary?

As alluded above, we need highly sensitive and standardized assays to measure aSyn, and aSyn PTMs in various biological fluids. This may require improved antibodies and detection methods.

We will also need to develop PET-tracers that can detect aSyn, to enable longitudinal studies in patients, and even in individuals at risk (based on genetic factors, for example). Attempts to develop radioligands able to bind to different forms of aSyn are ongoing, but we still face major challenges such as selectivity, or blood–brain barrier penetration (Cairns et al., 2018; Merchant et al., 2019; Verdurand et al., 2018). However, the coming years may bring novel developments in this area.

One of the most promising techniques for the detection aggregated, spreading competent forms of relies on the use of real time quaking induced complementation (RT-QuiC) and protein misfolding cyclic amplification. These techniques are based on the ability

of amyloid structures to bind the fluorescent dye thioflavin T, resulting in a characteristic emission spectrum. These assays, especially RT-QuiC, appears able to accurately detect PD in CSF samples. Therefore, it will be important to improve these methods further to ensure reproducibility and sensitivity, so they can be used as diagnostic tools (Manne et al., 2019; Saijo et al., 2019).

In summary, we need to develop, for example, (a) more specific tools, such as antibodies or mass spectrometry-based, (b) aSyn PET tracers, and (b) assays, such as RT-QuiC/protein misfolding cyclic amplification, for measuring aSyn in different samples and at different stages of synucleinopathy.

16 | OUTLOOK

The Synuclein Meeting 2019 constituted a unique forum to discuss ideas, to recognize limitations and sources of controversy, and to set the stage for where the field needs to go. Despite the many challenges, it is undeniable we have come a long way. We need to continue to address many basic questions for which we lack complete answers and, importantly, we need to be humbled by the fact that there is still a lot to discover. This will ensure we will maintain the open-minded spirit that will enable us to work as a team, sharing resources and data, to get to where patients and families need us to go, which is to have novel tools to diagnose and treat the various synucleinopathies.

ACKNOWLEDGEMENTS

TFO is supported by DFG SFB1286 (projects B6 and B8). TFO and DFL are supported by a grant from International Parkinson Fonds Deutschland. TFO and IB are supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 721802 SynDegen.

CONFLICT OF INTEREST

Tiago F. Outeiro was the Editor of the JNC Special Issue on Alpha-Synuclein dedicated to the Synuclein Meeting 2019. The other authors have no conflicts of interest to declare.

ORCID

Tiago Fleming Outeiro https://orcid.org/0000-0003-1679-1727

REFERENCES

Abati, E., Di Fonzo, A., & Corti, S. (2018). In vitro models of multiple system atrophy from primary cells to induced pluripotent stem cells. *Journal of Cellular and Molecular Medicine*, *22*, 2536–2546. https://doi.org/10.1111/jcmm.13563

Abeyawardhane, D. L., Fernandez, R. D., Heitger, D. R., Crozier, M. K., Wolver, J. C., & Lucas, H. R. (2018). Copper induced radical dimerization of alpha-synuclein requires histidine. *Journal of the American Chemical Society*, *140*, 17086–17094.

Anderson, J. P., Walker, D. E., Goldstein, J. M., De Laat, R., Banducci, K., Caccavello, R. J., … Diep, L. (2006). Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *The Journal of Biological Chemistry*, *281*, 29739–29752.

Baptista, M. J., O'Farrell, C., Daya, S., Ahmad, R., Miller, D. W., Hardy, J., … Cookson, M. R. (2003). Co-ordinate transcriptional regulation of dopamine synthesis genes by alpha-synuclein in human neuroblastoma cell lines. *Journal of Neurochemistry*, *85*, 957–968.

Barbour, R., Kling, K., Anderson, J. P., Banducci, K., Cole, T., Diep, L., … Chilcote, T. J. (2008). Red blood cells are the major source of alpha-synuclein in blood. *Neuro-degenerative Diseases*, *5*, 55–59. https://doi.org/10.1159/000112832

Bardien, S., Lesage, S., Brice, A., & Carr, J. (2011). Genetic characteristics of leucine-rich repeat kinase 2 (LRRK2) associated Parkinson's disease. *Parkinsonism & Related Disorders*, *17*(7), 501–508. https://doi.org/10.1016/j.parkreldis.2010.11.008.

Barrenschee, M., Zorenkov, D., Böttner, M., Lange, C., Cossais, F., Scharf, A. B., … Wedel, T. (2017). Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. *Acta Neuropathologica Communications*, *5*, 1. https://doi.org/10.1186/s40478-016-0408-2

Bartels, T., Choi, J. G., & Selkoe, D. J. (2011). alpha-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature*, *477*, 107–110.

Beach, T. G., Adler, C. H., Lue, L. F., Sue, L. I., Bachalakuri, J., Henry-Watson, J., … Walker, D. G. (2009). Unified staging system for Lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathologica*, *117*, 613–634. https://doi.org/10.1007/s00401-009-0538-8

Bergeron, C., Petrunka, C., Weyer, L., & Pollanen, M. S. (1996). Altered neurofilament expression does not contribute to Lewy body formation. *The American Journal of Pathology*, *148*, 267–272.

Bernstein, H. G., Johnson, M., Perry, R. H., LeBeau, F. E., Dobrowolny, H., Bogerts, B., & Perry, E. K. (2011). Partial loss of parvalbumin-containing hippocampal interneurons in dementia with Lewy bodies. *Neuropathology: Official Journal of the Japanese Society of Neuropathology*, *31*, 1–10. https://doi.org/10.1111/j.1440-1789.2010.01117.x

Binolfi, A., Rasia, R. M., Bertoncini, C. W., Ceolin, M., Zweckstetter, M., Griesinger, C., … Fernandez, C. O. (2006). Interaction of alpha-synuclein with divalent metal ions reveals key differences: A link between structure, binding specificity and fibrillation enhancement. *Journal of the American Chemical Society*, *128*, 9893–9901.

Blauwendraat, C., Nalls, M. A., & Singleton, A. B. (2019). The genetic architecture of Parkinson's disease. *The Lancet Neurology*, *19*, 170–178. https://doi.org/10.1016/S1474-4422(19)30287-X

Bousset, L., Pieri, L., Ruiz-Arlandis, G., Gath, J., Jensen, P. H., Habenstein, B., … Melki, R. (2013). Structural and functional characterization of two alpha-synuclein strains. *Nature Communications*, *4*, 2575. https://doi.org/10.1038/ncomms3575

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*, 197–211. https://doi.org/10.1016/S0197-4580(02)00065-9

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research*, *318*, 121–134. https://doi.org/10.1007/s00441-004-0956-9

Breda, C., Nugent, M. L., Estranero, J. G., Kyriacou, C. P., Outeiro, T. F., Steinert, J. R., & Giorgini, F. (2015). Rab11 modulates alpha-synuclein-mediated defects in synaptic transmission and behaviour. *Human Molecular Genetics*, *24*, 1077–1091.

Burre, J., Sharma, M., & Sudhof, T. C. (2012). Systematic mutagenesis of alpha-synuclein reveals distinct sequence requirements for physiological and pathological activities. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*, 15227–15242.

Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., & Sudhof, T. C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science*, *329*, 1663–1667. https://doi.org/10.1126/science.1195227

Butler, B., Saha, K., Rana, T., Becker, J. P., Sambo, D., Davari, P., … Khoshbouei, H. (2015). Dopamine transporter activity is modulated by alpha-synuclein. *The Journal of Biological Chemistry*, *290*, 29542–29554.

Cairns, A. G., Vazquez-Romero, A., Mahdi Moein, M., Ådén, J., Elmore, C. S., Takano, A., … Schou, M. (2018). Increased brain exposure of an alpha-synuclein fibrillization modulator by utilization of an activated ester prodrug strategy. *ACS Chemical Neuroscience*, *9*, 2542–2547. https://doi.org/10.1021/acschemneuro.8b00236

Caughey, B., & Lansbury, P. T. (2003). Protofibrils, pores, fibrils, and neurodegeneration: Separating the responsible protein aggregates from the innocent bystanders. *Annual Review of Neuroscience*, *26*, 267–298.

