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REVIEW

Initiation and Transmission of α-Synuclein Pathology in Parkinson's Disease

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Abstract

The pathogenesis of Parkinson's disease (PD) involves the accumulation of aggregated forms of α-synuclein in the body. The location for the initiation of misfolded forms of α-synuclein is now a contentious issue, what was once thought to be a disease of the central nervous system (CNS) now appears to involve multiple organs in the body. In particular, the two regions in the body where the nervous system is exposed to the environment, the olfactory bulb and the enteric nervous system, are now thought to play an important role in the initial phase of the disease. Epidemiological studies point to the gastrointestinal tract, including the appendix, as a potential site for the misfolding and transmission of α-synuclein, with the vagus nerve providing a conduit between the gut and brain. A growing body of animal studies also support this pathway, implicating the transmission of pathological α-synuclein from outside the CNS in the development of PD.

Keywords Gut · Appendix · Vagus nerve · Exosomes · Neurodegeneration

Introduction

Parkinson's disease (PD) has recently been identified as the fastest growing neurodegenerative disease worldwide [1], with 1–2 individuals per 1000 affected at any time [2]. Due to the chronic nature of PD, the prevalence increases with age and currently affects 1% of the world's population over the age of 60 [2]. Although a limited number of genes have been linked directly to PD, approximately 90% of cases have no identifiable genetic cause (idiopathic PD) [3]. As such, although genetics has been powerful in identifying important pathways linked to the disease, we still do not understand the cause for the majority of PD cases. Understanding the mechanisms behind PD progression and the relevant pathways will be key for uncovering therapeutic targets.

The damage caused throughout the progression of PD is brought about by the misfolding and aggregation of α-synuclein, a protein thought to play a role in neurotransmitter release [4]. Both pathogenic and genetic evidence supports the role of α-synuclein in PD, with mutations and multiplications in the gene being observed in familial forms of the disease. The aggregation of α-synuclein results in the pathology known as Lewy bodies, which are a hallmark of the disease. The progressive build-up of Lewy bodies was long thought to be obstructive to normal neuronal physiology, leading to cell injury and cell death [5]. Although Lewy bodies are required for the determination of PD post-mortem, recent evidence suggests that smaller fibril forms of α-synuclein aggregates are most likely pathogenic, with Lewy bodies themselves being a product of larger aggregates that may well be more benign in the disease process [6].

For nearly 200 years the brain has been the focus of PD pathology, with little regard to other organs and how they may be involved in the disease process. However, over the past 20 years our understanding of PD has changed rapidly. Analysis of post-mortem tissue revealed that the disease may have origins in the brain stem or olfactory bulbs, a finding that implied transmission of α-synuclein pathology in the body [7]. Since this possibility was realised, symptoms such as anosmia (loss of smell) and gastrointestinal problems have been increasingly regarded as prodromal to the development of PD [8]. Post-Parkinson's diagnosis, these regions have been found to be particularly resistant to anti-parkinsonian treatments, suggesting more progressed damage and thus supporting the early involvement of these areas in early stage PD [8]. Whilst changes in both the olfactory and gastrointestinal regions have been shown in prodromal

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stages, this review will focus on the gastrointestinal links to PD.

If the etiology of PD initiates outside the brain, then biochemical and epidemiology studies can potentially be used to identify this transmission hypothesis. Chronic inflammation, followed by oxidative stress, has been found to alter the cellular environment in such a way that is capable of inducing the misfolding of α-synuclein [9, 10]. As such, inflammatory bowel diseases (IBD) as well as infections within the gastrointestinal tract (GIT) are implicated in the pathogenesis of PD, as each of these conditions are characterized by their chronic inflammation of the GIT [11–14]. The vagus nerve which innervates the GIT is suggested to provide a direct pathway through which pathogenic α-synuclein is able to reach the midbrain. Together, this evidence suggests that stress factors initiating outside of the brain combined with the transmission of α-synuclein, are potentially involved in the etiology of PD. Here we review recent research findings investigating the transmission of α-synuclein in the body.

PD Staging and the Prion-Like Hypothesis of α-Synuclein Transmission

Prior to discussing the data and mechanisms which implicate the GIT and vagus nerve, it is important to explain the underlying theory that would allow us to consider these organs in the development of PD. Recent research has highlighted the potential for α-synuclein to act in a prion-like manner, as such, it is necessary to understand what defines prions and why the behavior of α-synuclein in PD is referred to as prion-like.

