How Does Parkinson's Disease Begin? Perspectives on Neuroanatomical Pathways, Prions, and Histology

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ABSTRACT: Parkinson's disease (PD) is a multisystem disorder with involvement of the peripheral nervous system. Misfolding and aggregation of α-synuclein is central to the pathogenesis of PD, and it has been postulated that the disease may originate in olfactory and gastrointestinal nerve terminals. The prion-like behavior of α-synuclein has been convincingly demonstrated in vitro and in animal models of PD. Lewy-type pathology have been detected in peripheral organs many years prior to PD diagnosis, and 2 independent studies have now suggested that truncal vagotomy may be protective against the disorder. Other lines of evidence are difficult to reconcile with a peripheral onset of PD, most importantly the relative scarcity of post mortem cases with isolated gastrointestinal α-synuclein pathology without concomitant CNS pathology. This Scientific Perspectives article revisits some important topics with implications for the dual-hit hypothesis. An account of the neuroanatomical pathways necessary for stereotypical α-synuclein spreading is presented. Parallels to the existing knowledge on true prion disorders, including Creutzfeld-Jakob disease, are examined. Finally, the vagotomy studies and the somewhat inconsistent findings in the growing literature on peripheral α-synuclein pathology are discussed. It is concluded that the dual-hit hypothesis remains a potential explanation for PD pathogenesis, but several issues need to be resolved before more firm conclusions can be drawn. © 2017 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; alpha-synuclein; Lewy; dual-hit hypothesis; prion

Parkinson's disease (PD) is a multisystem disorder that involves the central and peripheral nervous systems. Recent decades have witnessed a considerable expansion in our knowledge of the pathogenesis and molecular characteristics of PD. The neuronal inclusion bodies originally identified by Friedrich Lewy in 1912[1] contain pathological aggregates of the normally soluble small protein α-synuclein (α-syn).[2] The spatial distribution of Lewy neurites and bodies in the brain of PD patients is not random, but generally compatible with a staging scheme proposed in 2003 postulating that inclusions initially appear in the olfactory bulb and dorsal motor nucleus of the vagus (DMV) in the lower brain stem.[3] The pathology then seems to ascend in a predictable fashion with subsequent involvement of monoaminergic pontine nuclei and the dopaminergic neurons of the substantia nigra. The credibility of this idea has gained further support because α-syn exhibits prion-like properties, including the propensity to misfold and formation of aggregates, which display cell-to-cell transmission.[4,5]

The "dual-hit hypothesis" extended this explanatory framework by postulating that initial formation of α-syn aggregates occurs outside the brain, that is, in the terminal end fields of neurons residing in the olfactory bulb and the preganglionic parasympathetic neurons of the DMV.[6,7] This concept presents a potential explanation as to why the olfactory bulb and DMV shows early, severe involvement in most cases of PD, but is also controversial because it implies that PD could in part be caused by exterior insults such as toxins or microorganisms. Also, not all lines of evidence are supportive of the dual-hit hypothesis, which has led to alternative theories about the origin of PD.[5,8,9]

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In this Scientific Perspectives article, several important topics with implications for the dual-hit hypothesis will be examined. First, the stereotypical spreading pattern presupposes the existence of specific neuronal interconnections. An account of these neuroanatomical pathways is provided. Second, centripetal spreading of peripherally administered α-syn aggregates in transgenic rodent models of PD has been demonstrated. Such seeding experiments have been performed for several decades in models of true prion diseases such as bovine spongiform encephalopathy and scrapie. The similarities between these prion models and the α-syn PD models are discussed. Third, a number of studies have investigated the presence and distribution of pathological α-syn assemblies in theperipheral organs of PD patients. Recently, we and others have reported that Lewy-type pathology is detectable in the gastrointestinal tract up to 20 years prior to PD diagnosis.[10] However, the literature also shows inconsistencies and, in addition, some important findings go against the dual-hit hypothesis. Fourth, in 2015, we reported that truncal vagotomy substantially reduces the risk of PD.[11] The implications of this finding are discussed.

Neuro-Anatomical Pathways Subserving α-Syn Propagation

A number of neuro-anatomical tracer studies have demonstrated the existence of relevant neuronal interconnections[12-24] (summarized in Figure 1). Most studies employed classical retrograde and anterograde staining techniques such as horseradish peroxidase, fluorogold, and phaseolus vulgaris leucoagglutinin in rodents, cats, and monkeys. More advanced methods have also been used, including bidirectional tracers and barcode messenger RNA (mRNA) sequencing techniques.[15,20]

Several important observations arise from this literature. First, the most relevant mono-synaptic interconnections necessary for the hypothesized stereotypical α-syn spreading pattern[3] have been demonstrated. The DMV is connected to the raphe nuclei and locus coeruleus (LC),[12,17,19,22] which are in turn connected to the substantia nigra.[12,18,23] Importantly, no direct mono-synaptic pathways have been found between the DMV and the nigra,[12] which could conceivably introduce a time lag for the α-syn propagation from the lower brain stem to the mesencephalon.

Second, the spinal cord including the preganglionic sympathetic neurons of the intermediolateral cell column (IML) is directly connected to the LC and neighboring noradrenergic A5 nucleus[19,21] as well as to the caudal raphe complex and magnocellular nucleus of the reticular formation[25,26] (not shown in Fig 1). A small fraction of PD patients (<10%) do not show α-syn pathology in the DMV postmortem, but these cases most often exhibit involvement of the LC and raphe nuclei.[27,28] If pathological α-syn species truly reach the brain stem via autonomic nerves, these aberrant PD cases could therefore be explained by a predominantly sympathetic involvement and subsequent IML-to-LC spreading, which would bypass the DMV. However, autopsy cases with isolated α-syn pathology in the spinal cord but not in the CNS are rare,[25,29,30] which argues against this spreading route.