Chandra, S., Chen, X., Rizo, J., Jahn, R., & Sudhof, T. C. (2003). A broken alpha -helix in folded alpha-synuclein. *The Journal of Biological Chemistry*, *278*, 15313–15318.

Chen, R. H., Wislet-Gendebien, S., Samuel, F., Visanji, N. P., Zhang, G., Marsilio, D., … Tandon, A. (2013). alpha-Synuclein membrane association is regulated by the Rab3a recycling machinery and presynaptic activity. *The Journal of Biological Chemistry*, *288*, 7438–7449.

Chen, S. G., Stribinskis, V., Rane, M. J., Demuth, D. R., Gozal, E., Roberts, A. M., … Friedland, R. P. (2016). Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged Fischer 344 rats and *Caenorhabditis elegans*. *Scientific Reports*, *6*, 34477. https://doi.org/10.1038/srep34477

Chutna, O., Gonçalves, S., Villar-Piqué, A., Guerreiro, P., Marijanovic, Z., Mendes, T., … Barral, D. C. (2014). The small GTPase Rab11 co-localizes with alpha-synuclein in intracellular inclusions and modulates its aggregation, secretion and toxicity. *Human Molecular Genetics*, *23*, 6732–6745.

Cole, N. B., Murphy, D. D., Grider, T., Rueter, S., Brasaemle, D., & Nussbaum, R. L. (2002). Lipid droplet binding and oligomerization properties of the Parkinson's disease protein alpha-synuclein. *The Journal of Biological Chemistry*, *277*, 6344–6352.

Colla, E., Coune, P., Liu, Y., Pletnikova, O., Troncoso, J. C., Iwatsubo, T., … Lee, M. K. (2012). Endoplasmic reticulum stress is important for the manifestations of alpha-synucleinopathy in vivo. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*, 3306–3320.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Harper, J. D., Williamson, R. E., & Lansbury, P. T. Jr (2000). Accelerated oligomerization by Parkinson's disease linked alpha-synuclein mutants. *Annals of the New York Academy of Sciences*, *920*, 42–45.

Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., … Cao, S. (2006). Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science*, *313*, 324–328. https://doi.org/10.1126/science.1129462

Covell, D. J., Robinson, J. L., Akhtar, R. S., Grossman, M., Weintraub, D., Bucklin, H. M., … Lee, V. Y. (2017). Novel conformation-selective alpha-synuclein antibodies raised against different in vitro fibril forms show distinct patterns of Lewy pathology in Parkinson's disease. *Neuropathology and Applied Neurobiology*, *43*, 604–620.

Cullen, V., Lindfors, M., Ng, J., Paetau, A., Swinton, E., Kolodzeij, P., … Tyynela, J. (2009). Cathepsin D expression level affects alpha-synuclein processing, aggregation, and toxicity in vivo. *Molecular Brain*, *2*, 5. https://doi.org/10.1186/1756-6606-2-5

Dahmene, M., Berard, M., & Oueslati, A. (2017). Dissecting the molecular pathway involved in PLK2 kinase-mediated alpha-synuclein-selective autophagic degradation. *The Journal of Biological Chemistry*, *292*, 3919–3928.

Danzer, K. M., Kranich, L. R., Ruf, W. P., Cagsal-Getkin, O., Winslow, A. R., Zhu, L., … McLean, P. J. (2012). Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Molecular Neurodegeneration*, *7*, 42. https://doi.org/10.1186/1750-1326-7-42

de Oliveira, R. M., Vicente Miranda, H., Francelle, L., Pinho, R., Szegö, É. M., Martinho, R., … Outeiro, T. F. (2017). The mechanism of sirtuin 2-mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease. *PLoS Biology*, *15*, e2000374. https://doi.org/10.1371/journal.pbio.2000374

Delenclos, M., Burgess, J. D., Lamprokostopoulou, A., Outeiro, T. F., Vekrellis, K., & McLean, P. J. (2019). Cellular models of alpha-synuclein toxicity and aggregation. *Journal of Neurochemistry*, *150*, 566–576. https://doi.org/10.1111/jnc.14806

Devi, L., & Anandatheerthavarada, H. K. (2010). Mitochondrial trafficking of APP and alpha synuclein: Relevance to mitochondrial dysfunction in Alzheimer's and Parkinson's diseases. *Biochimica Et Biophysica Acta*, *1802*, 11–19. https://doi.org/10.1016/j.bbadis.2009.07.007

Devi, L., Raghavendran, V., Prabhu, B. M., Avadhani, N. G., & Anandatheerthavarada, H. K. (2008). Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *The Journal of Biological Chemistry*, *283*, 9089–9100.

Di Maio, R., Barrett, P. J., Hoffman, E. K., Barrett, C. W., Zharikov, A., Borah, A., … Hastings, T. G. (2016). alpha-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. *Science Translational Medicine*, *8*, 342ra378.

Dijkstra, A. A., Voorn, P., Berendse, H. W., Groenewegen, H. J., Rozemuller, A. J., & van de Berg, W. D. (2014). Stage-dependent nigral neuronal loss in incidental Lewy body and Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, *29*, 1244–1251. https://doi.org/10.1002/mds.25952

Doorn, K. J., Moors, T., Drukarch, B., van de Berg, W., Lucassen, P. J., & van Dam, A. M. (2014). Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. *Acta Neuropathologica Communications*, *2*, 90.

Duffy, P. E., & Tennyson, V. M. (1965). Phase and electron microscopic observations of Lewy bodies and melanin granules in the substantia nigra and locus caeruleus in Parkinson's disease. *Journal of Neuropathology and Experimental Neurology*, *24*, 398–414.

Dugger, B. N., & Dickson, D. W. (2010). Cell type specific sequestration of choline acetyltransferase and tyrosine hydroxylase within Lewy bodies. *Acta Neuropathologica*, *120*, 633–639. https://doi.org/10.1007/s00401-010-0739-1

Ebrahimi-Fakhari, D., Cantuti-Castelvetri, I., Fan, Z., Rockenstein, E., Masliah, E., Hyman, B. T., … Unni, V. K. (2011). Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of alpha-synuclein. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*, 14508–14520.

Ejlerskov, P., Rasmussen, I., Nielsen, T. T., Bergstrom, A. L., Tohyama, Y., Jensen, P. H., & Vilhardt, F. (2013). Tubulin polymerization-promoting protein (TPPP/p25alpha) promotes unconventional secretion of alpha-synuclein through exophagy by impairing autophagosome-lysosome fusion. *The Journal of Biological Chemistry*, *288*, 17313–17335.

El-Agnaf, O., Overk, C., Rockenstein, E., Mante, M., Florio, J., Adame, A., … Masliah, E. (2017). Differential effects of immunotherapy with antibodies targeting alpha-synuclein oligomers and fibrils in a transgenic model of synucleinopathy. *Neurobiology of Disease*, *104*, 85–96.

Eliezer, D., Kutluay, E., Bussell, R. Jr, & Browne, G. (2001). Conformational properties of alpha-synuclein in its free and lipid-associated states. *Journal of Molecular Biology*, *307*, 1061–1073.

Emmanouilidou, E., Melachroinou, K., Roumeliotis, T., Garbis, S. D., Ntzouni, M., Margaritis, L. H., … Vekrellis, K. (2010). Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*, 6838–6851.

Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., … Dawson, V. L. (1999). Synphilin-1 associates with

alpha-synuclein and promotes the formation of cytosolic inclusions. *Nature Genetics*, *22*, 110–114.