Prion diseases, commonly termed transmissible spongiform encephalopathies, encompass a whole family of fatal neurodegenerative diseases that can impact both humans and animals [15]. The most commonly known prion diseases include Creutzfeldt–Jakob disease and bovine spongiform encephalopathy (BSE, or 'mad cow disease'). These diseases share some symptoms with those affected by PD, including movement problems, cognitive and communication deficits [16]. In a similar manner to PD, symptoms are developed due to damage caused to the central nervous system (CNS), a result of aggregation and build-up of prion proteins within affected cells. Based on the believed mode of propagation, α-synuclein has been termed as 'prion-like' or more recently as 'prionoid' [17]. The distinct difference between prionoid proteins and typical prions has long been the lack of infectivity between either humans or animals, which still remains the case for α-synuclein and PD [18]. However, recently iatrogenic transmission in humans has been observed with another prionoid protein, amyloid-β [19, 20]. This protein is believed to be a cause of Alzheimer's disease, and this observation is perhaps an indicator that other previously thought to be non-infectious prionoids may in fact be infectious [19, 20]. However, currently there is no clear evidence that α-synuclein can transmit between individuals that would allow for re-classification as a prion protein.

Alpha-synuclein is believed to behave in a prion-like manner, referring to Stanley Prusiner's Nobel Prize winning 'protein-only' hypothesis. Prion-proteins are commonly found cellular proteins, contributing to normal bodily functions. These proteins normally exist primarily in an α-helical structure with a small portion of β-sheet structure [21]. However, these proteins are susceptible to conformational changes when exposed to certain conditions or pathogens [22]. Isoforms are created, shifting a proportion of their α-helical structure towards β-sheet structure, overall altering the proteins function within the body [21]. Alpha-synuclein is thought to exist primarily as an unfolded monomer within the cytoplasm, however, has been observed to undergo conformational changes when exposed to certain conditions [23–25]. Akin to prion-proteins, α-synuclein's transition from its native form towards a primarily β-sheet structure has been found to make the protein insoluble and protease resistant [23, 26]. This simultaneously causes a loss of the proteins original function and creates toxic fibrils, leading towards aggregation and the eventual damage of affected cells [23, 24, 27]. Once the structural shift occurs, this pathogenic protein begins to behave in a prionoid fashion, as has been observed in both cell culture and animal models [5, 28, 29]. It is through structural alterations such as this, that α-synuclein is believed to initiate the pathogenesis of PD.

According to the protein-only hypothesis, structurally altered prion-proteins utilize the body's own cell-to-cell communication pathways to propagate. Infectious prion molecules transfer from an infected cell to nearby naïve cells, utilizing the cell-to-cell mechanisms of communication, either through processes such as passive diffusion, endocytosis or by using membrane bound vesicles such as exosomes [30, 31]. Once these infectious prions have gained access to recipient cells, they create a template for the de novo folding of proteins, thereby altering the naïve cells to also contain misfolded proteins. This transmission process is believed to be cyclic, propagating between cells in the disease process [32, 33]. This is in contrast to normal cellular function, where the process of creating new proteins occurs from the transcription and translation of a cell's genetic information [34].

In Braak's 'dual hit' hypothesis, he postulated that an unknown foreign pathogen is responsible for initiating the misfolding of α-synuclein [35]. While the pathogen itself has not been identified, Braak proposes that the first Lewy-pathology is initiated within the olfactory bulb or the GIT, the latter site then allowing for the propagation along the vagus nerve towards the midbrain. From there, the pathology spreads in a caudal-rostral direction throughout the CNS, eventually causing the neurodegeneration in the brain observed in PD. The terming of PD as a prion-like disease originates here, as the movement of aggregated α-synuclein along the vagus nerve to the midbrain is believed to occur with the use of prion-like mechanisms [36, 37].

