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How strong is the evidence that Parkinson's disease is a prion disorder?

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Abstract

Purpose of review—We describe evidence supporting the hypothesis that α -synuclein has a prion-like role in Parkinson's disease and related α -synucleinopathies, and discuss how this novel thinking impacts the development of diagnostics and disease-modifying therapies.

Recent findings—Observations that immature dopamine neurons grafted to Parkinson's disease patients can develop Lewy bodies triggered a surge of interest in the putative prion-like properties of α -synuclein. We recount results from experiments which confirm that misfolded α -synuclein can exhibit disease-propagating properties, and describe how they relate to the spreading of α -synuclein aggregates in α -synucleinopathies. We share insights into the underlying molecular mechanisms and their relevance to novel therapeutic targets. Finally, we discuss what the initial triggers of α -synuclein misfolding might be, where in the body the misfolding events might take place and how this can instruct development of novel diagnostic tools. We speculate that differences in anatomical trigger sites and variability in α -synuclein fibril structure can contribute to clinical differences between α -synucleinopathies.

Summary—The realization that α -synuclein pathology can propagate between brain regions in neurodegenerative diseases has deepened and expanded our understanding of potential pathogenic processes which can lead to the development of novel diagnostic tools as well as the identification of new therapeutic targets.

Keywords

Parkinson's disease; α -synuclein; prion-like; Lewy body

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Conflicts of interest

Dr. Ma has no conflicts of interest relevant to this article.

Introduction

The word prion was established in 1982 by Stanley B. Prusiner. It is derived from **protein** and **infection** and is short for "proteinaceous infectious particle". Prions can self transform their shape and propagate. Thus they can be transmitted from cell to cell, between individuals, and even between animals of different species. For his discovery of prions, Prusiner was awarded the Nobel Prize in 1997.

The neuropathology of Parkinson's disease (PD) is characterized, in part, by severe loss of dopaminergic neurons in the substantia nigra and the development of intraneuronal α -synuclein aggregates called Lewy bodies and Lewy neurites (collectively Lewy pathology) in widespread brain regions (1). Patients with Dementia with Lewy bodies (DLB) also develop intraneuronal α -synuclein aggregates, while in Multiple System Atrophy (MSA) the majority of the α -synuclein aggregates are located in oligodendrocytes. Due to the shared pathological feature of α -synuclein aggregates, PD, DLB and MSA are known as α -synucleinopathies (1). Recently, several proteins that are prone to misfold, form aggregates in the brain and that each define specific neurodegenerative disorders have been shown to transmit from one cell to another in experimental disease models. This applies to α -synuclein in models of PD, DLB and MSA, as well as Tau in models of Alzheimer's disease; mutant huntingtin in Huntington's disease models, and mutant SOD in amyotrophic lateral sclerosis (ALS) models (2,3). The behavior of these disease-related proteins has been called "prion-like". In this minireview, we briefly describe 1) evidence that α -synucleinopathies are prion-like disorders; 2) mechanisms that underlie intercellular transfer of misfolded α -synuclein; and 3) the relevance to future therapies and diagnostics. We also discuss where the first misfolding events might occur, and controversies regarding the possibility that α -synucleinopathies might be communicable.

What triggered the idea that α -synuclein might be a prion-like protein?

In 2005 John Hardy speculated that "permissive templating" could play a key role in the pathogenesis α -synucleinopathies (4). This was an exciting concept, however, at the time there was no real clinical or experimental supporting evidence. Eventually, support for the 'prion hypothesis' came from a surprising place. We published two papers simultaneously showing that Lewy bodies were present, at autopsy, in 2–5% of dopamine neurons in transplants of fetal midbrain tissue implanted into the striatum of PD patients more than a decade before they had died (5,6). Interestingly, based on these first 3 cases and observations in 8 subsequent cases (operated in 5 different centers worldwide) that we are aware of, the Lewy bodies in the grafted neurons first develop after a lag period of about one decade and gradually increase in frequency up to the longest surviving case at 24 years after surgery (5–13). The aggregates stain for phosphorylated serine129- α -synuclein, ubiquitin and thioflavin S, akin to Lewy bodies found in PD brain. In 2008, when the two initial papers were published (5,6), we suggested that misfolded α -synuclein had spread from neurons in the diseased PD brain into the young dopamine neurons inside the transplant and had seeded misfolding of endogenous α -synuclein. We also proposed that these observations could explain neuropathology findings in PD that Braak and colleagues had reported 5 years

earlier (14). Braak and his team had mapped α -synuclein aggregates in the nervous system of a large series of post-mortem cases and suggested that Lewy pathology in PD can be divided into 6 distinct stages and that, over time, the pathology is propagated from one brain region to another via long unmyelinated pathways (15,16). While Braak and colleagues suggested that the transmitting agent might be a unknown pathogen, e.g. a neurotropic virus (17–19), following our observations in the transplants we instead proposed that it was a misfolded species of α -synuclein (14). At the time, α -synuclein was primarily viewed as an intraneuronal protein with a possible role in vesicular trafficking and synaptic transmission (20). A few studies, notably by El-Agnaf and Lee and coworkers, had already independently shown that α -synuclein could be secreted by neurons into the extracellular space (even be measured in human CSF and plasma) (21–23) and affect nearby cells, e.g. microglia (22). However, the idea that this α -synuclein might be taken up by nearby neurons and act as a template for misfolding was not generally discussed. Thus, the suggestion in 2008 that cell-to-cell transfer of α -synuclein was what caused the development of Lewy bodies in the transplants in PD patients received a mixed reception. In the research community there was both a great deal of excitement, as well as significant skepticism, with suggestions that e.g. neuroinflammation or accelerated cellular ageing inside the transplant had triggered the formation of the Lewy bodies (10,14). During the years immediately following the proposal that misfolded α -synuclein might behave in a prion-like fashion, a large number of experimental studies addressing this topic were published and we summarize them briefly in the next section.