Eusebi, P., Giannandrea, D., Biscetti, L., Abraha, I., Chiasserini, D., Orso, M., … Parnetti, L. (2017). Diagnostic utility of cerebrospinal fluid alpha-synuclein in Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders: Official Journal of the Movement Disorder Society*, *32*, 1389–1400.

Fares, M. B., Ait-Bouziad, N., Dikiy, I., Mbefo, M. K., Jovičić, A., Kiely, A., … Lashuel, H. A. (2014). The novel Parkinson's disease linked mutation G51D attenuates in vitro aggregation and membrane binding of alpha-synuclein, and enhances its secretion and nuclear localization in cells. *Human Molecular Genetics*, *23*, 4491–4509.

Fayyad, M., Salim, S., Majbour, N., Erskine, D., Stoops, E., Mollenhauer, B., & El-Agnaf, O. M. A. (2019). Parkinson's disease biomarkers based on alpha-synuclein. *Journal of Neurochemistry*, *150*, 626–636.

Feany, M. B., & Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature*, *404*, 394–398. https://doi.org/10.1038/35006074

Ferreira, D. G., Temido-Ferreira, M., Vicente Miranda, H., Batalha, V. L., Coelho, J. E., Szegö, É. M., … Zerr, I. (2017). alpha-Synuclein interacts with PrP(C) to induce cognitive impairment through mGluR5 and NMDAR2B. *Nature Neuroscience*, *20*, 1569–1579.

Forsyth, C. B., Shannon, K. M., Kordower, J. H., Voigt, R. M., Shaikh, M., Jaglin, J. A., … Keshavarzian, A. (2011). Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE*, *6*, e28032. https://doi.org/10.1371/journal.pone.0028032

Fortin, D. L., Troyer, M. D., Nakamura, K., Kubo, S., Anthony, M. D., & Edwards, R. H. (2004). Lipid rafts mediate the synaptic localization of alpha-synuclein. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *24*, 6715–6723.

Freundt, E. C., Maynard, N., Clancy, E. K., Roy, S., Bousset, L., Sourigues, Y., … Brahic, M. (2012). Neuron-to-neuron transmission of alpha-synuclein fibrils through axonal transport. *Annals of Neurology*, *72*, 517–524.

Friedman, L. G., Lachenmayer, M. L., Wang, J., He, L., Poulose, S. M., Komatsu, M., … Yue, Z. (2012). Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and LRRK2 in the brain. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*, 7585–7593.

Fukuda, T., Takahashi, J., & Tanaka, J. (1999). Tyrosine hydroxylase-immunoreactive neurons are decreased in number in the cerebral cortex of Parkinson's disease. *Neuropathology: Official Journal of the Japanese Society of Neuropathology*, *19*, 10–13. https://doi.org/10.1046/j.1440-1789.1999.00196.x

Gamache, J., Benzow, K., Forster, C., Kemper, L., Hlynialuk, C., Furrow, E., … Koob, M. D. (2019). Factors other than hTau overexpression that contribute to tauopathy-like phenotype in rTg4510 mice. *Nature Communications*, *10*, 2479. https://doi.org/10.1038/s41467-019-10428-1

Ghio, S., Kamp, F., Cauchi, R., Giese, A., & Vassallo, N. (2016). Interaction of alpha-synuclein with biomembranes in Parkinson's disease–role of cardiolipin. *Progress in Lipid Research*, *61*, 73–82.

Gitler, A. D., Chesi, A., Geddie, M. L., Strathearn, K. E., Hamamichi, S., Hill, K. J., … Lindquist, S. (2009). Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nature Genetics*, *41*, 308–315.

Goers, J., Manning-Bog, A. B., McCormack, A. L., Millett, I. S., Doniach, S., Di Monte, D. A., … Fink, A. L. (2003). Nuclear localization of alpha-synuclein and its interaction with histones. *Biochemistry*, *42*, 8465–8471.

Goncalves, S., & Outeiro, T. F. (2013). Assessing the subcellular dynamics of alpha-synuclein using photoactivation microscopy. *Molecular Neurobiology*, *47*, 1081–1092. https://doi.org/10.1007/s12035-013-8406-x

Grassi, D., Howard, S., Zhou, M., Diaz-Perez, N., Urban, N. T., Guerrero-Given, D., … Lasmézas, C. I. (2018). Identification of a highly neurotoxic alpha-synuclein species inducing mitochondrial damage and mitophagy in Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, E2634–E2643.

Grozdanov, V., & Danzer, K. M. (2018). Release and uptake of pathologic alpha-synuclein. *Cell and Tissue Research*, *373*, 175–182. https://doi.org/10.1007/s00441-017-2775-9

Guerreiro, P. S., Huang, Y., Gysbers, A., Cheng, D., Gai, W. P., Outeiro, T. F., & Halliday, G. M. (2013). LRRK2 interactions with alpha-synuclein in Parkinson's disease brains and in cell models. *Journal of Molecular Medicine*, *91*, 513–522.

Guo, J. T., Chen, A. Q., Kong, Q., Zhu, H., Ma, C. M., & Qin, C. (2008). Inhibition of vesicular monoamine transporter-2 activity in alpha-synuclein stably transfected SH-SY5Y cells. *Cellular and Molecular Neurobiology*, *28*, 35–47.

Guo, J. L., Covell, D. J., Daniels, J. P., Iba, M., Stieber, A., Zhang, B., … Lee, V. M. (2013). Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. *Cell*, *154*, 103–117.

Halliday, G. M., Li, Y. W., Blumbergs, P. C., Joh, T. H., Cotton, R. G., Howe, P. R., … Geffen, L. B. (1990). Neuropathology of immunohistochemically identified brainstem neurons in Parkinson's disease. *Annals of Neurology*, *27*, 373–385. https://doi.org/10.1002/ana.410270405

Hansson, O., Hall, S., Ohrfelt, A., Zetterberg, H., Blennow, K., Minthon, L., … Al-Hayani, A. (2014). Levels of cerebrospinal fluid alpha-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease. *Alzheimer's Research and Therapy*, *6*, 25.

Harding, A. J., Stimson, E., Henderson, J. M., & Halliday, G. M. (2002). Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. *Brain: A Journal of Neurology*, *125*, 2431–2445. https://doi.org/10.1093/brain/awf251

Heintz-Buschart, A., Pandey, U., Wicke, T., Sixel-Döring, F., Janzen, A., Sittig-Wiegand, E., … Wilmes, P. (2018). The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Movement Disorders: Official Journal of the Movement Disorder Society*, *33*, 88–98. https://doi.org/10.1002/mds.27105

Hill-Burns, E. M., Debelius, J. W., Morton, J. T., Wissemann, W. T., Lewis, M. R., Wallen, Z. D., … Knight, R. (2017). Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Movement Disorders: Official Journal of the Movement Disorder Society*, *32*, 739–749.

Hoffmann, A., Ettle, B., Bruno, A., Kulinich, A., Hoffmann, A. C., von Wittgenstein, J., … Schlachetzki, J. C. M. (2016). Alpha-synuclein activates BV2 microglia dependent on its aggregation state. *Biochemical and Biophysical Research Communications*, *479*, 881–886. https://doi.org/10.1016/j.bbrc.2016.09.109

Holmes, B. B., DeVos, S. L., Kfoury, N., Li, M., Jacks, R., Yanamandra, K., … Diamond, M. I. (2013). Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, E3138–3147. https://doi.org/10.1073/pnas.1301440110

Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., … Li, J.-Y. (2014). Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathologica*, *128*, 805–820. https://doi.org/10.1007/s00401-014-1343-6

Iannaccone, S., Cerami, C., Alessio, M., Garibotto, V., Panzacchi, A., Olivieri, S., … Perani, D. (2013). In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. *Parkinsonism and Related Disorders*, *19*, 47–52.