Support for the prion-like propagation of pathogenic α-synuclein was principally observed within the grafted dopaminergic neurons of patients who had undergone a trial of neuronal replacement therapy [38, 39]. The post-mortem analysis of these individuals' brain tissue saw the presence of Lewy-pathology within the newly grafted dopaminergic neurons, which were too young to have developed the pathology in situ. This data strongly supported the concept that Lewy-pathology is able to propagate between cells in the brain. Since then, both mouse and non-human primate experimental models of PD have had success in demonstrating the development and spread of Lewy-pathology using synthetic forms of α-synuclein. Injections of pre-formed fibrils of α-synuclein into the nasal and intraperitoneal regions of mice caused the eventual decline in motor control, through damage to dopaminergic neurons [5, 40, 41]. Studies assessing the impact on non-human primates noted that symptoms bore a close resemblance to that of diagnosed PD patients [5].

The culmination of these observations forms the basis behind the involvement of the vagus nerve in PD development. As the GIT is innervated by the vagus nerve, this creates a theoretical pathway for pathogenic α-synuclein to reach the midbrain. The following section will elaborate on the vagus nerve and its role in PD.

The Role of the Vagal Nerve in the Development of Parkinson's Disease

The innervation of the GIT and its organs by the vagus nerve is a proposed link between the gut and the brain, implicating the nerve as a highway for pathogenic α-synuclein in PD [42]. The vagus nerve is involved in carrying sensory fibres from the midbrain to innervated organs, which includes the heart, lower respiratory tract and the GIT [43]. Of particular interest to the development of PD are the myenteric and mesenteric plexuses of the vagus nerve, which are the branches supplying the organs of the GIT (Fig. 1) [43]. These connections include the stomach, duodenum, liver, gallbladder, pancreas and the inferior duodenum [43]. It is through this route that pathogenic α-synuclein may gain access to the midbrain, a hypothesis supported by several epidemiological studies.

The first of these studies utilised the Danish registry of patients, comparing two groups who had undergone vagotomy against a matched general population. Vagotomy is a surgical procedure formally used to treat peptic ulcers where the vagus nerve is resected. The two vagotomy groups differed in the degree to which their vagus nerve was resected, as their relative diagnoses required the procedure to be performed at different locations. The first group of patients underwent a full truncal vagotomy, wherein both of the vagal trunks innervating the GIT were severed. The second group only underwent a selective vagotomy, only cutting the branches which innervate the fundus and stomach [44]. After assessing the prevalence of PD between these groups, the meta-data showed a significantly lower risk of PD in patients which had undergone a truncal vagotomy [44]. In contrast, the super-selective vagotomy group was found to have a similar risk as the general population, demonstrating little to no reduction in PD risk [44].

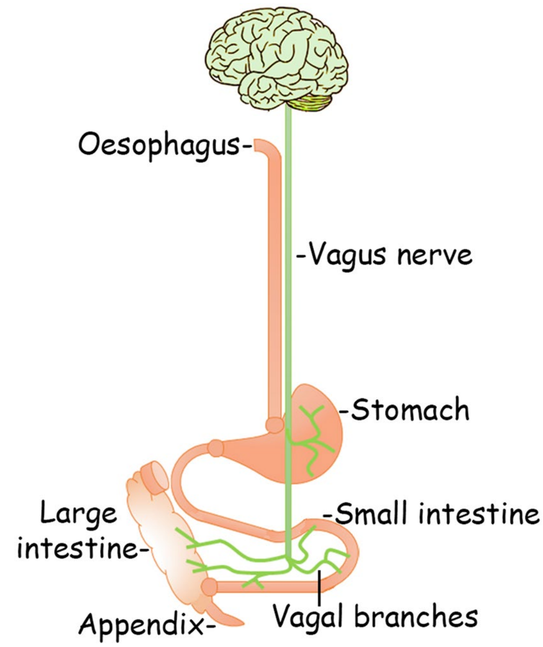

Fig. 1 The role of the gut-brain axis in Parkinson's disease. The vagus nerve innervates multiple regions of the gastrointestinal tract, providing a connection between the enteric nervous system and the central nervous system

These findings were later followed by a second study using data from the Swedish registry, assessing patients with the same procedures [45]. This study also found a lower risk of PD among truncal vagotomy patients. However, in contrast to the Danish study, the decrease in risk was reported as not statistically significant in the Swedish study, although they did report a possible protective effect from truncal vagotomies, with a stronger protective effect following at least 5 years post-vagotomy. [45]. Thus the evidence is not clear as to the protective effect of vagotomy, as various factors such as vagotomy type, timing of procedure and age when the surgery was performed must be considered for variability, especially considering the chronic nature of the PD.