Third, the nigra and especially the LC are directly connected to the olfactory bulb.[16,20] Indeed, a recent study using high throughput mapping of barcoded mRNA demonstrated that 23% of LC neurons directly innervate the olfactory bulb in mice.[15] At the initial formulation of the Braak staging scheme, the importance of early olfactory bulb pathology was questioned because few incursions into related olfactory regions were seen.[6] However, the direct pathways from the LC and nigra to the bulb provide yet another route of entry from a Braak stage 1 structure to the pons and mesencephalon—a pathway that also bypasses the DMV.

In summary, known neuro-anatomical pathways are compatible with a peripheral-to-central spreading pattern and the interconnections also provide potential explanations why not all PD cases strictly follow the original Braak staging system.[3] On the other hand, the existence of these pathways does not per se prove that PD originates in peripheral organs, only that it is a theoretical possibility.

Prions and PD

The prion concept in PD and Alzheimer's disease has been the subject of several recent reviews to which the reader is referred for a full account on this topic.[4,5]

The normally soluble protein α-syn is predominantly found in nerve terminals and may be involved interneuronal vesicle transport.[31,32] Interestingly, recent studies have shown that native α-syn may be a neuron-specific inhibitor preventing access of neuroinvasive viruses to the central nervous system perhaps by inhibiting viral exocytosis pathways.[33-35] The α-syn has the propensity for spontaneous misfolding and displays prion-like properties in vitro, including cell-to-cell propagation.[4,36,37] Both retro- and anterograde axonal transport of α-syn fibrils have been demonstrated in the vagal nerve,[38,39] and pathological aggregates were detected in the DMV 1 week after injection of α-syn fibrils in the duodenum of rats.[40] Recently, it was shown that a single intra-peritoneal injection of α-syn fibrils in A53T transgenic mice led to marked neurological symptoms after an incubation period of ~7 months. Severe widespread α-syn pathology was seen in the spinal cord, brain stem, and cerebrum.[41]

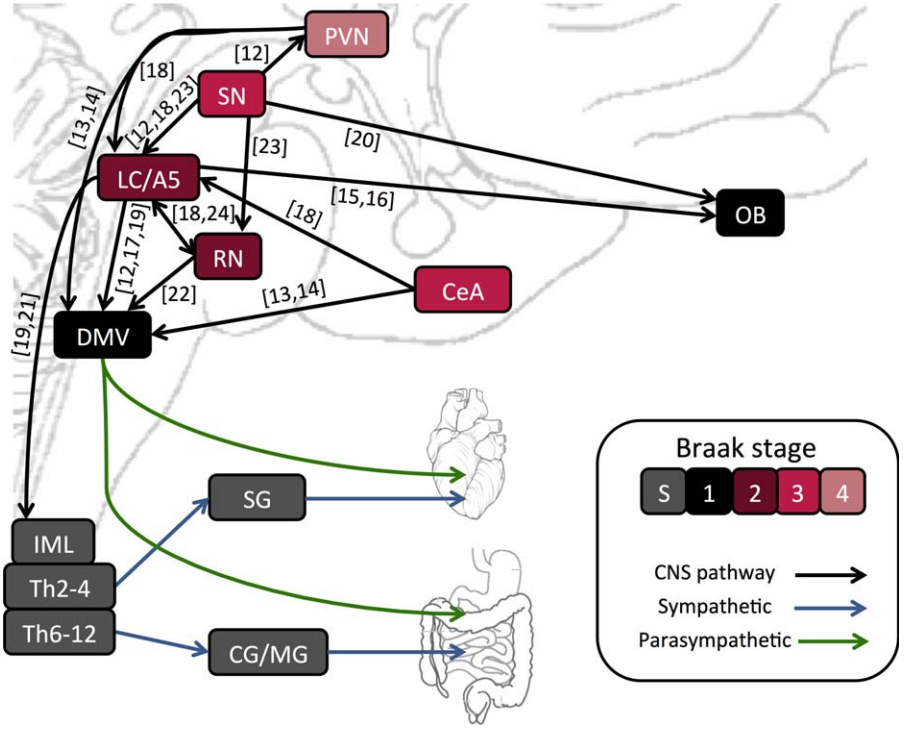

FIG. 1. Summary of relevant neuro-anatomical pathways necessary for α-syn propagation. Braak stages are color coded. Pre- and postganglionic cell groups of the sympathetic nervous system are shown in gray. Numbers in brackets designate references.[12-24] CeA, central nucleus of amygdala; CG/MG, celiac & superior mesenteric ganglia; DMV, dorsal motor nucleus of vagus; IML, intermediolateral cell column; LC/A5, locus coeruleus and noradrenergic A5 nucleus; OB, olfactory bulb; PVN, paraventricular nucleus; RN, raphe nuclei; SG, stellate ganglion; SN, substantia nigra. [Color figure can be viewed at wileyonlinelibrary.com]

Thus, there is little doubt about the prion-like properties of α-syn in animal models, but no formal evidence have demonstrated that similar prion-like spreading occurs in human PD patients. Interestingly, a recent report suggested that iatrogenic transmissible Alzheimer pathology may have developed decades after treatment with cadaveric pituitary-derived growth hormone in adolescence.[42]

Some observations and findings in true prion disorders such as Creutzfeldt-Jakob disease (CJD) may have relevance for PD. In the prion field, seeding experiments in animal models have been performed for decades, and Figure 2 summarizes the main findings.

After oral infection with bovine spongiform encephalopathy–associated or scrapie-associated prions in cattle, sheep, and hamsters, the initial prion aggregates are seen in the gut-associated lymphoid tissue of the tonsils and Peyer's patches.[43-47] The subsequent spreading of prions through nervous tissues shows similar spatial and temporal characteristics in these animal models. Often, the first prion aggregates are seen in the coeliac-mesenteric ganglia (CMG) followed by involvement of the DMV and IML (Figure 2A). From these initial entry points, the pathology spreads throughout the CNS, but the involvement of the telencephalon is a relatively late phenomenon. It should also be noted that prion aggregates are seen in efferent pathways before afferent, that is, the CMG and IML are affected before the dorsal root ganglia, and the DMV before the nodose ganglion.[43,44] A similar predilection for retrograde transport through efferent fibers may also be the case in PD, suggested by the much more severe involvement of the DMV compared to the sensory vagal nuclei[3] and also by the demonstration that virtually all vagal efferents to the gut express α-syn, whereas vagal afferents in the myenteric plexus and GI smooth muscle do not.[48] Thus, the initial spreading of prions through the autonomic nervous system in these animal models is highly similar to the hypothesized route of α-syn propagation in PD.