Experiments support the idea that misfolded α -synuclein behaves in a prion-like fashion

Numerous *in vitro* studies have convincingly shown that cultured neurons can both secrete α -synuclein and take it up from the extracellular space (22–26). In other experiments, recombinant α -synuclein protein added to the culture medium was found to be taken up by cultured neurons (27–29). Once inside a “new” neuron, “exogenous” α -synuclein (both when cell-derived and when added as recombinant protein) can oligomerize with endogenous α -synuclein and seed the formation of aggregates (24,27–29). Similarly, animal experiments have shown that α -synuclein can be released and taken up by neurons. For example, in different paradigms involving transplantation of rodent-derived neurons or neural stem cells into animals overexpressing human α -synuclein (either transgenically or via an injected viral vector), the grafted rodent neurons incorporate the human α -synuclein (24,30–32). Moreover, using strategies involving the intracerebral, intramuscular or intraperitoneal injections of oligomeric or fibrillar α -synuclein derived either from recombinant protein or from post-mortem brain samples obtained at autopsies of patients with α -synucleinopathies (e.g. PD, MSA and DLB), the injected material triggers accumulation of α -synuclein in the host rodent or non-human primate brain (33–43). This occurs within a few weeks of the injection both in rodent brains overexpressing α -synuclein and in normal wild-type brains, indicating that the seeding of aggregation is a powerful mechanism. It is clear, however, that the precise methods used (e.g. preparation of the α -synuclein fibrils and amount injected) strongly influence the outcome, which has been reviewed in detail recently (41). What is also clear is that different forms of α -synuclein

fibrils exist, with varying three-dimensional structures. Akin to “prion strains”, different α -synuclein fibril conformers exhibit varying degrees of cellular toxicity (44) and can cause different distributions (cellular and anatomic) of neuropathology when injected into animals (40,45).

While the demonstration of induced α -synucleinopathy in experimental animals is undeniably fascinating, critics have argued that the models are not relevant to the human diseases and comments such as “PD patients do not develop the disease due to an injection of α -synuclein fibrils into the striatum” are frequently heard. In the next section we discuss how animal models that more closely mimic human α -synucleinopathies have recently entered the stage.

Braak neuropathological staging supports the concept of propagation of α -synuclein aggregates

Based on the proposed six stages of Lewy pathology in PD, Braak and colleagues proposed two starting points for the disease process, namely the olfactory system and the enteric nervous system (18,19). The olfactory bulb and enteric nerves (including the dorsal motor nucleus of the vagal nerve) both exhibit a heavy load of Lewy pathology already in early disease (46–48). Therefore they formulated the “dual-hit hypothesis”, mentioned above, stating that an unknown toxic agent or pathogen was inhaled, affecting the olfactory bulb and the gut wall, after being swallowed (18,19). This hypothesis is also consistent with clinical observations that hyposmia and constipation are prevalent at the time of PD diagnosis and often precede the development of overt motor symptoms by several years (49–52). Braak’s team suggested that the offending agent spread along axonal pathways, not unlike a suggestion made 20 years earlier (long before the discovery of α -synuclein in Lewy bodies) by Saper et al. (53), who proposed that “*the processes of axonal and transneuronal transport may subserve the transmission from neuron to neuron of a toxic or infectious agent*”.

Mouse studies have shown that injections of recombinant α -synuclein fibrils into the olfactory bulb lead to rapid transport of the protein throughout the olfactory system and interconnected brain regions (54). When the injected material is a mixture of different sized α -synuclein fibrils, the Lewy body-like pathology spreads gradually to several additional brain areas over 6 months (41), validating the idea that α -synuclein aggregates can spread from the olfactory bulb along axonal pathways and transcend synaptic connections (46). The vagus nerve is a second potential entry point for Lewy pathology into the central nervous system. Interestingly, a epidemiological study on a large Danish cohort reported a controversial finding describing a significantly reduced future PD risk in patients who underwent truncal vagotomy as a treatment for peptic ulcer in 1977–1995 (55). Recently, it was speculated that changes in gut microbiome (56,57), e.g. as a consequence of smoking or coffee (known modifiers of PD risk), could reduce the likelihood of inflammatory events that might trigger α -synuclein misfolding in the gut wall (58,59). In rats, injections of monomeric, oligomeric or fibrillar α -synuclein or PD patient-derived brain lysates into the gut wall led to transport of α -synuclein retrogradely along the vagus nerve to the brainstem

(60). Providing further support for the gut being of interest in PD, injection of a viral vector encoding human α -synuclein into the vagal nerve lead to α -synuclein spreading beyond the cranial nerve nucleus to more rostral brain areas (61). When the brainstem neurons initially involved were damaged, the α -synuclein aggregates spread less rostrally, suggesting that neural connections are essential (62). These initial observations of α -synuclein spreading from the olfactory bulb and gut (via the vagal nerve) in mice are very exciting and await replication in larger animals. It is unclear if α -synuclein pathology can spread to nigral dopamine neurons, or if this takes so long that it cannot be studied in rodents with a lifespan of 2–3 years.

Cellular and molecular mechanisms of α -synuclein intercellular transfer and interregional transport

Several studies, mostly on cultured neurons, have defined molecular mechanisms of α -synuclein release, uptake and axonal transport. Recent reviews describe these areas in detail (3,63,64) and we only provide a short overview. It is important to emphasize that underlying mechanisms differ depending on whether the α -synuclein is present as a monomer, oligomer or fibril. A recent *in vivo* study indicated that monomeric α -synuclein is released from nerve terminals in the striatum in a process that is potassium-dependent and regulated by presynaptic GABA receptors (65). Monomers of α -synuclein appear to readily translocate across cellular membranes in a temperature-insensitive manner (22). For both α -synuclein oligomers and fibrils, the cellular release and uptake is likely regulated differently because they are not presumed to have normal physiological roles. In cell cultures, α -synuclein is released via exocytosis and the process is enhanced by cellular stress such as inhibition of lysosomes (66–70). A minor portion (frequently estimated at around 5%) of the extracellular α -synuclein is associated with exosomes and it can be present in oligomeric forms, both on the inside and surface of the microvesicles (68,69,71,72). It is unclear whether all of the α -synuclein found in the exosomal fraction is secreted with the exosomes or whether they associate with each other outside the cell. Exosomal lipids can accelerate the process of α -synuclein fibril formation and thereby they might stimulate the disease process (73).