Ihse, E., Yamakado, H., van Wijk, X. M., Lawrence, R., Esko, J. D., & Masliah, E. (2017). Cellular internalization of alpha-synuclein aggregates by cell surface heparan sulfate depends on aggregate

conformation and cell type. *Scientific Reports*, *7*, 9008. https://doi.org/10.1038/s41598-017-08720-5

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., & Hashizume, Y. (2003). Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathologica*, *106*, 518–526. https://doi.org/10.1007/s00401-003-0766-2

Ishizawa, T., Mattila, P., Davies, P., Wang, D., & Dickson, D. W. (2003). Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *Journal of Neuropathology and Experimental Neurology*, *62*, 389–397. https://doi.org/10.1093/jnen/62.4.389

Iwai, A., Masliah, E., Yoshimoto, M., Ge, N., Flanagan, L., de Silva, H. A., … Saitoh, T. (1995). The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron*, *14*, 467–475.

Jensen, P. H., Nielsen, M. S., Jakes, R., Dotti, C. G., & Goedert, M. (1998). Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. *The Journal of Biological Chemistry*, *273*, 26292–26294.

Jiang, P., Gan, M., Yen, S. H., McLean, P. J., & Dickson, D. W. (2017). Impaired endo-lysosomal membrane integrity accelerates the seeding progression of alpha-synuclein aggregates. *Scientific Reports*, *7*, 7690.

Jo, E., McLaurin, J., Yip, C. M., St George-Hyslop, P., & Fraser, P. E. (2000). alpha-Synuclein membrane interactions and lipid specificity. *The Journal of Biological Chemistry*, *275*, 34328–34334.

Kahle, P. J., Neumann, M., Ozmen, L., Müller, V., Jacobsen, H., Schindzielorz, A., … Kremmer, E. (2000). Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha -synuclein in human and transgenic mouse brain. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *20*, 6365–6373.

Kalia, L. V., Lang, A. E., Hazrati, L. N., Fujioka, S., Wszolek, Z. K., Dickson, D. W., … Alcalay, R. N. (2015). Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurology*, *72*, 100–105.

Karpowicz, R. J. Jr, Haney, C. M., Mihaila, T. S., Sandler, R. M., Petersson, E. J., & Lee, V. M. (2017). Selective imaging of internalized proteopathic alpha-synuclein seeds in primary neurons reveals mechanistic insight into transmission of synucleinopathies. *The Journal of Biological Chemistry*, *292*, 13482–13497.

Katsuse, O., Iseki, E., Marui, W., & Kosaka, K. (2003). Developmental stages of cortical Lewy bodies and their relation to axonal transport blockage in brains of patients with dementia with Lewy bodies. *Journal of the Neurological Sciences*, *211*, 29–35. https://doi.org/10.1016/S0022-510X(03)00037-6

Katzeff, J. S., Phan, K., Purushothuman, S., Halliday, G. M., & Kim, W. S. (2019). Cross-examining candidate genes implicated in multiple system atrophy. *Acta Neuropathologica Communications*, *7*, 117. https://doi.org/10.1186/s40478-019-0769-4

Keshavarzian, A., Green, S. J., Engen, P. A., Voigt, R. M., Naqib, A., Forsyth, C. B., … Shannon, K. M. (2015). Colonic bacterial composition in Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, *30*, 1351–1360. https://doi.org/10.1002/mds.26307

Khalaf, O., Fauvet, B., Oueslati, A., Khalaf, O., Bruno, F. B., Oueslati, A., … Lashuel, H. A. (2014). The H50Q mutation enhances alpha-synuclein aggregation, secretion, and toxicity. *The Journal of Biological Chemistry*, *289*, 21856–21876.

Killinger, B. A., & Kordower, J. H. (2019). Spreading of alpha-synuclein - relevant or epiphenomenon? *Journal of Neurochemistry*, *150*, 605–611. https://doi.org/10.1111/jnc.14779

Killinger, B. A., Madaj, Z., Sikora, J. W., Rey, N., Haas, A. J., Vepa, Y., … Labrie, V. (2018). The vermiform appendix impacts the risk of developing Parkinson's disease. *Science Translational Medicine*, *10*, eaar5280.

Kim, C., Lv, G., Lee, J. S., Jung, B. C., Masuda-Suzukake, M., Hong, C. S., … Masliah, E. (2016). Exposure to bacterial endotoxin generates a distinct strain of alpha-synuclein fibril. *Scientific Reports*, *6*, 30891.

Kim, S., Kwon, S. H., Kam, T. I., Panicker, N., Karuppagounder, S. S., Lee, S., … Shen, C. (2019). Transneuronal propagation of pathologic alpha-synuclein from the Gut to the brain models Parkinson's disease. *Neuron*, *103*(627–641), e627.

Klucken, J., Poehler, A. M., Ebrahimi-Fakhari, D., Schneider, J., Nuber, S., Rockenstein, E., … Winkler, J. (2012). Alpha-synuclein aggregation involves a bafilomycin A 1-sensitive autophagy pathway. *Autophagy*, *8*, 754–766.

Kontopoulos, E., Parvin, J. D., & Feany, M. B. (2006). Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. *Human Molecular Genetics*, *15*, 3012–3023.

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B., & Olanow, C. W. (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature Medicine*, *14*, 504–506. https://doi.org/10.1038/nm1747

Kotzbauer, P. T., Tu, Z., & Mach, R. H. (2017). Current status of the development of PET radiotracers for imaging alpha synuclein aggregates in Lewy bodies and Lewy neurites. *Clinical and Translational Imaging*, *5*, 3–14. https://doi.org/10.1007/s40336-016-0217-4

Kragh, C. L., Lund, L. B., Febbraro, F., Hansen, H. D., Gai, W. P., El-Agnaf, O., … Jensen, P. H. (2009). Alpha-synuclein aggregation and Ser-129 phosphorylation-dependent cell death in oligodendroglial cells. *The Journal of Biological Chemistry*, *284*, 10211–10222.

Kramer, M. L., & Schulz-Schaeffer, W. J. (2007). Presynaptic alpha-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*, 1405–1410.

Kubo, S., Nemani, V. M., Chalkley, R. J., Anthony, M. D., Hattori, N., Mizuno, Y., … Fortin, D. L. (2005). A combinatorial code for the interaction of alpha-synuclein with membranes. *The Journal of Biological Chemistry*, *280*, 31664–31672.

Kuzuhara, S., Mori, H., Izumiyama, N., Yoshimura, M., & Ihara, Y. (1988). Lewy bodies are ubiquitinated. A light and electron microscopic immunocytochemical study. *Acta Neuropathologica*, *75*, 345–353. https://doi.org/10.1007/BF00687787

Lautenschlager, J., Stephens, A. D., Fusco, G., Ströhl, F., Curry, N., Zacharopoulou, M., … Pinotsi, D. (2018). C-terminal calcium binding of alpha-synuclein modulates synaptic vesicle interaction. *Nature Communications*, *9*, 712.

Lazaro, D. F., Pavlou, M. A. S., & Outeiro, T. F. (2017). Cellular models as tools for the study of the role of alpha-synuclein in Parkinson's disease. *Experimental Neurology*, *298*, 162–171. https://doi.org/10.1016/j.expneurol.2017.05.007

Lázaro, D. F., Rodrigues, E. F., Langohr, R., Shahpasandzadeh, H., Ribeiro, T., Guerreiro, P., … Outeiro, T. F. (2014). Systematic comparison of the effects of alpha-synuclein mutations on its oligomerization and aggregation. *PLoS Genetics*, *10*, e1004741. https://doi.org/10.1371/journal.pgen.1004741

Lee, H. J., Shin, S. Y., Choi, C., Lee, Y. H., & Lee, S. J. (2002). Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *The Journal of Biological Chemistry*, *277*, 5411–5417.

Lee, H. J., Suk, J. E., Bae, E. J., & Lee, S. J. (2008a). Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochemical and Biophysical Research Communications*, *372*, 423–428.