The most common human prion disease is CJD, in which ~1% of cases represent the acquired variant CJD (vCJD); 85% to 90% are sporadic (sCJD), and approximately 10% are familial.[49] Several postmortem studies have revealed striking differences between acquired and sporadic/familial CJD. In vCJD autopsies, prions were detected in the lympho-reticular system of the tonsils, spleen, and lymph nodes,[50] and the appendix also showed consistent involvement—in 1 vCJD case, 8 months prior to disease onset.[51] Prions have also been detected in the coeliac and stellate ganglia and were found to colocalize with sympathetic postganglionic neurons.[52] In contrast, such peripheral prion pathology is never seen in sCJD, but is confined to the CNS.

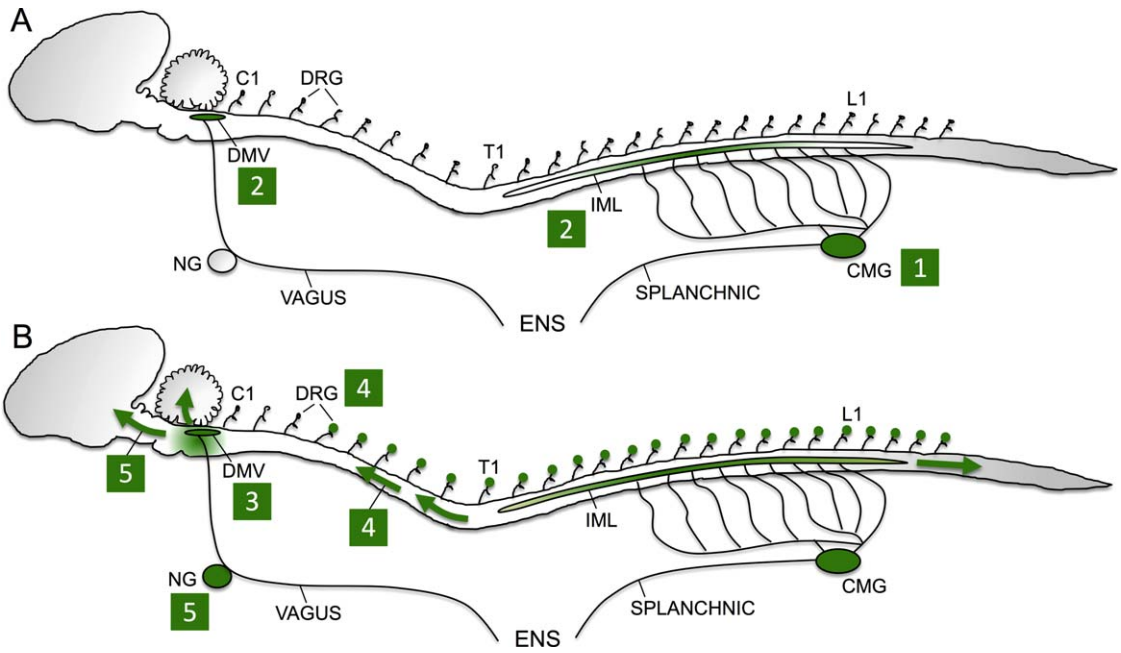

FIG. 2. Summary of temporal and spatial prion spreading patterns in hamster-, sheep-, and cattle-transmissible spongiform encephalopathy models. **A**. After oral infection with scrapie/bovine spongiform encephalopathy strains, the initial neuronal pathology is often seen in abdominal ganglia (1) followed by the IML and DMV (2). **B**. From these initial entry points, the pathology spreads throughout the CNS (3) and at later time points involve structures such as the nodose and dorsal root ganglia (4, 5), perhaps as a result of anterograde axonal transport of prions. Extensive involvement of the telencephalon occurs relatively late (5). CMG, coeliac and mesenteric ganglia; DMV, dorsal motor nucleus of vagus; DRG, dorsal root ganglia; ENS, enteric nervous system; IML, intermediolateral cell column; NG, nodose ganglion. [Color figure can be viewed at wileyonlinelibrary.com]

These findings in vCJD support the experimental prion animal literature and suggest that initial disease propagation in some acquired prion disorders occurs via the autonomic nervous system. In contrast, the initial misfolding event in the much more frequent sporadic/familial prion disorders probably arises through a spontaneous stochastic event within the CNS.[53]

In PD, it was originally suggested that α-syn pathology may preferentially originate in the terminals of neurons with long, nonmyelinated, hyper-branched axons.[5,6] This proposition would partly explain why autonomic nerves are so prone to formation of Lewy pathology, as these axons are among the longest and most hyper-branched in the body. One possibility is that the initiation point in a given PD case may be a stochastic event—similarly to sCJD. In other words, the pathology in most PD patients may originate in olfactory, gastrointestinal, and/or cardiac nerve endings because these are statistically the most likely sites of stochastic α-syn misfolding. In addition, the propensity to misfold could be further enhanced by the close proximity of these nerve endings to exogenous toxins and microorganisms. Whether such exogenous factors are of major importance in the pathogenesis of PD is at present unknown, but pesticides have been linked to increased risk of PD,[54] and gastric lavage with rotenone was shown to induce α-syn aggregation and secondary spreading to the CNS in a mouse model.[55] Perturbations in the gut microbiota might also predispose toward enhanced α-syn misfolding and aggregation.[56,57] Finally, the purported protective effect of tobacco use[58] could be related to a direct anti-aggregation effect on the olfactory and gastrointestinal nerve terminals.

Pathological α-Syn in Peripheral Organs

In diagnosed PD patients, pathological α-syn aggregations have been detected in various peripheral tissues, including the nervous plexuses of the gastrointestinal tract,[59-63], heart,[64] salivary glands,[63,65,66] and skin.[67,68] However, to assess whether pathological α-syn appears during the preclinical phase, studies of prodromal cases, archived tissues, and autopsy material from neurologically intact individuals with or without incidental Lewy body disease (ILBD) are necessary.