A common trend presents in both these studies, wherein an increased protective effect is observed the earlier in life the vagotomy is performed. This observation is significant as it is in line with the chronic progression and development of PD. The data implies that severing this theoretical highway for pathogenic α-synuclein may slow or halt the movement of the protein to the midbrain. In addition to this, the results also stated that there was little to no impact on the risk of PD for patients with a selective vagotomy. This may indicate that α-synuclein pathology is able to enter the vagus nerve through different locations, as the vagus nerve innervates the majority of the GIT. However, it is important to keep in mind that these epidemiological studies do not show any direct cause of the disease, or the movement of pathological α-synuclein through the vagus nerve, but rather implicate the vagus nerve as a pathway in the disease mechanism.

The transmission of α-synuclein through the vagus nerve is supported by several animal studies. These models demonstrated the ability of exogenous pathogenic α-synuclein to be internalised by cells and thereby seed nearby naïve cells to misfold endogenous α-synuclein [5, 46]. Intrastriatal inoculations of synthesised and human Lewy body tissue lysates into mice and monkeys saw the eventual spread of PD pathology to anatomically interconnected regions, followed by progressive neurodegeneration and Parkinsonian symptoms [5, 46]. A study by Holmqvist et al. has since provided direct evidence of retrograde movement of α-synuclein along the vagus nerve. To confirm that forms of α-synuclein were transported from the GIT to the brain, fluoro-tagged monomeric, oligomeric or fibrillar α-synuclein were injected into the intestinal wall of mice. Following 12, 48 and 72 h incubation periods, the fluorescent α-synuclein was detected within the dorsal motor nucleus of the vagus nerve [47]. This study was also able to show the time-dependent transmission of α-synuclein as it progressed along the vagus nerve.

A recent study by Kim et al. set out to investigate the direct gut-brain movement of α-synuclein, providing further support for the involvement of the vagus nerve in PD development. This study injected pre-formed α-synuclein fibrils into the pylorus and duodenum of mice, both of which are innervated by the vagus nerve. Following a 10-month incubation period, a reduction in fine motor control and dexterity was observed, representing Parkinson-like symptoms. These results were compared against two groups, mice which had a vagotomy performed above the injected site and against α-synuclein knockout mice. While PD was observed in the wild type group, the other groups did not develop Parkinson-like symptoms and no α-synuclein pathology was found beyond the resected section of the vagus nerve [48]. The movement of PD pathology along the vagus nerve is clearly demonstrated in this study and has since been supported by studies using transgenic rats and mice [49, 50]. This is also reinforced by the demonstration that the movement of pathogenic α-synuclein is halted when this proposed route is severed, stopping the development of Parkinson-like symptoms.

The culmination of the protective effect observed through epidemiological studies, as well as the representation of α-synuclein movement along the vagal nerve through the animal studies, strongly support the involvement of the vagus nerve in PD. Following on from this, it is important to assess the organs innervated by the vagus nerve and how their physiology may impact the later development of PD.

Does the Appendix Have a Role in Initiating Parkinson's Disease?

The appendix is located within the GIT, attached to the end of the cecum wherein the small intestine empties its contents [51]. It is currently unknown whether this organ serves any useful function to the human body. There is a long-held belief that the appendix is slowly being phased out as an organ through the evolutionary process. However, there is evidence for the role of the appendix in both the maintenance of gut microbiota and in modulating immune reactions within the GIT [52]. Thus, this believed to be vestigial organ may in fact have important physiological functions, with new data also suggesting it may be instrumental in the development of PD.

The presence of pathologic α-synuclein has been detected in multiple sites within the GIT, including the duodenum, pylorus, small intestine and the appendix. This was assessed through the analysis of tissue biopsies acquired from patients believed to be in the prodromal stages of PD, with the results showing that PD pathology can appear up to 20 years prior to diagnosis [53]. Follow-up investigations have found that the appendix is rich with endogenous α-synuclein. Most notably it was found that α-synuclein pathology is present in healthy appendixes of both young and old individuals [54]. Assessments into PD risk following an appendectomy was conducted using both the Swedish National Patient Registry and the Parkinson's Progression Markers Initiative. This analysis identified a reduction in PD risk and a delayed age of onset in appendectomy patients when compared to the general population [54]. Interestingly, this reduction in risk was higher for populations that live in a rural setting, where the use of pesticides is higher, supporting the impact of environmental factors on PD risk [40].