The uptake of extracellular α -synuclein, whether it is oligomeric or fibrillar, is believed to be mediated via endocytosis. Several pharmacologic and genetic experiments have demonstrated that endocytosis is major mechanism for uptake of extracellular α -synuclein (24,30,74–79). How α -synuclein binds the outer cellular membrane is not fully understood, but heparan sulphate proteoglycans appear to play a role because uptake of α -synuclein is reduced when they are blocked (75). What also remains unclear is how fibrillar α -synuclein contained in an endosome can gain access to the cytoplasm so that it can seed the aggregation of the endogenous protein, and it has been suggested that it has the capacity to penetrate lysosomal membrane akin to what viral peptides are capable of (80). *In vitro* experiments have shown that α -synuclein that has been taken up from the extracellular space undergo bidirectional fast and slow axonal transport, i.e. to and from the cell body (60,81,82). This can explain how α -synuclein pathology spreads in PD between brain regions over considerable distances.

While we have primarily considered transfer of from neuron-to-neuron, astrocytes, microglia and oligodendrocytes can also all take up extracellular α -synuclein (79,83–86). It is beyond the scope of this brief review to describe this in detail but it warrants a few comments. Uptake of α -synuclein has been suggested to activate astrocytes, which can explain the astrocytosis sometimes observed in α -synucleinopathies (87). Uptake of α -synuclein by microglia is enhanced by immunoglobulins binding to α -synuclein, which is relevant to ongoing immunotherapy trials in PD and MSA (88–93). Further, uptake of α -synuclein by oligodendrocytes (79,83) might explain why these cells exhibit α -synuclein aggregates in MSA (1), although they also can synthesize α -synuclein endogenously (94,95).

New treatment strategies and diagnostics based on α -synuclein acting like a prion

If the cell-to-cell propagation of α -synuclein aggregates is linked to clinical progression of α -synucleinopathies, then preventing its spread may be a powerful clinical target. Four principally different strategies are evident: inhibition of α -synuclein release or its uptake by neurons (for PD and DLB) or oligodendroglia (for MSA); prevention of α -synuclein seeding and promotion of α -synuclein clearance from the extracellular space. It is conceivable that α -synuclein can be reduced by promoting its intracellular clearance by enhancing the lysosomal-autophagy system through inhibition of the mammalian target of rapamycin (mTOR), using, e.g., drugs such as rapamycin or metoformin (96,97). As mentioned previously, endocytic uptake of α -synuclein can be reduced by in cell cultures by pharmacologically interfering with heparan sulphate proteoglycans (75). Further, oligomerization, the initial crucial steps in the seeding process, can be inhibited by certain small molecules such as Anle138b (98,99). Finally, as mentioned above, immunotherapy is currently being pursued both in the laboratory and clinically in attempts to clear α -synuclein from the extracellular space (88–93).

The therapeutic potential of any of these approaches will be maximized once diagnostic and progression biomarkers have been established. Such biomarkers would potentially allow for the identification of PD prior to the onset of the motor symptoms (52). Related to the prion hypothesis for α -synucleinopathies, it is notable that extensive efforts are being made to identify pathological expression of α -synuclein in peripheral tissues, e.g. the submaxillary gland (100,101) or enteric nervous system biopsies (102,103), although the field remains controversial with contradicting results (104).

No evidence that α -synucleinopathy is communicable except in experimental paradigms

The experimental transmission of α -synuclein leads to the question: Is PD (and/or related α -synucleinopathies) a communicable disease? The epidemiology studies conducted so far do not support that PD is a communicable disease. Thus, a retrospective analysis of patients who received cadaveric human growth hormone (c-hGH), the same cohort that hosted an outbreak of more than 200 cases of iatrogenic Creutzfeldt-Jakob disease (iCJD), revealed no cases of PD (105). A caveat of the study is that the follow up period might not have been

long enough. The incubation time for iCJD can be as long as 42 years (106) and it might take several decades for misfolded α -synuclein to cause a widespread aggregation. Another caveat is the reliance on death certificates, but not neuropathological analyses, to ascertain if the subjects had PD or not. Finally, we do not know if the protocol used to extract growth hormone affects the stability of the putatively pathogenic α -synuclein conformers. While currently available evidence does not support that PD is communicable disease, the limited observation period and lack of neuropathology data in this key study means that we cannot be certain.

Conclusions

Experimental studies demonstrating cell-to-cell transfer of α -synuclein pathology firmly establish that, in addition to its normal conformation, α -synuclein can exist in altered, self-propagating conformation(s). Once inside animals, the altered α -synuclein conformers are able to propagate to host α -synuclein, resulting in α -synuclein aggregation and, e.g., degeneration of nigral dopaminergic neurons, both of which are pathological hallmarks of PD. These properties of α -synuclein are exactly as those of prions, i.e. 1) able to stably exist in at least two conformational states (one being the self-perpetuating conformer); and 2) able to replicate and cause neurodegeneration when it is inside the body.

Despite several similarities at the cellular level, current evidence does not support that PD or other α -synucleinopathies are communicable disease like several other prion diseases. As mentioned above, it might still be too early to exclude the possibility that we will eventually identify cases of contracted PD, but that the need for a 'high dose' of pathogenic α -synuclein protein make them rare and the putatively long incubation times will make them difficult to detect. That said, the lack of communicability is not an exclusion criterion *per se* for a prion disease. Prion diseases are a group of neurodegenerative disorders and only some animal prion diseases are classic communicable diseases, e.g. scrapie in sheep and chronic wasting disease in deer and elk. Further, some human prion diseases are communicable via man-made transmission routes, such as Kuru and iCJD, but only in rare situations (cannibalism and transplantation procedures). On the other hand, certain prion diseases are not communicable, e.g. sporadic and inherited CJD. Thus, even if PD and other α -synucleinopathies are non-communicable diseases, this should not exempt them from membership in the family of prion diseases. The final take home message, however, should be that the most important issue is not classification or not of α -synucleinopathies as prion diseases, but the realization that prion properties of α -synuclein contribute to the pathogenic process and further understanding of mechanistic underpinnings can lead to new therapeutic strategies.