Several autopsy studies have, in addition to PD patients, included a number of ILBD cases and reported pathological α-syn aggregates in peripheral organs, including gastrointestinal nerve plexuses and sympathetic ganglia.[25,30,61,63,69,70] However, the prevalence of peripheral α-syn pathology is generally lower in ILBD than in diagnosed PD and DLB cases.

Studies of patients with idiopathic rapid eye movement sleep behavior disorder reported that 56% of cases displayed phosphorylated α-syn in dermal fibres, and 8 of 9 cases were positive in the salivary glands.[71,72] A few studies examined archived tissue

specimens removed from PD patients years prior to diagnosis. In one study, 9% of PD cases (3/33) were positive for phosphorylated α-syn in the gastrointestinal tract, whereas another reported 67% (2/3) positive cases.[73,74] No positive controls were reported in these studies. We found that 56% (22/39) of PD cases harbored phosphorylated α-syn aggregates in the gut up to 20 years prior to diagnosis, but 26% (23/90) of matched control tissues were also positive.[10]

An important criticism of the peripheral onset hypothesis in PD stems from the relative lack of cases with isolated peripheral α-syn pathology in the absence of CNS involvement. A total of 466 whole-body autopsies from the Arizona study of Aging and Neurodegenerative Disorders suggest that the olfactory bulb is the most common site of isolated α-syn pathology.[75] However, this group of researchers has not encountered a single case in which pathological α-syn was present in a peripheral body region but not in the CNS.[66] In contrast, Japanese studies have reported a number of cases with isolated α-syn pathology in the myenteric plexus of the esophagus and small intestine, the paraspinal sympathetic ganglia, adrenal glands, and also some cases with pathology restricted to the ganglia and thoracic IML.[30,76-78] It should be noted that 1 of these studies was published prior to the era of α-syn immunohistochemistry, and the findings should be interpreted with caution. Put together, these data are compatible with the olfactory bulb being a probable entry point. At face value, the scarcity of cases with isolated gastrointestinal α-syn pathology could be considered a coup de grâce for the "gut part" of the dual-hit hypothesis. Still, several factors need to be taken into account.

First, in a brain-stem nucleus such as the DMV, thousands of neurons are situated closely together, which maximizes the probability of detecting pathology. In contrast, the terminal fields of the DMV neurons are widely distributed across the thoracic and most abdominal organs. If the terminal axonal branches are heterogeneously affected by α-syn pathology, it follows that the false negative rate could be considerably higher for detection of peripheral pathology, whereas central lesions will be much easier to find because the pathology is siphoned toward the same localized, compact nuclei.

Second, the time window from terminal α-syn misfolding to the appearance of aggregates in the soma located in the CNS could in theory be relatively short (weeks), as suggested by animal evidence.[40] Also, sparse and highly localized terminal pathology may be sufficient to initiate retrograde transport of α-syn aggregates. Detecting such limited and localized peripheral pathology in just the right time window before central pathology appears may be like finding the proverbial needle in the hay stack. This concept is supported by observations from the prion literature. In cattle, which were orally challenged with bovine spongiform encephalopathy, prions were detected in the CMG, IML, and DMV, whereas the animals were negative in the myenteric or submucosal plexuses.[46] Thus, prion pathology, which with certainty have entered through the gastrointestinal tract and subsequently spread to the CNS, can remain below detection threshold in the periphery while being detectable in the brain. Interestingly, a similar finding was reported in a single autopsy case of kuru, a prion disorder transmitted via endocannibalism among the native population on Papua New Guinea. Here, spongiosis and prions were seen in the cortex, basal ganglia, thalamus, and brain stem. Limited pathology was seen in the spinal cord and roots, and none was found in peripheral tissues, including the ileum and spleen.[79]

Third, the most commonly used immunohistochemical methods are optimized toward detection of macroaggregates, that is, inclusion bodies and neurites. However, the formation of mature aggregates is preceded by oligomeric and proto-fibril species.[80] Several studies have shown that α-syn oligomers disseminate more efficiently than higher molecular weight assemblies,[81-83] and retrograde vagus-mediated transport of α-syn oligomers has been demonstrated in an in vivo rat model.[40] Therefore, it cannot be ruled out that retrograde axonal transmission of α-syn oligomers and proto-fibrils may precede the formation of mature α-syn aggregates in the periphery. Current immunohistochemical techniques may lack the sensitivity to detect such early aggregates. Interestingly, a recent study used a proximity ligation assay to detect α-syn oligomer species in brain tissue and was able to detect significantly more pale bodies and extra-somal Lewy bodies than standard immunohistochemistry, suggesting greater sensitivity to earlier lesions.[84] Another technique termed *real-time quaking-induced conversion* exploits the ability of prion protein to self-aggregate and showed a diagnostic accuracy of >97% for diagnosing sCJD patients using nasal brushings.[85] This assay has now been adapted for α-syn detection and has shown high diagnostic accuracy for diagnosing PD in CSF samples.[86] Such techniques could conceivably also improve detection rates of α-syn pathology in peripheral tissues.

Fourth, it is now recognized that current immunohistochemical methods for detecting α-syn pathology in peripheral organs suffer from lack of standardization and difficulties of interpretation. This is underscored by published rates of positive colonic staining in neurologically intact individuals, which varies from 0%[59,87] to nearly 100%.[88,89] Also, skin biopsies from PD patients were positive in 100% of cases in one study, but 0% in another.[63,67] Consequently, multicenter studies are now being carried out to optimize

staining protocols and homogenize interpretation.[90-93] An important goal will be to define robust criteria for what constitutes pathological staining in peripheral organs, but this task is made difficult by the lack of definite negative controls. It is known that 10% to 30% of aged subjects without clinical parkinsonism or dementia display Lewy pathology in the brain postmortem.[94-97] Also, the prevalence of incidental Alzheimer-type amyloid pathology increases from 15% to 44% in the 60-to-90-year range in cognitively intact individuals.[98] In other words, it cannot automatically be expected that healthy aged individuals without ILBD do not harbor pathological α-syn aggregates in peripheral organs. For this reason, staining patterns seen in aged non-ILBD subjects cannot automatically be designated as physiological or nonspecific. Further validation studies may require inclusion of tissue from very young subjects, where pathological α-syn aggregates are probably less frequent. To my knowledge, this is currently not an active field of investigation.