Lee, J. T., Wheeler, T. C., Li, L., & Chin, L. S. (2008b). Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. *Human Molecular Genetics*, *17*, 906–917.

Leverenz, J. B., Hamilton, R., Tsuang, D. W., Schantz, A., Vavrek, D., Larson, E. B., … Kaye, J. (2008). Empiric refinement of the pathologic assessment of Lewy-related pathology in the dementia patient. *Brain Pathology (Zurich, Switzerland)*, *18*, 220–224.

Lewy, F. (1912). Paralysis agitans. I. Pathologische Anatomie, In *Handbuch Der Neurologie*, Vol. *3* (pp. 920–933). Berlin: Springer-Verlag.

Li, J.-Y., Englund, E., Holton, J. L., Soulet, D., Hagell, P., Lees, A. J., … Brundin, P. (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nature Medicine*, *14*, 501–503. https://doi.org/10.1038/nm1746

Li, W., Wu, X., Hu, X., Wang, T., Liang, S., Duan, Y., … Qin, B. (2017). Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Science China. Life Sciences*, *60*, 1223–1233.

Li, W. W., Yang, R., Guo, J. C., Ren, H. M., Zha, X. L., Cheng, J. S., & Cai, D. F. (2007). Localization of alpha-synuclein to mitochondria within midbrain of mice. *NeuroReport*, *18*, 1543–1546.

Lindersson, E., Beedholm, R., Hojrup, P., Moos, T., Gai, W., Hendil, K. B., & Jensen, P. H. (2004). Proteasomal inhibition by alpha-synuclein filaments and oligomers. *The Journal of Biological Chemistry*, *279*, 12924–12934.

Liu, B., Fang, F., Pedersen, N. L., Tillander, A., Ludvigsson, J. F., Ekbom, A., … Wirdefeldt, K. (2017). Vagotomy and Parkinson disease: A Swedish register-based matched-cohort study. *Neurology*, *88*, 1996–2002. https://doi.org/10.1212/WNL.0000000000003961

Liu, C. W., Corboy, M. J., DeMartino, G. N., & Thomas, P. J. (2003). Endoproteolytic activity of the proteasome. *Science*, *299*, 408–411. https://doi.org/10.1126/science.1079293

Lopes da Fonseca, T., Pinho, R., & Outeiro, T. F. (2016). A familial ATP13A2 mutation enhances alpha-synuclein aggregation and promotes cell death. *Human Molecular Genetics*, *25*, 2959–2971. https://doi.org/10.1093/hmg/ddw147

Loria, F., Vargas, J. Y., Bousset, L., Syan, S., Salles, A., Melki, R., & Zurzolo, C. (2017). alpha-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. *Acta Neuropathologica*, *134*, 789–808.

Lothian, A., Lago, L., Mukherjee, S., Connor, A. R., Fowler, C., McLean, C. A., … Roberts, B. R. (2019). Characterization of the metal status of natively purified alpha-synuclein from human blood, brain tissue, or recombinant sources using size exclusion ICP-MS reveals no significant binding of Cu, Fe or Zn. *Metallomics: Integrated Biometal Science*, *11*, 128–140. https://doi.org/10.1039/C8MT00223A

Lowe, J., Blanchard, A., Morrell, K., Lennox, G., Reynolds, L., Billett, M., … Mayer, R. J. (1988). Ubiquitin is a common factor in intermediate filament inclusion bodies of diverse type in man, including those of Parkinson's disease, Pick's disease, and Alzheimer's disease, as well as Rosenthal fibres in cerebellar astrocytomas, cytoplasmic bodies in muscle, and mallory bodies in alcoholic liver disease. *The Journal of Pathology*, *155*, 9–15. https://doi.org/10.1002/path.1711550105

Luk, K. C., Kehm, V. M., Zhang, B., O'Brien, P., Trojanowski, J. Q., & Lee, V. M. (2012). Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. *The Journal of Experimental Medicine*, *209*, 975–986.

Luk, K. C., Song, C., O'Brien, P., Stieber, A., Branch, J. R., Brunden, K. R., … Lee, V. M. (2009). Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 20051–20056.

Lwin, A., Orvisky, E., Goker-Alpan, O., LaMarca, M. E., & Sidransky, E. (2004). Glucocerebrosidase mutations in subjects with parkinsonism. *Molecular Genetics and Metabolism*, *81*(1), 70–73. https://doi.org/10.1016/j.ymgme.2003.11.004.

Maass, F., Schulz, I., Lingor, P., Mollenhauer, B., & Bahr, M. (2019). Cerebrospinal fluid biomarker for Parkinson's disease: An overview. *Molecular and Cellular Neurosciences*, *97*, 60–66. https://doi.org/10.1016/j.mcn.2018.12.005

Magdalinou, N. K., Noyce, A. J., Pinto, R., Lindstrom, E., Holmén-Larsson, J., Holtta, M., … Gobom, J. (2017). Identification of candidate cerebrospinal fluid biomarkers in parkinsonism using quantitative proteomics. *Parkinsonism and Related Disorders*, *37*, 65–71. https://doi.org/10.1016/j.parkreldis.2017.01.016

Mak, S. K., McCormack, A. L., Manning-Bog, A. B., Cuervo, A. M., & Di Monte, D. A. (2010). Lysosomal degradation of alpha-synuclein in vivo. *The Journal of Biological Chemistry*, *285*, 13621–13629.

Manne, S., Kondru, N., Hepker, M., Jin, H., Anantharam, V., Lewis, M., … Kanthasamy, A. G. (2019). Ultrasensitive detection of aggregated alpha-synuclein in glial cells, human cerebrospinal fluid, and brain tissue using the RT-QuIC assay: New high-throughput neuroimmune biomarker assay for parkinsonian disorders. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *14*, 423–435.

Mao, X., Ou, M. T., Karuppagounder, S. S., Kam, T. I., Yin, X., Xiong, Y., … Kang, H. C. (2016) Pathological alpha-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science*, *353*, aah3374.

Maroteaux, L., Campanelli, J. T., & Scheller, R. H. (1988). Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *8*, 2804–2815.

Martin, L. J., Pan, Y., Price, A. C., Sterling, W., Copeland, N. G., Jenkins, N. A., … Lee, M. K. (2006). Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *26*, 41–50.

Martinez-Vicente, M., Talloczy, Z., Kaushik, S., Massey, A. C., Mazzulli, J., Mosharov, E. V., … Dauer, W. (2008). Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *The Journal of Clinical Investigation*, *118*, 777–788.

Marui, W., Iseki, E., Kato, M., & Kosaka, K. (2003). Degeneration of tyrosine hydroxylase-immunoreactive neurons in the cerebral cortex and hippocampus of patients with dementia with Lewy bodies. *Neuroscience Letters*, *340*, 185–188. https://doi.org/10.1016/S0304-3940(03)00101-0

Marvian, A. T., Koss, D. J., Aliakbari, F., Morshedi, D., & Outeiro, T. F. (2019). In vitro models of synucleinopathies: Informing on molecular mechanisms and protective strategies. *Journal of Neurochemistry*, *150*, 535–565.

Masaracchia, C., Hnida, M., Gerhardt, E., Lopes da Fonseca, T., Villar-Pique, A., Branco, T., … Outeiro, T. F. (2018). Membrane binding, internalization, and sorting of alpha-synuclein in the cell. *Acta Neuropathologica Communications*, *6*, 79. https://doi.org/10.1186/s40478-018-0578-1

Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., … Mucke, L. (2000). Dopaminergic loss and inclusion body formation in alpha-synuclein mice: Implications for neurodegenerative disorders. *Science*, *287*, 1265–1269.

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Oikawa, T., Arai, T., Akiyama, H., … Hasegawa, M. (2013). Prion-like spreading of pathological alpha-synuclein in brain. *Brain: A Journal of Neurology*, *136*, 1128–1138.