The suggested link between appendectomy and PD risk is due to the innervation of the appendix by the vagus nerve. If the role of the appendix as an immune organ is accurate, this would imply that readily available endogenous α-synuclein within the appendix is exposed to conditions created by inflammation. The typical reactive immune process releases pro-inflammatory cytokines to trigger a systemic inflammatory response against infection, injury, irritants or toxic proteins

[55]. However, issues arise when inflammation becomes chronic, as cells are unable to reach homeostasis, resulting in oxidative stress and the overproduction of harmful reactive oxidative species (ROS) [55]. These ROS are usually a normal by-product of oxidative phosphorylation, the process of creating energy, and are cleared by the body's antioxidant systems [56]. However, when imbalances occur between the amounts of ROS created and removed, this leads to oxidative stress and cytotoxicity.

Experimental models have also shown that conformational changes and aggregation of α-synuclein can occur during periods of chronic oxidative stress. The aggregation of α-synuclein into fibrils is highly pH sensitive, and oxidative stress has been shown to lower pH surrounding the mitochondria [10, 57]. At the body's physiological pH of 7.0–7.5, α-synuclein exists in a monomeric state and is negatively charged [9, 25, 57]. Experimental models have been able to demonstrate that α-synuclein's net charge falls to zero as the surrounding environment's pH drops, nullifying the refractive forces between α-synuclein molecules. This facilitates conformational changes within α-synuclein and accelerates the aggregation process [9, 57]. In addition, morphological differences of the protein are observed at different pH values, with fibrils appearing more bundled at lower pH values [9]. Together, this data indicates that local inflammation potentially creates an environment that can alter the conformation of α-synuclein.

Further support for the role of the appendix in PD onset was observed in individuals who had appendectomies earlier in life, with these individuals demonstrating a delay in the age of PD onset [54]. When considering the proposed role of the appendix in the human body and the organ's innervation by the vagus nerve, the removal of the appendix may be beneficial for several reasons. Firstly, the early removal of this α-synuclein rich organ would potentially limit the amount of available endogenous α-synuclein. In the long term, this would theoretically limit the protein's exposure to the inflammatory environment created through either inflammatory bowel disease (IBD) or infection, which have been shown to induce misfolding of α-synuclein. In addition, the removal of the appendix would reduce the surface area innervated by the vagus nerve and further limit the access routes through which misfolded α-synuclein could gain access to the proposed pathway to the midbrain. However, the early removal of the appendix is potentially detrimental to the health of the GIT, as is explored later. Therefore, further studies are required on the role of the appendix, including long term impacts resulting from removal of the organ.

Inflammatory Bowel Diseases and Parkinson's Disease

Due to the observed interactions between inflammation and α-synuclein, inflammatory conditions which impact the GIT should be considered in the overall aetiology of PD. Crohn's disease (CD) and ulcerative colitis (UC) are two diseases which possess these prerequisite symptoms and both fall under the IBD umbrella term. These conditions are typically characterised by chronic inflammation of the GIT, with patients affected as early as young adulthood [58, 59]. Ulcerative colitis only causes superficial inflammation of the mucosa of the colon and anus. However, CD impacts multiple organs: the ileum, colon and areas along the intestinal wall with the inflammation penetrating beyond the mucosal layer [60, 61].

Nationwide cohort studies on Taiwanese, Danish and Swedish populations were conducted to ascertain the associated risk of PD in patients diagnosed with IBD [12–14]. Divergent results were observed, with the Taiwanese study reporting a higher PD risk for patients with CD, while the Danish study reported a higher risk to patients with UC. Nonetheless, all these studies agreed that IBD was associated with a higher risk of developing PD. It is however important to note the reservations presented by Weimers from the Swedish cohort study. Here, they noted that the associations found between IBD and PD may in part be attributed to surveillance bias, a factor which may have impacted the other epidemiological studies [14]. In relation to the Danish study produced by Villumsen et al., Weimers suggested that adjusting their data by the number of patient follow-up visits to the doctors would result in the disappearance of the observed increased risk of PD as a result of IBD diagnosis [62]. In response to these observations, Villumsen et al. conducted follow up analyses of their data, also adjusting for the number of healthcare visits [63]. The results of this analysis showed that the overall risk of PD in patients with IBD did not decrease, suggesting that the association between IBD and PD is not explained by surveillance bias [63].