In summary, the relative lack of postmortem cases with isolated peripheral α-syn pathology for the moment weakens the case for a gastrointestinal onset in PD. However, several issues need to be resolved before more firm conclusions can be drawn.

Protective Effect of Vagotomy

If the vagal nerve constitutes a major highway for centripetal spreading of α-syn pathology, it follows that vagotomy could be protective against PD. Prior to the *helicobacter pylori* era, vagotomy was a commonly used treatment for peptic ulcer. Three principal types of vagotomy were employed: full truncal vagotomy; selective vagotomy where only vagal branches to the stomach were cut, and the most refined super-selective vagotomy, where only the corpus and fundus were denervated.[99] Only truncal vagotomy would be expected to significantly prevent retrograde propagation of α-syn aggregates.

Observations from animal models support that sectioning parts of the autonomic nervous system delays the neuroinvasion of peripherally seeded protein aggregates. In a hamster scrapie model, chemical or immunological sympathectomy significantly delayed (but did not prevent) the spreading of scrapie-type prions to the CNS after intraperitoneal injection.[100] Similar findings were seen in the rotenone mouse model of PD, although concerns have been raised about the reproducibility of this model. When the stomach of mice is exposed to the mitochondrial poison rotenone, α-syn aggregation and subsequent spreading to the brain stem via the vagus has been demonstrated.[55,101] However, both partial sympathectomy and hemivagotomy significantly delayed the development of motor symptoms in the animals. Also, partial sympathectomy delayed the appearance and diminished the amount of pathological α-syn in the IML, and hemivagotomy prevented accumulation of α-syn in the ipsilateral DMV. Intriguingly, hemivagotomy also seemed to prevent cell death in the ipsilateral SNc.

In 2015, we published the first epidemiological study to test whether vagotomy modifies the risk of PD.[11] Cohorts of patients, who had undergone vagotomy during 1977-1995 were constructed and compared to matched controls. Of note, the statistical power of our study may have been blunted because truncal and selective vagotomy patients were binned into 1 group because of concerns of registry code validity. Only super-selective vagotomy patients could be isolated and analyzed separately. After >20 years of follow-up, the truncal/selective vagotomy cohort showed a significantly decreased risk of PD compared to the background population (hazard ratio [HR] = 0.53; 95% confidence interval [95% CI] 0.28-0.99). A nonsignificantly decreased risk of PD was also seen when comparing the truncal/selective group directly to the super-selective group (HR = 0.58; 95% CI 0.28-1.20).

This finding has now been reproduced by Swedish researchers.[102] Importantly, here the truncal vagotomy patients were uniquely identifiable, and the selective and super-selective cases were binned into 1 group. The truncal vagotomy group exhibited a decreased risk of PD after >5 years of follow-up when compared with the background population (HR = 0.59; 95% CI 0.37-0.93), and also when compared with the selective/super-selective group (HR = 0.54; 95% CI 0.32-0.91). Thus, the risk-modifying effect of truncal vagotomy has been detected in 2 independent datasets.

Of note, the Danish vagotomy data set was independently analyzed by a Norwegian research group.[103] The study was published as a brief letter and contains sparse details on the statistical methodology making a critical evaluation difficult. The Norwegian group included all 15,079 vagotomy subjects operated during 1977-2011. We identified nearly the same number, that is, 14,893 subjects operated during the golden era of vagotomy (1977-1995) prior to the *H. pylori* treatments. The follow-up period was 1 year longer in our study. The Norwegian group analyzed truncal and selective vagotomies in 2 separate groups, which was not completely valid because surgical codes of truncal and selective vagotomy are known to be unreliable in the Danish registers.[104] However, both operations require pyloroplasty, so a combined truncal/selective group can be unequivocally identified based on the concomitant pyloroplasty code, which was the strategy we employed. Tysnes and colleagues[103] focused only on significance testing and found no significant

protective effect of vagotomy. They did not report important estimates of relative risk with confidence intervals in vagotomy patients stratified on follow-up time (<10 years and 10-20 years). In contrast to our study, they also did not impose a minimum follow-up criterion on the data, but accepted PD cases in the vagotomy cohorts even if patients were diagnosed immediately after vagotomy. The prodromal phase in PD is thought to span 5 to 20 years, with early involvement of the brain stem. Thus, if a patient develops PD within a few years after vagotomy, the pathology was conceivably already in the brain stem at the time of vagotomy. By not imposing a minimum required follow-up time from vagotomy to PD diagnosis, the protective effect of vagotomy will likely be more difficult to detect. This concept is underscored by the Swedish findings. When using the entire follow-up period (0-30 years) a smaller protective trend was observed (HR = 0.78; 95% CI 0.55-1.09), but when restricting the follow-up period to >5 years a larger effect was seen (HR = 0.59; 95% CI 0.37-0.93). In summary, the analysis of Tysnes and colleagues[103] differed from ours in a number of important ways, and the findings in the 2 studies cannot be directly compared.

A number of epidemiological studies have reported an increased risk of PD risk following various types of infections and inflammation, including hepatitis C, herpes viruses, influenza, and *H. pylori*.[105-109] Interestingly, both vagotomy studies reported an increased PD risk (HR = ~1.1-1.2) among the selective/super-selective vagotomy patients compared to the background population. Although these findings were not statistically significant, they are nevertheless supported by the observation in a Danish register study that *H. pylori* treatment is associated with a 45% increased risk of PD.[107]

One intriguing possibility is that gastrointestinal inflammation caused by infections or ulcers could predispose to α-syn aggregation. Recently, we and another research group showed that appendectomy carries an increased risk of subsequent PD.[110,111] Again, a possible interpretation is that the inflammatory state (ie, appendicitis) preceding the appendectomy increases the risk of initiating the α-syn aggregation cascade. At the moment, this hypothesized association between gut inflammation/infection and α-syn aggregation remains speculative, but the novel findings that native α-syn may inhibit the access of neuro-invasive viruses to the CNS could be involved.[33,34] Such infection/α-syn aggregation interactions could potentially be studied in the novel transgenic rodent models of PD. Also, more epidemiological studies are needed to elucidate if respiratory, gastrointestinal, and urogenital infections in general may predispose to PD.