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References

1. Halliday GM, Holton JL, Revesz T, Dickson DW. Neuropathology underlying clinical variability in patients with synucleinopathies. *Acta Neuropathol.* 2011 Jul 1; 122(2):187–204. [PubMed: 21720849]
2. Brundin P, Melki R, Kopito R. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat Rev Mol Cell Biol.* 2010 Apr; 11(4):301–307. [PubMed: 20308987]
3. Guo JL, Lee VM-Y. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nature Medicine.* 2014 Feb; 20(2):130–138.
4. Hardy J. Expression of normal sequence pathogenic proteins for neurodegenerative disease contributes to disease risk: "permissive templating" as a general mechanism underlying neurodegeneration. *Biochem Soc T.* 2005 Aug; 33(Pt 4):578–581.
5. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. 2008 May 6; 14(5):504–506.
6. Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. 2008 May; 14(5):501–503.
7. Kurowska Z, Englund E, Widner H, Lindvall O, Li J-Y, Brundin P. Signs of degeneration in 12–22-year old grafts of mesencephalic dopamine neurons in patients with Parkinson's disease. *J Parkinsons Dis.* 2011 Jun 7.1:83–92. [PubMed: 23939259]
8. Li J-Y, Englund E, Widner H, Rehnström S, Björklund A, Lindvall O, et al. Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease. *Mov Disord.* 2010 Jun 15; 25(8):1091–1096. [PubMed: 20198645]
9. Ahn T-B, Langston JW, Aachi VR, Dickson DW. Relationship of neighboring tissue and gliosis to α -synuclein pathology in a fetal transplant for Parkinson's disease. *Am J Neurodegener Dis.* 2012; 1(1):49–59. [PubMed: 23383381]
10. Cooper O, Astradsson A, Hallett P, Robertson H, Mendez I, Isacson O. Lack of functional relevance of isolated cell damage in transplants of Parkinson's disease patients. *J Neurol.* 2009 Aug 1; 256(Suppl 3):310–316. [PubMed: 19711122]
11. Chu Y, Kordower JH. Lewy body pathology in fetal grafts. *Ann N Y Acad Sci.* 2010; 1184:55–67. [PubMed: 20146690]
12. Brundin P, Kordower JH. Functional Neural Transplantation III. 1st. Vol. 200. Elsevier B.V; 2012. Chapter 11 - Neuropathology in transplants in Parkinson's disease: Implications for disease pathogenesis and the future of cell therapy; p. 21
13. Li W, Englund E, Widner H, Mattsson B, van Westen D, Lätt J, et al. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating Parkinsonian brain. *Proc Natl Acad Sci USA.* 2016 Feb 18; x-x. in press. *Paper describing one patient who exhibited gradual loss of graft-induced clinical benefits 14 years post-transplantation, presumably due to the development of widespread α -synuclein pathology in the host brain and in 12% of the grafted dopamine neurons
14. Brundin P, Li J-Y, Holton JL, Lindvall O, Revesz T. Research in motion: the enigma of Parkinson's disease pathology spread. *Nat Rev Neurosci.* 2008 Oct; 9(10):741–745. [PubMed: 18769444]
15. Braak H, Bohl JR, Müller CM, Rüb U, de Vos RAI, Del Tredici K. Stanley Fahn Lecture 2005: The staging procedure for the inclusion body pathology associated with sporadic Parkinson's disease reconsidered. *Mov Disord.* 2006; 21(12):2042–2051. [PubMed: 17078043]
16. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K. *Cell Tissue Res.* Vol. 318. Springer-Verlag; 2004 Oct. Stages in the development of Parkinson's disease-related pathology; p. 121–134.
17. Braak H, Rüb U, Gai WP, Del Tredici K. *Journal of neural transmission (Vienna, Austria : 1996).* Vol. 110. Springer-Verlag; 2003 May. Idiopathic Parkinson's disease: possible routes by

which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen; p. 517-536.

18. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* 2007 Dec; 33(6):599–614. [PubMed: 17961138]
19. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. *Ann N Y Acad Sci.* 2009 Jul.1170:615–622. [PubMed: 19686202]
20. Cookson MR, van der Brug M. Cell systems and the toxic mechanism (s) of α -synuclein. *Exp Neurol.* 2008; 209(1):5–11. [PubMed: 17603039]
21. El-Agnaf OMA, Salem SA, Paleologou KE, Cooper LJ, Fullwood NJ, Gibson MJ, et al. Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J Federation of American Societies for Experimental Biology.* 2003 Oct; 17(13):1945–1947.
22. Lee S-J. Origins and effects of extracellular alpha-synuclein: implications in Parkinson's disease. *J Mol Neurosci.* 2008; 34(1):17–22. [PubMed: 18157654]
23. Lee H-J, Patel S, Lee S-J. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci.* 2005 Jun 22; 25(25):6016–6024. [PubMed: 15976091]
24. Hansen C, Angot E, Bergström A-L, Steiner JA, Pieri L, Paul G, et al. α -Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest.* 2011 Jan.18:1–11.
25. Reyes JF, Olsson TT, Lamberts JT, Devine MJ, Kunath T, Brundin P. A cell culture model for monitoring α -synuclein cell-to-cell transfer. *Neurobiol Dis.* 2015 May.77:266–275. [PubMed: 25046995]
26. Bae, E-J.; Lee, H-J.; Lee, S-J. *Methods Mol Biol.* Vol. 1345. New York, NY: Springer New York; 2016. *Cell Models to Study Cell-to-Cell Transmission of α -Synuclein*; p. 291-298.
27. Luk, KC.; Song, C.; O'Brien, P.; Stieber, A.; Branch, JR.; Brunden, KR., et al. *Proc Natl Acad Sci USA.* Vol. 106. National Acad Sciences; 2009 Nov 24. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells; p. 20051-20056.
28. Volpicelli-Daley, LA.; Luk, KC.; Patel, TP.; Tanik, SA.; Riddle, DM.; Stieber, A., et al. *Neuron.* Elsevier Inc; 2011 Oct 2. Exogenous alpha-synuclein fibrils induce Lewy Body pathology leading to synaptic dysfunction and neuron death; p. 1-15.
29. Nonaka, T.; Watanabe, ST.; Iwatsubo, T.; Hasegawa, M. *J Biol Chem.* Vol. 285. American Society for Biochemistry and Molecular Biology; 2010 Nov 5. Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases; p. 34885-34898.
30. Desplats P, Lee H-J, Bae E-J, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci USA.* 2009 Aug 4; 106(31):13010–13015. [PubMed: 19651612]
31. Angot, E.; Steiner, JA.; Lema Tomé, CM.; Ekström, P.; Mattsson, B.; Björklund, A., et al. Alpha-Synuclein Cell-to-Cell Transfer and Seeding in Grafted Dopaminergic Neurons In Vivo. In: Mosley, RL., editor. *PLoS ONE.* Vol. 7. 2012 Jun 21. p. e39465
32. Kordower, JH.; Dodiya, HB.; Kordower, AM.; Terpstra, B.; Paumier, K.; Madhavan, L., et al. *Neurobiol Dis.* Vol. 31. Elsevier Inc; 2011. Transfer of host-derived alpha synuclein to grafted dopaminergic neurons in rat; p. 1-6.
33. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM-Y. Intracerebral inoculation of pathological α -synuclein initiates a rapidly progressive neurodegenerative α -synucleinopathy in mice. *J Exp Med.* 2012 May 7; 209(5):975–986. [PubMed: 22508839]
34. Mougenot, AL.; Nicot, S.; Bencsik, A.; Morignat, E.; Verchère, J.; Lakhdar, L., et al. *Neurobiol Aging.* Elsevier Inc; 2011 Aug 1. Prion-like acceleration of a synucleinopathy in a transgenic mouse model; p. 1-4.
35. Recasens A, Dehay B, Bové J, Carballo-Carbajal I, Dovero S, Pérez-Villalba A, et al. Lewy body extracts from Parkinson disease brains trigger α -synuclein pathology and neurodegeneration in mice and monkeys. *Ann Neurol.* 2014 Feb 18; 75(3):351–362. [PubMed: 24243558] **This paper demonstrates for the first time the induction of α -synuclein pathology in a non-human primate brain following intracerebral injection of a brain extract from a Parkinson disease patient

36. Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, et al. Evidence for α -synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc Natl Acad Sci USA*. 2015 Sep 22; 112(38):National Acad Sciences:E5308–E5317. [PubMed: 26324905] *The study highlights that brain extracts from Multiple System Atrophy patients can induce α -synuclein aggregation when injected into mouse brains and suggests that these extracts trigger pathology more effectively than extracts prepared from Parkinson disease brains. The authors claim that α -synuclein fulfill the criteria of a bona fide prion
37. Watts, JC.; Giles, K.; Oehler, A.; Middleton, L.; Dexter, DT.; Gentleman, SM., et al. *Proc Natl Acad Sci USA*. Vol. 110. National Acad Sciences; 2013 Nov 26. Transmission of multiple system atrophy prions to transgenic mice; p. 19555-19560.
38. Paumier KL, Luk KC, Manfredsson FP, Kanaan NM, Lipton JW, Collier TJ, et al. Intrastriatal injection of pre-formed mouse α -synuclein fibrils into rats triggers α -synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiol Dis*. 2015 Jun 17.82:185–199. [PubMed: 26093169] *These experiments use rats to confirm prior findings in mice demonstrating that intrastriatal injections of preformed α -synuclein trigger degeneration of the substantia nigra dopamine neurons, and add the novel finding that there is some spread of pathology to the contralateral substantia nigra.
39. Sacino AN, Brooks M, Thomas MA, McKinney AB, Lee S, Regenhardt RW, et al. Intramuscular injection of α -synuclein induces CNS α -synuclein pathology and a rapid-onset motor phenotype in transgenic mice. *Proc Natl Acad Sci USA*. 2014 Jul 22; 111(29):National Acad Sciences:10732–10737. [PubMed: 25002524] **This study reports for the first time that intramuscular injections of preformed α -synuclein fibrils in mice overexpressing α -synuclein can trigger widespread α -synuclein aggregate pathology in the central nervous system, eventually leading to early death of the mouse.
40. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, et al. α -Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature*. 2015 Jun 18; 522(7556):340–344. [PubMed: 26061766] **This report describes several experiments highlighting interesting differences between different forms of α -synuclein assemblies regarding their capacities to seed aggregates and spread in vivo after injection into the brain or intraperitoneal cavity of rodents. The findings are particularly important in relation to the idea that different α -synucleinopathies in humans might be caused by a variety of distinct and disease-specific α -synuclein assemblies
41. Rey NL, George S, Brundin P. Review: Spreading the word: precise animal models and validated methods are vital when evaluating prion-like behaviour of alpha-synuclein. *Neuropathol Appl Neurobiol*. 2016 Feb 29; 42(1):51–76. [PubMed: 26666838]
42. Sacino AN, Brooks M, McKinney AB, Thomas MA, Shaw G, Golde TE, et al. Brain injection of α -synuclein induces multiple proteinopathies, gliosis, and a neuronal injury marker. *J Neurosci. Society for Neuroscience*. 2014 Sep 10; 34(37):12368–12378.
43. Masuda-Suzukake, M.; Nonaka, T.; Hosokawa, M.; Oikawa, T.; Arai, T.; Akiyama, H., et al. *Brain*. Vol. 136. Oxford University Press; 2013 Apr. Prion-like spreading of pathological α -synuclein in brain; p. 1128-1138.
44. Bousset L, Pieri L, Ruiz-Arlandis G, Gath J, Jensen PH, Habenstein B, et al. Structural and functional characterization of two alpha-synuclein strains. *Nature Communications*. 2013; 4:2575.
45. Melki R. Role of Different Alpha-Synuclein Strains in Synucleinopathies, Similarities with other Neurodegenerative Diseases. *J Parkinsons Dis*. 2015; 5(2):IOS Press:217–227. [PubMed: 25757830] *A review summarizing current concepts related to the possible existence of different strains of α -synuclein aggregates in human neurodegenerative diseases
46. Angot E, Steiner JA, Hansen C, Li J-Y, Brundin P. Are synucleinopathies prion-like disorders? *Lancet Neurol*. 2010 Nov; 9(11):1128–1138. [PubMed: 20846907]
47. Braak H, de Vos RAI, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neuroscience Letters*. 2006 Mar 20; 396(1):67–72. [PubMed: 16330147]
48. Beach TG, White CL, Hladik CL, Sabbagh MN, Connor DJ, Shill HA, et al. Olfactory bulb alpha-synucleinopathy has high specificity and sensitivity for Lewy body disorders. *Acta Neuropathol*. 2009 Feb; 117(2):169–174. [PubMed: 18982334]

49. Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W, et al. Identifying prodromal Parkinson's disease: Pre-Motor disorders in Parkinson's disease. *Mov Disord.* 2012 Apr 16; 27(5):617–626. [PubMed: 22508280]
50. Duda JE. Olfactory system pathology as a model of Lewy neurodegenerative disease. 2010 Jan 2; 289(1–2):49–54.
51. Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley Des Varannes S, Neunlist M, et al. The second brain and Parkinson's disease. 2009 Sep 1; 30(5):735–741.
52. Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. *Mov Disord.* 2015 Oct; 30(12):1600–1611. [PubMed: 26474317]
 **This is an important paper providing clinical criteria that define prodromal Parkinson's disease i.e. a condition with non-motor signs and symptoms (possibly coupled, at least in part, to α -synuclein aggregation in certain neurons) that precedes the development of the motor symptoms in Parkinson's disease.
53. Saper CB, Wainer BH, German DC. Axonal and transneuronal transport in the transmission of neurological disease: potential role in system degenerations, including Alzheimer's disease. *NSC.* 1987 Nov; 23(2):389–398.
54. Rey NL, Petit GH, Bousset L, Melki R, Brundin P. Transfer of human α -synuclein from the olfactory bulb to interconnected brain regions in mice. *Acta Neuropathol.* 2013 Aug 8.
55. Svensson E, Horváth-Puhó E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol.* 2015 Oct; 78(4):522–529. [PubMed: 26031848] **A debated epidemiological study suggesting that the risk for Parkinson's disease is reduced in people who underwent vagotomy surgery in 1977–1995, which would be consistent with the idea that α -synuclein pathology could propagate from the gut to the central nervous system via the vagus nerve.
56. Derkinderen P, Shannon KM, Brundin P. Gut feelings about smoking and coffee in Parkinson's disease. *Mov Disord.* 2014 Jul; 29(8):976–979. [PubMed: 24753353] *A review articulating a novel hypothesis that reduced Parkinson's disease risk in smokers and people who consume large amounts of coffee could be due to changes in the gut microbiome, such that the gut environment becomes less permissive for α -synuclein aggregation.
57. Scheperjans, F.; Pekkonen, E.; Kaakkola, S.; Auvinen, P. *J Parkinsons Dis.* Vol. 5. IOS Press; 2015. Linking Smoking, Coffee, Urates, and Parkinson's Disease - A Role for Gut Microbiota?; p. 255-262.
58. Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, et al. Colonic inflammation in Parkinson's disease. *Neurobiol Dis.* 2013 Feb; 50:42–48. [PubMed: 23017648]
59. Forsyth, CB.; Shannon, KM.; Kordower, JH.; Voigt, RM.; Shaikh, M.; Jaglin, JA., et al. Increased Intestinal Permeability Correlates with Sigmoid Mucosa α -Synuclein Staining and Endotoxin Exposure Markers in Early Parkinson's Disease. In: Oreja-Guevara, C., editor. *PLoS ONE.* Vol. 6. 2011 Dec 1. p. e28032
60. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Björklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* 2014 Oct 9. *A demonstration that different forms of α -synuclein can be transported along the vagal nerve to the brainstem, lending support to the idea that Parkinson's disease could be triggered by misfolding of α -synuclein in enteric nerves
61. Ulusoy A, Rusconi R, Pérez-Revuelta BI, Musgrove RE, Helwig M, Winzen-Reichert B, et al. Caudo-rostral brain spreading of α -synuclein through vagal connections. *EMBO Mol Med.* 2013 Jul; 5(7):EMBO Press:1051–1059. [PubMed: 23666744] *A study using viral vector-mediated expression of α -synuclein in the vagal nerve to provide experimental support for spreading of α -synuclein pathology to more rostral brain regions in rodents.
62. Ulusoy, A.; Musgrove, RE.; Rusconi, R.; Klinkenberg, M.; Helwig, M.; Schneider, A., et al. *BioMed Central.* 2nd. Vol. 3. *Acta Neuropathologica Communications*; 2015. Neuron-to-neuron α -synuclein propagation in vivo is independent of neuronal injury; p. 13
63. Tyson T, Steiner JA, Brundin P. Sorting Out Release, Uptake and Processing of Alpha-Synuclein During Prion-Like Spread of Pathology. *J Neurochem.* 2015 Nov 30. n/a–n/a.
64. Vekrellis, K.; Xilouri, M.; Emmanouilidou, E.; Rideout, HJ.; Stefanis, L. *Lancet Neurol.* Vol. 10. Elsevier; 2011 Nov. Pathological roles of α -synuclein in neurological disorders; p. 1015-1025.