Mattila, P. M., Rinne, J. O., Helenius, H., Dickson, D. W., Röyttä, M., & Mattila, P. M. (2000). Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. *Acta Neuropathologica*, *100*, 285–290. https://doi.org/10.1007/s004019900168

Mazzulli, J. R., Zunke, F., Isacson, O., Studer, L., & Krainc, D. (2016). alpha-Synuclein-induced lysosomal dysfunction occurs through disruptions in protein trafficking in human midbrain synucleinopathy models. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 1931–1936.

McKeith, I. G., Boeve, B. F., Dickson, D. W., Halliday, G., Taylor, J.-P., Weintraub, D., … Kosaka, K. (2017). Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. *Neurology*, *89*, 88–100. https://doi.org/10.1212/WNL.0000000000004058

McLean, P. J., Kawamata, H., & Hyman, B. T. (2001). Alpha-synuclein-enhanced green fluorescent protein fusion proteins form proteasome sensitive inclusions in primary neurons. *Neuroscience*, *104*, 901–912.

McLean, P. J., Kawamata, H., Ribich, S., & Hyman, B. T. (2000). Membrane association and protein conformation of alpha-synuclein in intact neurons. Effect of Parkinson's disease-linked mutations. *The Journal of Biological Chemistry*, *275*, 8812–8816.

Mendes, A., Gonçalves, A., Vila-Chã, N., Moreira, I., Fernandes, J., Damásio, J., … Cavaco, S. (2015). Appendectomy may delay Parkinson's disease Onset. *Movement Disorders: Official Journal of the Movement Disorder Society*, *30*, 1404–1407. https://doi.org/10.1002/mds.26311

Merchant, K. M., Cedarbaum, J. M., Brundin, P., Dave, K. D., Eberling, J., Espay, A. J., … Weiner, D. M. (2019). A Proposed roadmap for Parkinson's disease proof of concept clinical trials investigating compounds targeting alpha-synuclein. *Journal of Parkinson's Disease*, *9*, 31–61. https://doi.org/10.3233/JPD-181471

Milber, J. M., Noorigian, J. V., Morley, J. F., Petrovitch, H., White, L., Ross, G. W., & Duda, J. E. (2012). Lewy pathology is not the first sign of degeneration in vulnerable neurons in Parkinson disease. *Neurology*, *79*, 2307–2314. https://doi.org/10.1212/WNL.0b013e318278fe32

Mollenhauer, B., Bowman, F. D., Drake, D., Duong, J., Blennow, K., El-Agnaf, O., … Dunty, J. M. (2019a). Antibody-based methods for the measurement of alpha-synuclein concentration in human cerebrospinal fluid - method comparison and round robin study. *Journal of Neurochemistry*, *149*, 126–138.

Mollenhauer, B., Caspell-Garcia, C. J., Coffey, C. S., Taylor, P., Singleton, A., Shaw, L. M., … Oertel, W. (2019b). Longitudinal analyses of cerebrospinal fluid alpha-Synuclein in prodromal and early Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, *34*, 1354–1364.

Moors, T. E., Maat, C. A., Niedieker, D., Mona, D., Petersen, D., Timmermans-Huisman, E., … Mundigl, O. (2018). Detailed structural orchestration of Lewy pathology in Parkinson's disease as revealed by 3D multicolor. STED Microscopy. *Biorxiv*, 470476.

Nakhjavani, M., Morteza, A., Khajeali, L., Esteghamati, A., Khalilzadeh, O., Asgarani, F., & Outeiro, T. F. (2010). Increased serum HSP70 levels are associated with the duration of diabetes. *Cell Stress and Chaperones*, *15*, 959–964. https://doi.org/10.1007/s12192-010-0204-z

Nathan, J. A., Kim, H. T., Ting, L., Gygi, S. P., & Goldberg, A. L. (2013). Why do cellular proteins linked to K63-polyubiquitin chains not associate with proteasomes? *The EMBO Journal*, *32*, 552–565. https://doi.org/10.1038/emboj.2012.354

Oaks, A. W., Marsh-Armstrong, N., Jones, J. M., Credle, J. J., & Sidhu, A. (2013). Synucleins antagonize endoplasmic reticulum function to modulate dopamine transporter trafficking. *PLoS ONE*, *8*, e70872. https://doi.org/10.1371/journal.pone.0070872

O'Farrell, C., Murphy, D. D., Petrucelli, L., Singleton, A. B., Hussey, J., Farrer, M., … Cookson, M. R. (2001). Transfected synphilin-1 forms cytoplasmic inclusions in HEK293 cells. *Brain Research. Molecular Brain Research*, *97*, 94–102. https://doi.org/10.1016/S0169-328X(01)00292-3

Olsen, A. L., & Feany, M. B. (2019). Glial alpha-synuclein promotes neurodegeneration characterized by a distinct transcriptional program in vivo. *Glia*, *67*, 1933–1957.

Orme, T., Guerreiro, R., & Bras, J. (2018). The genetics of dementia with Lewy bodies: Current understanding and future directions. *Current Neurology and Neuroscience Reports*, *18*, 67. https://doi.org/10.1007/s11910-018-0874-y

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., & Torizuka, T. (2005). Microglial activation and dopamine terminal loss in early Parkinson's disease. *Annals of Neurology*, *57*, 168–175. https://doi.org/10.1002/ana.20338

Oueslati, A. (2016). Implication of alpha-synuclein phosphorylation at S129 in synucleinopathies: What have we learned in the last decade? *Journal of Parkinson's Disease*, *6*, 39–51. https://doi.org/10.3233/JPD-160779

Outeiro, T. F., Klucken, J., Strathearn, K. E., Liu, F., Nguyen, P., Rochet, J. C., … McLean, P. J. (2006). Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. *Biochemical and Biophysical Research Communications*, *351*, 631–638.

Outeiro, T. F., & Lindquist, S. (2003). Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science*, *302*, 1772–1775. https://doi.org/10.1126/science.1090439

Outeiro, T. F., & Mestre, T. A. (2019). Synuclein Meeting 2019: Where we are and where we need to go. *Journal of Neurochemistry*, *150*, 462–466. https://doi.org/10.1111/jnc.14825

Outeiro, T. F., Putcha, P., Tetzlaff, J. E., Spoelgen, R., Koker, M., Carvalho, F., … McLean, P. J. (2008). Formation of toxic oligomeric alpha-synuclein species in living cells. *PLoS ONE*, *3*, e1867.

Paleologou, K. E., Oueslati, A., Shakked, G., Rospigliosi, C. C., Kim, H. Y., Lamberto, G. R., … Chiappe, D. (2010). Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*, 3184–3198.

Parnetti, L., Chiasserini, D., Persichetti, E., Eusebi, P., Varghese, S., Qureshi, M. M., … Calabresi, P. (2014). Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, *29*, 1019–1027. https://doi.org/10.1002/mds.25772

Peng, C., Gathagan, R. J., Covell, D. J., Medellin, C., Stieber, A., Robinson, J. L., … Trojanowski, J. Q. (2018). Cellular milieu imparts distinct pathological alpha-synuclein strains in alpha-synucleinopathies. *Nature*, *557*, 558–563.

Perez, R. G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., & Zigmond, M. J. (2002). A role for alpha-synuclein in the regulation of dopamine biosynthesis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *22*, 3090–3099.

Pinho, R., Paiva, I., Jerčić, K. G., Fonseca-Ornelas, L., Gerhardt, E., Fahlbusch, C., … Outeiro, T. F. (2019). Nuclear localization and phosphorylation modulate pathological effects of alpha-synuclein. *Human Molecular Genetics*, *28*, 31–50. https://doi.org/10.1093/hmg/ddy326

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., … Stenroos, E. S. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*, *276*, 2045–2047.