In summary, the vagotomy findings support the dual-hit hypothesis, but alternative explanations cannot be ruled out. For instance, the vagus is critically involved in a cholinergic anti-inflammatory pathway with the ability to suppress both local and systemic inflammation.[112] It is at present largely unknown how truncal vagotomy affects inflammatory responses in humans, and especially how such modifications of inflammatory mechanisms might affect cellular capabilities to deal with α-syn aggregates.

Conclusions—Where Does PD Begin?

At the time of its conception, the "dual-hit" hypothesis[6,7,113] was controversial and to a certain extent it still is. Nevertheless, it cannot be denied that a range of observations and findings in PD is supportive of this hypothesis. Hyposmia and constipation are frequent nonmotor symptoms appearing years or decades prior to PD diagnosis. Lewy-type pathology in peripheral tissues has been detected up to 20 years prior to diagnosis. Two independent studies reported that the risk of PD may be substantially decreased following truncal vagotomy. Finally, accumulating evidence from animal studies clearly demonstrates the prion-like properties of α-syn, including centripetal spreading following peripheral administration of pathological aggregates. Of note, the spreading pattern is similar to that seen in animal models of true prion disorders such as scrapie and also similar to autopsy findings in patients with acquired CJD.

Other lines of evidence are difficult to reconcile with a peripheral onset of PD, most notably the relative lack of autopsy cases with isolated peripheral α-syn pathology in the absence of CNS involvement. However, the field of α-syn staining of peripheral tissues is still immature. More studies are needed to refine staining methods and define robust criteria on how to interpret staining patterns in the gastro-intestinal tract and other tissues. At the moment, we have insufficient knowledge about what constitutes a pathological α-syn aggregate in peripheral tissues. Also, it is unclear whether α-syn oligomers and proto-fibrils formed in peripheral tissues are sufficient for retrograde transport to the brain stem or spinal cord, where peripheral pathology is still sparse and difficult to detect. It may be necessary to implement methods for detecting and quantifying oligomeric and proto-fibrillar α-syn species in peripheral tissues to answer some of these questions. In addition, a "brain first" or a "nose first" hypothesis of PD etiopathogenesis also needs to account for why truncal vagotomy seems to be protective against the disease.

Multiple system atrophy and limbic-predominant DLB are also α-synucleinoathies, but with different

distributions of the pathological α-syn aggregates in comparison to PD.[94] It appears more difficult to argue that these disorders originate in the gut, although an olfactory bulb entry hypothesis has been suggested for DLB.[114-116] In any case, CJD constitutes an interesting parallel. Many fundamental aspects of sporadic and variant CJD are similar,[117] but one is an acquired prion disease travelling through the autonomic nervous system—the other arises much later in life probably as a result of an "unfortunate" stochastic event within the CNS. Perhaps the α-synucleinoathies will eventually be categorized into similarly discrete categories with distinct initiation points of pathological protein aggregation.

Finally, if α-syn aggregation is initiated in peripheral nerve terminals of the olfactory and gastrointestinal lining, this presents some interesting prospects for therapeutic interventions. Even small amounts of ingested or inhaled agents would reach these target zones in high concentrations, and the ability to cross the blood–brain barrier would not necessarily be a requirement. ■