65. Emmanouilidou E, Minakaki G, Keramioti MV, Xylaki M, Balafas E, Chrysanthou-Piterou M, et al. GABA transmission via ATP-dependent K⁺ channels regulates α -synuclein secretion in mouse striatum. *Brain*. 2016 Mar; 139(Pt 3):871–890. [PubMed: 26912647]
66. Lee, H-J.; Cho, E-D.; Lee, K-W.; Kim, J-H.; Cho, S-G.; Lee, S-J. *Exp Mol Med*. Vol. 45. Nature Publishing Group; 2013. Autophagic failure promotes the exocytosis and intercellular transfer of α -synuclein; p. e22
67. Lee H-J, Khoshaghideh F, Patel S, Lee S-J. Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. *J Neurosci. Society for Neuroscience*. 2004 Feb 25; 24(8): 1888–1896.
68. Alvarez-Erviti, L.; Seow, Y.; Schapira, AH.; Gardiner, C.; Sargent, IL.; Wood, MJA., et al. *Neurobiol Dis*. Vol. 42. Elsevier Inc; 2011 Jun 1. Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission; p. 360-367.
69. Emmanouilidou E, Vekrellis K. Exocytosis and spreading of normal and aberrant α -synuclein. *Brain Pathol*. 2016 Mar 3. n/a–n/a.
70. Bae EJ, Yang NY, Lee C, Kim S, Lee HJ, Lee SJ. Haploinsufficiency of cathepsin D leads to lysosomal dysfunction and promotes cell-to-cell transmission of α -synuclein aggregates. *Cell Death and Disease*. 2015; 6(10)Nature Publishing Group:e1901. [PubMed: 26448324] *Important findings showing that lysosomal dysfunction leads to enhanced cell-to-cell transfer of α -synuclein aggregates
71. Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, et al. Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Mol Neurodegener*. 2012; 7(1):42. [PubMed: 22920859]
72. Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, et al. Cell-Produced Alpha-Synuclein Is Secreted in a Calcium-Dependent Manner by Exosomes and Impacts Neuronal Survival. *J Neurosci*. 2010 May 19; 30(20):6838–6851. [PubMed: 20484626]
73. Grey M, Dunning CJ, Gaspar R, Grey C, Brundin P, Sparr E, et al. Acceleration of α -Synuclein Aggregation by Exosomes. *J Biol Chem*. 2015 Jan 30; 290(5):2969–2982. [PubMed: 25425650] *Study demonstrating that lipids found in exosomes can promote the aggregation of α -synuclein, further highlighting the possible role of lipids in the pathogenesis of α -synucleinopathies.
74. Lee H-J, Suk J-E, Bae E-J, Lee J-H, Paik SR, Lee S-J. Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. *Int J Biochem Cell Biol*. 2008; 40(9):1835–1849. [PubMed: 18291704]
75. Holmes BB, Devos SL, Kfoury N, Li M, Jacks R, Yanamandra K, et al. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci USA*. 2013 Aug 13; 110(33):E3138–E3147. [PubMed: 23898162]
76. Clayton EL, Evans GJO, Cousin MA. Bulk Synaptic Vesicle Endocytosis Is Rapidly Triggered during Strong Stimulation. *J Neurosci*. 2008 Jun 25; 28(26):6627–6632. [PubMed: 18579735]
77. Sung, JY.; Kim, J.; Paik, SR.; Park, JH.; Ahn, YS.; Chung, KC. *J Biol Chem*. Vol. 276. American Society for Biochemistry and Molecular Biology; 2001 Jul 20. Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein; p. 27441-27448.
78. Spencer B, Kim C, Gonzalez T, Bisquertt A, Patrick C, Rockenstein E, et al. α -Synuclein interferes with the ESCRT-III complex contributing to the pathogenesis of Lewy body disease. *Hum Mol Genet*. 2016 Mar 15; 25(6):1100–1115. [PubMed: 26740557] *Detailed experiments demonstrating, e.g., that disruption of the endosomal sorting complex required for transport (ESCRT) pathway leads to increased cell to cell transfer of α -synuclein.
79. Konno M, Hasegawa T, Baba T, Miura E, Sugeno N, Kikuchi A, et al. Suppression of dynamin GTPase decreases α -synuclein uptake by neuronal and oligodendroglial cells: a potent therapeutic target for synucleinopathy. *Mol Neurodegener. BioMed Central*. 2012; 7(1):38.
80. Freeman, D.; Cedillos, R.; Choyke, S.; Lukic, Z.; McGuire, K.; Marvin, S., et al. Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. In: Kahle, PJ., editor. *PLoS ONE*. Vol. 8. Public Library of Science; 2013. p. e62143
81. Brahic M, Bousset L, Bieri G, Melki R, Gitler AD. Axonal transport and secretion of fibrillar forms of α -synuclein, A β 42 peptide and HTTexon 1. *Acta Neuropathol*. 2016 Jan 25. Springer

- Berlin Heidelberg:1–10. *Study characterizing anterograde and retrograde axonal transport, and extracellular release, of α -synuclein fibrils in cultured mouse cortical neurons.
82. Lamberts JT, Hildebrandt EN, Brundin P. Spreading of α -synuclein in the face of axonal transport deficits in Parkinson's disease: A speculative synthesis. *Neurobiol Dis.* 2015 May;77:276–283. [PubMed: 25046996]
 83. Reyes JF, Rey NL, Bousset L, Melki R, Brundin P, Angot E. Alpha-synuclein transfers from neurons to oligodendrocytes. *Glia.* 2014 Mar; 62(3):387–398. [PubMed: 24382629] *Experiments both in cell culture and in mice showing that oligodendrocytes can take up extracellular forms of α -synuclein which might be relevant to the pathogenesis of Multiple System Atrophy.
 84. Lee H-J, Suk J-E, Patrick C, Bae E-J, Cho J-H, Rho S, et al. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J Biol Chem.* 2010 Mar 19; 285(12):9262–9272. [PubMed: 20071342]
 85. Lee H-J, Suk J-E, Bae E-J, Lee S-J. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochem Biophys Res Commun.* 2008 Aug 1; 372(3):423–428. [PubMed: 18492487]
 86. Park J-Y, Kim KS, Lee S-B, Ryu J-S, Chung KC, Choo Y-K, et al. On the mechanism of internalization of α -synuclein into microglia: Roles of ganglioside GM1 and lipid-raft. *J Neurochem.* 2009:1–38.
 87. Fellner L, Jellinger KA, Wenning GK, Stefanova N. Glial dysfunction in the pathogenesis of α -synucleinopathies: emerging concepts. *Acta Neuropathol.* 2011 Jun; 121(6):675–693. [PubMed: 21562886]
 88. Bae E-J, Lee H-J, Rockenstein E, Ho D-H, Park E-B, Yang N-Y, et al. Antibody-aided clearance of extracellular α -synuclein prevents cell-to-cell aggregate transmission. *J Neurosci. Society for Neuroscience.* 2012 Sep 26; 32(39):13454–13469.
 89. Tran, HT.; Chung, CH-Y.; Iba, M.; Zhang, Bin; Trojanowski, JQ.; Luk, KC., et al. *CellReports. The Authors;* 2014 Jun 10. a-synuclein immunotherapy blocks uptake and templated propagation of misfolded a-synuclein and neurodegeneration; p. 1-29.
 90. George, S.; Brundin, P. *J Parkinsons Dis. Vol. 5. IOS Press;* 2015 Sep 14. Immunotherapy in Parkinson's Disease: Micromanaging Alpha-Synuclein Aggregation; p. 413-424.
 91. Lee, JS.; Lee, S-J. *J Mov Disord. Vol. 9. The Korean Movement Disorders Society;* 2016 Jan. Mechanism of Anti- α -Synuclein Immunotherapy; p. 14-19.
 92. Lindström V, Ihse E, Fagerqvist T, Bergström J, Nordström E, Möller C, et al. Immunotherapy targeting α -synuclein, with relevance for future treatment of Parkinson's disease and other Lewy body disorders. *Immunotherapy.* 2014 Feb; 6(2):141–153. [PubMed: 24491088]
 93. Games D, Valera E, Spencer B, Rockenstein E, Mante M, Adame A, et al. Reducing C-Terminal-Truncated Alpha-Synuclein by Immunotherapy Attenuates Neurodegeneration and Propagation in Parkinson's Disease-Like Models. *J Neurosci.* 2014 Jul 9; 34(28):9441–9454. [PubMed: 25009275]
 94. Asi YT, Simpson JE, Heath PR, Wharton SB, Lees AJ, Revesz T, et al. Alpha-synuclein mRNA expression in oligodendrocytes in MSA. *Glia.* 2014 Mar 3; 62(6):964–970. [PubMed: 24590631] *Studies on human brain tissue indicating that oligodendrocytes can express mRNA for α -synuclein which could contribute to the glial intracellular α -synuclein inclusions in Multiple System Atrophy.
 95. Djelloul M, Holmqvist S, Boza-Serrano A, Azevedo C, Yeung MS, Goldwurm S, et al. Alpha-Synuclein Expression in the Oligodendrocyte Lineage: an In Vitro and In Vivo Study Using Rodent and Human Models. *Stem Cell Reports.* 2015 Aug 11; 5(2)Elsevier:174–184. [PubMed: 26235891] *Further evidence, in several model systems, that oligodendrocytes can express α -synuclein protein which is relevant to our understanding of Multiple System Atrophy neuropathology.
 96. Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Björklund A. TFEB-mediated autophagy rescues midbrain dopamine neurons from α -synuclein toxicity. *Proc Natl Acad Sci USA.* 2013 May 7; 110(19):E1817–E1826. [PubMed: 23610405]