Porro, C., Panaro, M. A., Lofrumento, D. D., Hasalla, E., & Trotta, T. (2019). The multiple roles of exosomes in Parkinson's disease: An overview. *Immunopharmacology and Immunotoxicology*, *41*, 469–476. https://doi.org/10.1080/08923973.2019.1650371

Poulopoulos, M., Levy, O. A., & Alcalay, R. N. (2012). The neuropathology of genetic Parkinson's disease. *Movement Disorders*, *27*(7), 811–933. https://doi.org/10.1002/mds.24962.

Prusiner, S. B. (1991). Molecular biology of prion diseases. *Science*, *252*, 1515–1522. https://doi.org/10.1126/science.1675487

Prusiner, S. B., Woerman, A. L., Mordes, D. A., Watts, J. C., Rampersaud, R., Berry, D. B., … Geschwind, D. H. (2015). Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, E5308–5317.

Qiao, L., Hamamichi, S., Caldwell, K. A., Caldwell, G. A., Yacoubian, T. A., Wilson, S., … Zhang, J. (2008). Lysosomal enzyme cathepsin D

protects against alpha-synuclein aggregation and toxicity. *Molecular Brain*, *1*, 17. https://doi.org/10.1186/1756-6606-1-17

Qing, H., Zhang, Y., Deng, Y., McGeer, E. G., & McGeer, P. L. (2009). Lrrk2 interaction with alpha-synuclein in diffuse Lewy body disease. *Biochemical and Biophysical Research Communications*, *390*, 1229–1234.

Rasia, R. M., Bertoncini, C. W., Marsh, D., Hoyer, W., Cherny, D., Zweckstetter, M., … Fernandez, C. O. (2005). Structural characterization of copper(II) binding to alpha-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 4294–4299.

Rodriguez, L., Marano, M. M., & Tandon, A. (2018). Import and export of misfolded alpha-synuclein. *Frontiers in Neuroscience*, *12*, 344.

Rott, R., Szargel, R., Haskin, J., Shani, V., Shainskaya, A., Manov, I., … Engelender, S. (2008). Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. *The Journal of Biological Chemistry*, *283*, 3316–3328.

Rott, R., Szargel, R., Shani, V., Hamza, H., Savyon, M., Abd Elghani, F., … Engelender, S. (2017). SUMOylation and ubiquitination reciprocally regulate alpha-synuclein degradation and pathological aggregation. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 13176–13181.

Roy, S., & Wolman, L. (1969). Ultrastructural observations in Parkinsonism. *The Journal of Pathology*, *99*, 39–44. https://doi.org/10.1002/path.1710990106

Sacino, A. N., Brooks, M. M., Chakrabarty, P., Saha, K., Khoshbouei, H., Golde, T. E., & Giasson, B. I. (2017). Proteolysis of alpha-synuclein fibrils in the lysosomal pathway limits induction of inclusion pathology. *Journal of Neurochemistry*, *140*, 662–678.

Sahin, C., Lorenzen, N., Lemminger, L., Christiansen, G., Møller, I. M., Vesterager, L. B., … Otzen, D. E. (2017). Antibodies against the C-terminus of α-synuclein modulate its fibrillation. *Biophysical Chemistry*, *220*, 34–41.

Saijo, E., Groveman, B. R., Kraus, A., Metrick, M., Orru, C. D., Hughson, A. G., & Caughey, B. (2019). Ultrasensitive RT-QuIC seed amplification assays for disease-associated tau, alpha-synuclein, and prion aggregates. *Methods in Molecular Biology*, *1873*, 19–37.

Salvador, N., Aguado, C., Horst, M., & Knecht, E. (2000). Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. *The Journal of Biological Chemistry*, *275*, 27447–27456. https://doi.org/10.1074/jbc.M001394200

Schaser, A. J., Osterberg, V. R., Dent, S. E., Stackhouse, T. L., Wakeham, C. M., Boutros, S. W., … Unni, V. K. (2019). Alpha-synuclein is a DNA binding protein that modulates DNA repair with implications for Lewy body disorders. *Scientific Reports*, *9*, 10919. https://doi.org/10.1038/s41598-019-47227-z

Schlossmacher, M. G., Frosch, M. P., Gai, W. P., Medina, M., Sharma, N., Forno, L., … Kosik, K. S. (2002). Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. *The American Journal of Pathology*, *160*, 1655–1667. https://doi.org/10.1016/S0002-9440(10)61113-3

Schmid, A. W., Fauvet, B., Moniatte, M., & Lashuel, H. A. (2013). Alpha-synuclein post-translational modifications as potential biomarkers for Parkinson disease and other synucleinopathies. *Molecular and Cellular Proteomics: MCP*, *12*, 3543–3558. https://doi.org/10.1074/mcp.R113.032730

Scott, D. A., Tabarean, I., Tang, Y., Cartier, A., Masliah, E., & Roy, S. (2010). A pathologic cascade leading to synaptic dysfunction in alpha-synuclein-induced neurodegeneration. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*, 8083–8095.

Shahmoradian, S. H., Lewis, A. J., Genoud, C., Hench, J., Moors, T. E., Navarro, P. P., … Lauer, M. E. (2019). Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. *Nature Neuroscience*, *22*, 1099–1109. https://doi.org/10.1038/s41593-019-0423-2

Shavali, S., Brown-Borg, H. M., Ebadi, M., & Porter, J. (2008). Mitochondrial localization of alpha-synuclein protein in alpha-synuclein overexpressing cells. *Neuroscience Letters*, *439*, 125–128. https://doi.org/10.1016/j.neulet.2008.05.005

Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R., … Ziegler, S. G. (2009). Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *The New England Journal of Medicine*, *361*(17), 1651–1656. https://doi.org/10.1056/NEJMoa0901281.

Spencer, B., Valera, E., Rockenstein, E., Overk, C., Mante, M., Adame, A., … Games, D. (2017). Anti-alpha-synuclein immunotherapy reduces alpha-synuclein propagation in the axon and degeneration in a combined viral vector and transgenic model of synucleinopathy. *Acta Neuropathologica Communications*, *5*, 7.

Spencer, B., Williams, S., Rockenstein, E., Valera, E., Xin, W., Mante, M., … Sierks, M. R. (2016). α-Synuclein conformational antibodies fused to penetratin are effective in models of Lewy body disease. *Ann Clin Transl Neurol*, *3*, 588–606.

Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. *Nature*, *388*, 839–840.

Svensson, E., Horvath-Puho, E., Thomsen, R. W., Djurhuus, J. C., Pedersen, L., Borghammer, P., & Sorensen, H. T. (2015). Vagotomy and subsequent risk of Parkinson's disease. *Annals of Neurology*, *78*, 522–529. https://doi.org/10.1002/ana.24448

Swant, J., Goodwin, J. S., North, A., Ali, A. A., Gamble-George, J., Chirwa, S., & Khoshbouei, H. (2011). alpha-Synuclein stimulates a dopamine transporter-dependent chloride current and modulates the activity of the transporter. *The Journal of Biological Chemistry*, *286*, 43933–43943.

Szegő, É. M., Dominguez-Meijide, A., Gerhardt, E., König, A., Koss, D. J., Li, W., … Outeiro, T. F. (2019). Cytosolic trapping of a mitochondrial heat shock protein is an early pathological event in synucleinopathies. *Cell Reports*, *28*(65–77), e66. https://doi.org/10.1016/j.celrep.2019.06.009

Tanaka, M., Kim, Y. M., Lee, G., Junn, E., Iwatsubo, T., & Mouradian, M. M. (2004). Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. *The Journal of Biological Chemistry*, *279*, 4625–4631.

Tenreiro, S., Reimão-Pinto, M. M., Antas, P., Rino, J., Wawrzycka, D., Macedo, D., … Outeiro, T. F. (2014). Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. *PLoS Genetics*, *10*, e1004302. https://doi.org/10.1371/journal.pgen.1004302

Tokuda, T., Salem, S. A., Allsop, D., Mizuno, T., Nakagawa, M., Qureshi, M. M., … El-Agnaf, O. M. (2006). Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. *Biochemical and Biophysical Research Communications*, *349*, 162–166.