Strengthening the link between IBD and PD, there is also a common genetic mutation found between CD and PD. A missense mutation of the leucine-rich repeat kinase 2 (LRRK2) gene has been linked to both conditions [64, 65]. It is hypothesised that this gene has cell-type specific interactions, with functional roles including vesicular trafficking and immune response regulation [66]. Within the scope of the lymphatic system, one of the functions of the LRRK2 gene is to be a negative regulator of T cells, modulating immune responses during foreign insults such as pathogens [67]. However, mutations of the LRRK2 gene have been linked to excessive inflammatory responses, further promoting the secretion of pro-inflammatory cytokines and thus in this manner may be a contributor to the progression of PD [67, 68].

The LRRK2 gene has also been found to play a role in regulating vesicular trafficking within neuronal cell types [69]. The movement of biological material between cells is generally controlled by endocytosis and exocytosis, with both processes being highly regulated. Dysregulation of these pathways has been found to occur in PD patients, with mutations of LRRK2 being linked to increased secretion of neurotransmitters and proteins [69]. Dysregulation of this pathway has been observed to alter neuronal cell physiology, with hyper-signalling of microglia within the CNS further promoting inflammation [69, 70].

All these factors are potentially part of a cascade leading to PD development. Genetic predisposition, such as the LRRK2 mutation, further potentiates inflammatory responses and increases the motility of vesicular transport, possibly facilitating the prion-like movement of pathogenic α-synuclein. While there is clearly a genetic contribution to PD development, as is observed through the LRRK2 mutation in both CD and PD, it is important to note that only approximately 10% of all PD cases have been attributed to genetic factors [3].

Conflicting Studies for the Role of the Gut-Brain Axis in Parkinson's Disease

The mechanisms behind PD development are a highly controversial area, with many studies reporting opposing views on the involvement of the GIT and enteric nervous system (ENS). For example, the cohort studies analysed in this review reported a decrease in PD risk following an appendectomy. However, other studies exploring the significance of the appendix in other inflammatory diseases report that the removal of the appendix results in a higher risk of developing CD later in life [71, 72]. These studies indicate that the function of the appendix in microbiota maintenance and immune response is essential to the overall wellbeing of the GIT [71]. It is thought that the appendix stores natural flora and replenishes the GIT system following damage which disturbs the balance of intestinal microbiota [73]. Here, we have discussed the potential significance of the appendix in immune responses and how it may increase PD risk via the endogenous α-synuclein it contains. As such, it is believed that an appendectomy would remove a significant lymphatic organ within the GIT and in turn impact the mechanisms responsible for maintaining homeostasis in the GIT, supposedly increasing risk of CD [71]. As the pathway for the transmission of aggregated α-synuclein is suggested to be promoted by inflammation, this data challenges the notion of a reduction in PD risk following an appendectomy. However, there are conflicting opinions regarding the impacts of an appendectomy on IBD. A cohort study on this topic concluded that the increased risk of CD following an appendectomy was most likely explained by diagnostic bias [74]. This occurred as patients were prematurely diagnosed with appendicitis and underwent an appendectomy, however were in fact in the early stages of developing CD, a matter that became apparent to their physicians at a later time [74]. Further, an Australian study from 2002 found that an appendectomy performed early in life, resulted in a delay in disease onset as well as milder symptoms for CD and UC [75]. However, the fact remains that there is data suggesting that an appendectomy causes an increase in CD risk.