97. Pérez-Revuelta BI, Hettich MM, Ciociaro A, Rotermund C, Kahle PJ, Krauss S, et al. Metformin lowers Ser-129 phosphorylated α -synuclein levels via mTOR-dependent protein phosphatase 2A activation. *Cell Death and Disease*. 2014; 5:e1209. [PubMed: 24810045]
98. Wagner J, Ryazanov S, Leonov A, Levin J, Shi S, Schmidt F, et al. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. *Acta Neuropathol*. 2013 Apr 19; 125(6):795–813. [PubMed: 23604588]
99. Levin J, Schmidt F, Boehm C, Prix C, Bötzel K, Ryazanov S, et al. The oligomer modulator anle138b inhibits disease progression in a Parkinson mouse model even with treatment started after disease onset. *Acta Neuropathol*. 2014 Mar 11; 127(5):779–780. [PubMed: 24615514]
100. Adler, CH.; Dugger, BN.; Hinni, ML.; Lott, DG.; Driver Dunckley, E.; Hidalgo, J., et al. *Neurology*. Vol. 82. Lippincott Williams & Wilkins; 2014 Mar 11. Submandibular gland needle biopsy for the diagnosis of Parkinson disease; p. 858-864.
101. Beach TG, Adler CH, Serrano G, Sue LI, Walker DG, Dugger BN, et al. Prevalence of Submandibular Gland Synucleinopathy in Parkinson's Disease, Dementia with Lewy Bodies and other Lewy Body Disorders. *J Parkinsons Dis*. 2016 Jan 9; 6(1):153–163. [PubMed: 26756744]
*Confirmatory evidence that tissue obtained from submandibular gland needle biopsies contains aggregated α -synuclein and that it can be considered as a diagnostic tool for Parkinson's disease and Dementia with Lewy Bodies
102. Corbillé A-G, Clairembault T, Coron E, Leclair-Visonneau L, Preterre C, Neunlist M, et al. What a gastrointestinal biopsy can tell us about Parkinson's disease? *Neurogastroenterol Motil*. 2016 Feb 23. n/a–n/a.
103. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's Disease? Evidence from 3 cases. *Mov Disord*. 2012 May 1; 27(6):716–719. [PubMed: 22550057]
104. Visanji NP, Marras C, Kern DS, Dakheel AlA, Gao A, Liu LWC, et al. Colonic mucosal α -synuclein lacks specificity as a biomarker for Parkinson disease. *Neurology*. 2015 Feb 10; 84(6): 609–616. [PubMed: 25589666] * Study questioning the specificity of α -synuclein aggregates in biopsies of colonic mucosa as a biomarker of Parkinson's disease, and indicating that they can also be found in control subjects.
105. Irwin, DJ.; Abrams, JY.; Schonberger, LB.; Leschek, EW.; Mills, JL.; Lee, VM-Y., et al. *JAMA Neurol*. Vol. 70. American Medical Association; 2013 Apr. Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone; p. 462-468.
106. Brown P, Brandel J-P, Sato T, Nakamura Y, MacKenzie J, Will RG, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. *Emerg Infect Dis*. 2012 Jun; 18(6):901–907. [PubMed: 22607808]

Key bullet points that summarize the article

- Intraneuronal α -synuclein aggregates are a key feature of Parkinson's disease neuropathology
- Emerging evidence suggests that α -synuclein pathology can propagate from cell-to-cell in a prion-like manner progressively engaging additional brain regions, and that this spreading might contribute to the symptomatic progression.
- The molecular mechanisms underpinning cell-to-cell spreading of α -synuclein pathology are currently being elucidated and are leading to the identification of new therapeutic targets
- Animal models of Parkinson's disease that are based on α -synuclein acting in a prion-like fashion offer a powerful test bed for novel therapeutic interventions