Uversky, V. N. (2003). A protein-chameleon: Conformational plasticity of alpha-synuclein, a disordered protein involved in neurodegenerative disorders. *Journal of Biomolecular Structure and Dynamics*, *21*, 211–234.

Vaikath, N. N., Hmila, I., Gupta, V., Erskine, D., Ingelsson, M., & El-Agnaf, O. M. A. (2019). Antibodies against alpha-synuclein: Tools and therapies. *Journal of Neurochemistry*, *150*, 612–625. https://doi.org/10.1111/jnc.14713

Vaikath, N. N., Majbour, N. K., Paleologou, K. E., Ardah, M. T., van Dam, E., van de Berg, W. D. J., … El-Agnaf, O. M. A. (2015). Generation and characterization of novel conformation-specific monoclonal antibodies for α-synuclein pathology. *Neurobiology of Disease*, *79*, 81–99. https://doi.org/10.1016/j.nbd.2015.04.009

Vasili, E., Dominguez-Meijide, A., & Outeiro, T. F. (2019). Spreading of alpha-Synuclein and Tau: A systematic comparison of the mechanisms involved. *Frontiers in Molecular Neuroscience*, *12*, 107.

Verdurand, M., Levigoureux, E., Zeinyeh, W., Berthier, L., Mendjel-Herda, M., Cadarossanesaib, F., … Chauveau, F. (2018). In silico, in vitro, and

in vivo evaluation of new candidates for alpha-synuclein PET imaging. *Molecular Pharmaceutics*, *15*, 3153–3166.

Vicente Miranda, H., Cássio, R., Correia-Guedes, L., Gomes, M. A., Chegão, A., Miranda, E., … Outeiro, T. F. (2017a). Posttranslational modifications of blood-derived alpha-synuclein as biochemical markers for Parkinson's disease. *Scientific Reports*, *7*, 13713. https://doi.org/10.1038/s41598-017-14175-5

Vicente Miranda, H., Szego, E. M., Oliveira, L. M. A., Breda, C., Darendelioglu, E., de Oliveira, R. M., … Munari, F. (2017b). Glycation potentiates alpha-synuclein-associated neurodegeneration in synucleinopathies. *Brain: A Journal of Neurology*, *140*, 1399–1419.

Villar-Pique, A., Lopes da Fonseca, T., Sant'Anna, R., Szegö, É. M., Fonseca-Ornelas, L., Pinho, R., … Rossetti, G. (2016). Environmental and genetic factors support the dissociation between alpha-synuclein aggregation and toxicity. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, E6506–E6515.

Vogiatzi, T., Xilouri, M., Vekrellis, K., & Stefanis, L. (2008). Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. *The Journal of Biological Chemistry*, *283*, 23542–23556.

Volpicelli-Daley, L. A., Gamble, K. L., Schultheiss, C. E., Riddle, D. M., West, A. B., & Lee, V. M. (2014). Formation of alpha-synuclein Lewy neurite-like aggregates in axons impedes the transport of distinct endosomes. *Molecular Biology of the Cell*, *25*, 4010–4023.

Wakabayashi, K., Engelender, S., Yoshimoto, M., Tsuji, S., Ross, C. A., & Takahashi, H. (2000). Synphilin-1 is present in Lewy bodies in Parkinson's disease. *Annals of Neurology*, *47*, 521–523. https://doi.org/10.1002/1531-8249(200004)47:4<521:AID-ANA18>3.0.CO;2-B

Wakabayashi, K., Hansen, L. A., & Masliah, E. (1995). Cortical Lewy body-containing neurons are pyramidal cells: Laser confocal imaging of double-immunolabeled sections with anti-ubiquitin and SMI32. *Acta Neuropathologica*, *89*, 404–408. https://doi.org/10.1007/BF00307643

Wakabayashi, K., Tanji, K., Odagiri, S., Miki, Y., Mori, F., & Takahashi, H. (2013). The Lewy body in Parkinson's disease and related neurodegenerative disorders. *Molecular Neurobiology*, *47*, 495–508. https://doi.org/10.1007/s12035-012-8280-y

Walsh, N. C., Kenney, L. L., Jangalwe, S., Aryee, K. E., Greiner, D. L., Brehm, M. A., & Shultz, L. D. (2017). Humanized mouse models of clinical disease. *Annual Review of Pathology*, *12*, 187–215. https://doi.org/10.1146/annurev-pathol-052016-100332

Wang, Q., Liu, Y., & Zhou, J. (2015). Neuroinflammation in Parkinson's disease and its potential as therapeutic target. *Translational Neurodegeneration*, *4*, 19. https://doi.org/10.1186/s40035-015-0042-0

Wersinger, C., Rusnak, M., & Sidhu, A. (2006). Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. *The European Journal of Neuroscience*, *24*, 55–64. https://doi.org/10.1111/j.1460-9568.2006.04900.x

Xilouri, M., Brekk, O. R., Polissidis, A., Chrysanthou-Piterou, M., Kloukina, I., & Stefanis, L. (2016). Impairment of chaperone-mediated autophagy induces dopaminergic neurodegeneration in rats. *Autophagy*, *12*, 2230–2247. https://doi.org/10.1080/15548627.2016.1214777

Xiong, Y., & Yu, J. (2018). Modeling Parkinson's disease in Drosophila: What have we learned for dominant traits? *Frontiers in Neurology*, *9*, 228. https://doi.org/10.3389/fneur.2018.00228

Yan, J. Q., Yuan, Y. H., Chu, S. F., Li, G. H., & Chen, N. H. (2018). E46K mutant alpha-synuclein is degraded by both proteasome and macroautophagy pathway. *Molecules*, *23*, 2839.

Yin, G., Lopes da Fonseca, T., Eisbach, S. E., Anduaga, A. M., Breda, C., Orcellet, M. L., … Fernandez, C. O. (2014). alpha-Synuclein interacts with the switch region of Rab8a in a Ser129 phosphorylation-dependent manner. *Neurobiology of Disease*, *70*, 149–161.

Yu, S., Zuo, X., Li, Y., Zhang, C., Zhou, M., Zhang, Y. A., … Chan, P. (2004). Inhibition of tyrosine hydroxylase expression in alpha-synuclein-transfected dopaminergic neuronal cells. *Neuroscience Letters*, *367*, 34–39.

Zaltieri, M., Grigoletto, J., Longhena, F., Navarria, L., Favero, G., Castrezzati, S., … Bellucci, A. (2015). alpha-synuclein and synapsin III cooperatively regulate synaptic function in dopamine neurons. *Journal of Cell Science*, *128*, 2231–2243. https://doi.org/10.1242/jcs.157867

Zhu, X., Babar, A., Siedlak, S. L., Yang, Q., Ito, G., Iwatsubo, T., … Chen, S. G. (2006). LRRK2 in Parkinson's disease and dementia with Lewy bodies. *Molecular Neurodegeneration*, *1*, 17.

Zigoneanu, I. G., Yang, Y. J., Krois, A. S., Haque, E., & Pielak, G. J. (2012). Interaction of alpha-synuclein with vesicles that mimic mitochondrial membranes. *Biochimica Et Biophysica Acta*, *1818*, 512–519.

Zondler, L., Miller-Fleming, L., Repici, M., Gonçalves, S., Tenreiro, S., Rosado-Ramos, R., … Outeiro, T. F. (2014). DJ-1 interactions with alpha-synuclein attenuate aggregation and cellular toxicity in models of Parkinson's disease. *Cell Death & Disease*, *5*, e1350.

How to cite this article: Brás IC, Dominguez-Meijide A, Gerhardt E, et al. Synucleinopathies: Where we are and where we need to go. *J. Neurochem*. 2020;153:433-454. https://doi.org/10.1111/jnc.14965