References

1. Rodrigues e Silva AM, Geldsetzer F, Holdorff B, et al. Who was the man who discovered the "Lewy bodies"? Mov Disord 2010; 25(12):1765-1773.
2. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997; 388(6645):839-840.
3. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24(2):197-211.
4. Goedert M, Masuda-Suzukake M, Falcon B. Like prions: the propagation of aggregated tau and alpha-synuclein in neurodegeneration. Brain 2017;140(Pt 2):266-278.
5. Uchihara T, Giasson BI. Propagation of alpha-synuclein pathology: hypotheses, discoveries, and yet unresolved questions from experimental and human brain studies. Acta Neuropathol 2016; 131(1):49-73.
6. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm (Vienna) 2003;110(5):517-536.
7. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol 2007;33(6):599-614.
8. Engelender S, Isacson O. The Threshold Theory for Parkinson's Disease. Trends Neurosci 2017;40(1):4-14.
9. Adler CH, Beach TG. Neuropathological basis of nonmotor manifestations of Parkinson's disease. Mov Disord 2016;31(8):1114-1119.
10. Stokholm MG, Danielsen EH, Hamilton-Dutoit SJ, Borghammer P. Pathological alpha-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. Ann Neurol 2016;79(6): 940-949.
11. Svensson E, Horvath-Puho E, Thomsen RW, et al. Vagotomy and subsequent risk of Parkinson's disease. Ann Neurol 2015;78(4): 522-529.
12. Wang ZY, Lian H, Cai QQ, et al. No direct projection is observed from the substantia nigra to the dorsal vagus complex in the rat. Journal of Parkinson's disease 2014;4(3):375-383.
13. Banihashemi L, O'Neill EJ, Rinaman L. Central neural responses to restraint stress are altered in rats with an early life history of repeated brief maternal separation. Neuroscience 2011;192:413-428.
14. Gray TS, Magnuson DJ. Neuropeptide neuronal efferents from the bed nucleus of the stria terminalis and central amygdaloid nucleus to the dorsal vagal complex in the rat. J Comp Neurol 1987;262(3):365-374.
15. Kebschull JM, Garcia da Silva P, Reid AP, Peikon ID, Albeanu DF, Zador AM. High-Throughput Mapping of Single-Neuron Projections by Sequencing of Barcoded RNA. Neuron 2016;91(5): 975-987.
16. Shipley MT, Halloran FJ, de la Torre J. Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. Brain Res 1985;329(1-2):294-299.
17. Ter Horst GJ, Toes GJ, Van Willigen JD. Locus coeruleus projections to the dorsal motor vagus nucleus in the rat. Neuroscience 1991;45(1):153-160.
18. Cedarbaum JM, Aghajanian GK. Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J Comp Neurol 1978;178(1):1-16.
19. Westlund KN, Coulter JD. Descending projections of the locus coeruleus and subcoeruleus/medial parabrachial nuclei in monkey: axonal transport studies and dopamine-beta-hydroxylase immunocytochemistry. Brain Res 1980;2(3):235-264.
20. Hoglinger GU, Alvarez-Fischer D, Arias-Carrion O, et al. A new dopaminergic nigro-olfactory projection. Acta Neuropathol 2015; 130(3):333-348.
21. Bruinstroop E, Cano G, Vanderhorst VG, et al. Spinal projections of the A5, A6 (locus coeruleus), and A7 noradrenergic cell groups in rats. J Comp Neurol 2012;520(9):1985-2001.
22. Rogers RC, Kita H, Butcher LL, Novin D. Afferent projections to the dorsal motor nucleus of the vagus. Brain Res Bull 1980;5(4): 365-373.
23. Lee HS, Kim MA, Waterhouse BD. Retrograde double-labeling study of common afferent projections to the dorsal raphe and the nuclear core of the locus coeruleus in the rat. J Comp Neurol 2005;481(2):179-193.
24. Kim MA, Lee HS, Lee BY, Waterhouse BD. Reciprocal connections between subdivisions of the dorsal raphe and the nuclear core of the locus coeruleus in the rat. Brain Res 2004;1026(1):56-67.
25. Del Tredici K, Braak H. Spinal cord lesions in sporadic Parkinson's disease. Acta Neuropathol 2012;124(5):643-664.
26. Hornung JP. The human raphe nuclei and the serotonergic system. J Chem Neuroanat 2003;26(4):331-343.
27. Kalaitzakis ME, Graeber MB, Gentleman SM, Pearce RK. The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease: a critical analysis of alpha-synuclein staging. Neuropathol Appl Neurobiol 2008;34(3):284-295.
28. Attems J, Jellinger KA. The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease. Neuropathol Appl Neurobiol 2008;34(4):466-467.
29. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol 2006;32(3):284-295.
30. Sumikura H, Takao M, Hatsuta H, et al. Distribution of alpha-synuclein in the spinal cord and dorsal root ganglia in an autopsy cohort of elderly persons. Acta Neuropathol Commun 2015;3:57.
31. Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 2010;329(5999):1663-1667.
32. Burre J, Sharma M, Sudhof TC. alpha-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proc Natl Acad Sci U S A 2014; 111(40):E4274-4283.
33. Beatman EL, Massey A, Shives KD, et al. Alpha-Synuclein Expression Restricts RNA Viral Infections in the Brain. J Virol 2015;90(6):2767-2782.
34. Massey AR, Beckham JD. Alpha-Synuclein, a Novel Viral Restriction Factor Hiding in Plain Sight. DNA Cell Biol 2016;35(11): 643-645.
35. Tomlinson JJ, Shutinoski B, Dong L, et al. Holocranohistochemistry enables the visualization of alpha-synuclein expression in the murine olfactory system and discovery of its systemic antimicrobial effects. J Neural Transm (Vienna) 2017;124(6):721-738.