Other contradictions arise against the proposed pathway, ones which argue against Braak's hypothesised routes of entry to the midbrain. One such study assessed the presence of Lewy pathology in the brain and spinal cord of 71 samples retrieved from the UK Parkinson's Disease Society Tissue Bank. Post-analysis, the study reported that not all the samples contained Lewy pathology within the dorsal motor nucleus of the vagus nerve, despite the pathology existing within the substantia nigra and cortical regions of the CNS [76]. In addition, a recent study by Ulosoy et al. injected human α-synuclein proteins into the midbrain of rats. Following a period of up to 12 months, they were able to observe that this human α-synuclein had found its way down the vagus nerve and imbedded within the gastric wall of injected rats [42]. This was also supported by Van Den Berge and colleagues, who demonstrated that infectious α-synuclein is able to proliferate bidirectionally [49]. The study noted that the infectious α-synuclein propagated initially from the duodenum towards the dorsal motor nucleus of the vagus nerve. However, once reaching this location the protein also had secondary anterograde propagation towards the stomach and the heart [49]. The studies by Ulosoy et al. and Van Den Berge et al. demonstrate alternative routes through which α-synuclein pathology can travel, suggesting that the movement of pathogenic α-synuclein is not always consistent with clinical presentations and Braak's initiation hypothesis. Within the clinical setting, another study noted that the hallmark Lewy bodies were not detected in the neighboring regions to the brain stem of post-mortem brain analyses of PD patients [77, 78]. These observations demonstrate that pathologic α-synuclein may not simply follow along synaptic connections but is influenced by other cell specific factors.

The lack of Lewy pathology within the dorsal motor nucleus of the vagus nerve of some patients and the alternative pathways for α-synuclein pathology identified in animal studies potentially challenge Braak's hypothesis. The idiopathic misfolding of α-synuclein in locations outside of the GIT or CNS would go against the proposed incubation period within the GIT and subsequent direction of travel along the vagus nerve. In addition, the lack of Lewy pathology within anatomically connected regions of the brain indicates that there is more to the transport of pathogenic α-synuclein than merely unidirectional trafficking along nerve fibers. Clearly more research is required to uncover the nuances behind the movement of pathological α-synuclein in the body.

How Does α-Synuclein Transmit Between Cells and Why Doesn't Everyone Get PD After Infection?

Currently little is known about the transmission of α-synuclein in the body. Extracellular α-synuclein can be found in the CSF, blood and saliva of both the general population and PD patients [79]. The vast majority of extracellular α-synuclein appears as a 'free' form (note, it is highly unlikely that α-synuclein would ever be present as a single molecule given its association with lipids and other proteins, free here refers to the extracellular protein not being enclosed in a biological membrane), although a small percentage is also encapsulated in extracellular vesicles (EVs) called exosomes [80]. Exosomes are nanometer-sized vesicles derived from multivesicular bodies in the cell, which have been shown to be involved in cell-to-cell communication [81]. Molecules of interest, including proteins, are encapsulated within a lipid membrane, moved to the cellular membrane and are secreted to recipient cells [81]. Interestingly, the exosome microenvironment has been shown to induce the misfolding and aggregation of α-synuclein, implicating this mechanism as a potential pathogenic pathway for α-synuclein transmission [57]. Exosomes have also been shown to increase the uptake of α-synuclein to recipient cells compared to the free form of the protein, suggesting they may play a larger role in the disease process than indicated by their low abundance of total extracellular α-synuclein [19]. Other methods for the transmission of α-synuclein between cells include tunneling nanotubes that allow direct transmission between two cells [82] (Fig. 2). Further research is required to identify the pathological basis for the transmission of α-synuclein in the body.

Given that the GIT undergoes many inflammatory challenges as we age, and the appendix of healthy people has been shown to contain misfolded and aggregated forms of α-synuclein [54], the question remains as to why everyone does not develop PD over time if the GIT is the site of origin? A potential regulation pathway limiting the transmission of extracellular α-synuclein is the efficient clearance of the protein from bodily fluids (Fig. 2). Free extracellular α-synuclein can be broken down by proteases, whilst exosomes have been shown to be efficiently cleared by the liver when in circulation, or through the action of macrophages in the bloodstream [83]. Other clearance mechanisms also exist in the brain, such as the lymphatic system that has recently been shown to have a role in clearance of waste products from the cerebrospinal and interstitial fluids [84]. Importantly, this pathway has been shown to play a potential role in neurodegenerative disease and has been observed to be impaired with aging [85, 86]. Failure of these clearance mechanisms is a potential pathway allowing for the accumulation of pathological α-synuclein in the body that over time can develop into PD.

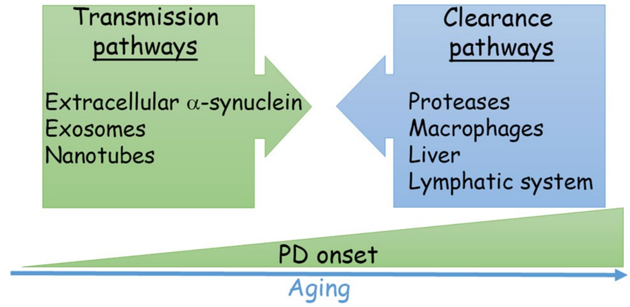

Fig. 2 Factors that can promote the transmission of pathogenic α-synuclein in the body and those that can prevent the spread of pathology. The transmission of Parkinson's disease is thought to occur through either (i) the diffusion of pathogenic extracellular α-synuclein, (ii) trafficking in extracellular vesicles such as exosomes or (iii) through cell to cell contacts using tunnelling nanotubes. There are multiple types of clearance mechanisms in the body that may prevent the transmission of pathogenic α-synuclein, these include (i) proteases that can break down the protein, (ii) macrophages in the bloodstream or (iii) organs such as the liver. Newly discovered lymphatic networks that allow clearance from the brain are also possible routes for removal, however these lymphatic pathways have not yet been shown in humans. It is postulated that during aging these clearance pathways may be inhibited allowing for the progression of disease

Conclusion

There is a growing body of evidence pointing towards the GIT and its involvement in the pathogenesis of PD. The findings link the innervation of the GIT by the vagus nerve, the existence of misfolded α-synuclein within the appendix, and the impact of inflammation on α-synuclein aggregation to the pathogenesis of PD. As inflammation is a common symptom associated with PD and has been shown to contribute to the misfolding of α-synuclein through both in vivo and in vitro studies, it should be considered as a potential contributor towards PD development.

One of the puzzling elements regarding the propagation of PD pathology is that the progression is somewhat structured (although not all cases follow this progression), and traverses along specific routes. One possible explanation for this, as presented by Braak, was that projection neurons are the most susceptible to Parkinson's pathology [87]. These neurons are described as having long, thin and unmyelinated axons and make up most of the vagus nerve [87]. However, the exact reasons behind this vulnerability are not fully understood [88]. It is unknown why cells of different structures, proximal to impacted cells, remain unaffected by Parkinson's pathology and continue to function as normal [88].

The findings presented by Kim et al., Van Den Berge et al. and Lohmann et al. in 2019, in combination with the decrease in PD risk observed in the epidemiological studies, suggest that the vagus nerve can potentially be a therapeutic target. If pathologic α-synuclein is detected early enough within an individual, future therapies may aim to slow or stop the movement of pathogenic α-synuclein along this pathway. While a full truncal vagotomy may appear to be a therapeutic option, it is an invasive procedure and the negative drawbacks such as diarrhea, regurgitation issues and dysphagia must be taken into consideration [89]. More research is required into both the early detection of PD and its relevant pathology, as well as the mechanisms involved in the transmission of α-synuclein in the body. Further, due to the presence of α-synuclein within a supposed vestigial appendix, an appendectomy may present itself as a potential procedure to limit PD onset. However, more research is required into the true role of the appendix within the human body, and the long-term impacts following an appendectomy. This is especially important considering the potential increases in IBD following the removal of the appendix. It is just as important to consider this organs role in immune response, as gastrointestinal infections have also been linked to increase PD risk [11].

As can be seen, there is a multitude of factors which have the potential to culminate in the development of PD, with a newly defined role of α-synuclein pathology initiating outside of the CNS. However, while most of the population possesses an appendix and undergoes inflammatory events throughout their life, only a subset of individuals develop PD. Therefore, multiple factors are important in determining the risk of PD, these include genetic factors, environmental exposure, α-synuclein aggregation and transmission, as well as clearance and protective mechanisms in the body. As convincing as current data may be in explaining the methods of PD development and propagation, there also exists contrary data suggesting alternate routes of α-synuclein propagation in the body. As such, more research is required to elucidate a full picture of the aetiology of PD, with the now expanded view that the disease may start or have a role in regions outside the CNS.

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