36. Luk KC, Kehm V, Carroll J, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in non-transgenic mice. Science 2012;338(6109):949-953.
37. Masuda-Suzukake M, Nonaka T, Hosokawa M, et al. Prion-like spreading of pathological alpha-synuclein in brain. Brain 2013; 136(Pt 4):1128-1138.
38. Ulusoy A, Rusconi R, Perez-Revuelta BI, et al. Caudo-rostral brain spreading of alpha-synuclein through vagal connections. EMBO molecular medicine 2013;5(7):1051-1059.
39. Ulusoy A, Phillips RJ, Helwig M, Klinkenberg M, Powley TL, Di Monte DA. Brain-to-stomach transfer of alpha-synuclein via vagal preganglionic projections. Acta Neuropathol 2017;133(3):381-393.
40. Holmqvist S, Chutna O, Bousset L, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. Acta Neuropathol 2014;128(6):805-820.
41. Breid S, Bernis ME, Babila JT, Garza MC, Wille H, Tamguney G. Neuroinvasion of alpha-Synuclein Prionoids after Intraperitoneal and Intraglossal Inoculation. J Virol 2016;90(20):9182-9193.
42. Jaunmuktane Z, Mead S, Ellis M, et al. Evidence for human transmission of amyloid-beta pathology and cerebral amyloid angiopathy. Nature 2015;525(7568):247-250.
43. McBride PA, Schulz-Schaeffer WJ, Donaldson M, et al. Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves. J Virol 2001;75(19):9320-9327.
44. van Keulen LJ, Vromans ME, Dolstra CH, Bossers A, van Zijderveld FG. Pathogenesis of bovine spongiform encephalopathy in sheep. Arch Virol 2008;153(3):445-453.
45. Wells GA, Dawson M, Hawkins SA, et al. Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy. Vet Rec 1994;135(2):40-41.
46. Hoffmann C, Ziegler U, Buschmann A, et al. Prions spread via the autonomic nervous system from the gut to the central nervous system in cattle incubating bovine spongiform encephalopathy. J Gen Virol 2007;88(Pt 3):1048-1055.
47. Baldauf E, Beekes M, Diringer H. Evidence for an alternative direct route of access for the scrapie agent to the brain bypassing the spinal cord. J Gen Virol 1997;78 (Pt 5):1187-1197.
48. Phillips RJ, Walter GC, Wilder SL, Baronowsky EA, Powley TL. Alpha-synuclein-immunopositive myenteric neurons and vagal preganglionic terminals: autonomic pathway implicated in Parkinson's disease? Neuroscience 2008;153(3):733-750.
49. Chen C, Dong XP. Epidemiological characteristics of human prion diseases. Infect Dis Poverty 2016;5(1):47.
50. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 1999;353(9148):183-189.
51. Hilton DA, Fathers E, Edwards P, Ironside JW, Zajicek J. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. Lancet 1998;352(9129):703-704.
52. Haik S, Faucheux BA, Sazdovitch V, et al. The sympathetic nervous system is involved in variant Creutzfeldt-Jakob disease. Nat Med 2003;9(9):1121-1123.
53. Head MW, Ritchie D, Smith N, et al. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: an immunohistochemical, quantitative, and biochemical study. Am J Pathol 2004;164(1):143-153.
54. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. Lancet neurology 2016; 15(12):1257-1272.
55. Pan-Montojo F, Anichtchik O, Dening Y, et al. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. PLoS One 2010;5(1):e8762.
56. Sampson TR, Debelius JW, Thron T, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell 2016;167(6):1469-1480 e1412.
57. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord 2015;30(3):350-358.
58. Li X, Li W, Liu G, Shen X, Tang Y. Association between cigarette smoking and Parkinson's disease: A meta-analysis. Arch Gerontol Geriatr 2015;61(3):510-516.
59. Lebouvier T, Neunlist M, Bruley des Varannes S, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 2010;5(9):e12728.
60. Devos D, Lebouvier T, Lardeux B, et al. Colonic inflammation in Parkinson's disease. Neurobiol Dis 2013;50:42-48.
61. Annerino DM, Arshad S, Taylor GM, Adler CH, Beach TG, Greene JG. Parkinson's disease is not associated with gastrointestinal myenteric ganglion neuron loss. Acta Neuropathol 2012; 124(5):665-680.
62. Gelpi E, Navarro-Otano J, Tolosa E, et al. Multiple organ involvement by alpha-synuclein pathology in Lewy body disorders. Mov Disord 2014;29(8):1010-1018.
63. Beach TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010;119(6):689-702.
64. Orimo S, Uchihara T, Nakamura A, et al. Axonal alpha-synuclein aggregates herald centripetal degeneration of cardiac sympathetic nerve in Parkinson's disease. Brain 2008;131(Pt 3):642-650.
65. Adler CH, Dugger BN, Hinni ML, et al. Submandibular gland needle biopsy for the diagnosis of Parkinson disease. Neurology 2014.
66. Beach TG, Adler CH, Serrano G, et al. Prevalence of Submandibular Gland Synucleinopathy in Parkinson's Disease, Dementia with Lewy Bodies and other Lewy Body Disorders. Journal of Parkinson's disease 2016;6(1):153-163.
67. Donadio V, Incensi A, Piccinini C, et al. Skin nerve misfolded alpha-synuclein in pure autonomic failure and Parkinson disease. Ann Neurol 2016;79(2):306-316.
68. Gibbons CH, Garcia J, Wang N, Shih LC, Freeman R. The diagnostic discrimination of cutaneous alpha-synuclein deposition in Parkinson disease. Neurology 2016;87(5):505-512.
69. Saito Y, Shioya A, Sano T, Sumikura H, Murata M, Murayama S. Lewy body pathology involves the olfactory cells in Parkinson's disease and related disorders. Mov Disord 2016; 31(1):135-138.
70. Del Tredici K, Duda JE. Peripheral Lewy body pathology in Parkinson's disease and incidental Lewy body disease: four cases. J Neurol Sci 2011;310(1-2):100-106.
71. Doppler K, Jentschke HM, Schulmeyer L, et al. Dermal phospho-alpha-synuclein deposits confirm REM sleep behaviour disorder as prodromal Parkinson's disease. Acta Neuropathol 2017;133(4): 535-545.
72. Vilas D, Iranzo A, Tolosa E, et al. Assessment of alpha-synuclein in submandibular glands of patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. Lancet neurology 2016;15(7):708-718.
73. Hilton D, Stephens M, Kirk L, et al. Accumulation of alpha-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. Acta Neuropathol 2014;127(2):235-241.
74. Ito S, Takao M, Hatsuta H, et al. Alpha-synuclein immunohistochemistry of gastrointestinal and biliary surgical specimens for diagnosis of Lewy body disease. Int J Clin Exp Pathol 2014;7(4): 1714-1723.
75. Adler CH, Dugger BN, Hentz JG, et al. Peripheral Synucleinopathy in Early Parkinson's Disease: Submandibular Gland Needle Biopsy Findings. Mov Disord 2016;31(2):250-256.
76. Miki Y, Mori F, Wakabayashi K, Kuroda N, Orimo S. Incidental Lewy body disease restricted to the heart and stellate ganglia. Mov Disord 2009;24(15):2299-2301.
77. Fumimura Y, Ikemura M, Saito Y, et al. Analysis of the adrenal gland is useful for evaluating pathology of the peripheral autonomic nervous system in lewy body disease. J Neuropathol Exp Neurol 2007;66(5):354-362.
78. Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol 1988;76(3):217-221.
79. Collinge J, Whitfield J, McKintosh E, et al. A clinical study of kuru patients with long incubation periods at the end of the epidemic in Papua New Guinea. Philos Trans R Soc Lond B Biol Sci 2008;363(1510):3725-3739.
80. Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med 1998;4(11):1318-1320.

81. Peelaerts W, Bousset L, Van der Perren A, et al. alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature 2015;522(7556):340-344.
82. Reyes JF, Rey NL, Bousset L, Melki R, Brundin P, Angot E. Alpha-synuclein transfers from neurons to oligodendrocytes. Glia 2014;62(3):387-398.
83. Rey NL, Petit GH, Bousset L, Melki R, Brundin P. Transfer of human alpha-synuclein from the olfactory bulb to interconnected brain regions in mice. Acta Neuropathol 2013;126(4):555-573.
84. Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. Brain 2015;138(Pt 6):1642-1657.
85. Orru CD, Bongianni M, Tonoli G, et al. A test for Creutzfeldt-Jakob disease using nasal brushings. N Engl J Med 2014;371(6): 519-529.
86. Fairfoul G, McGuire LI, Pal S, et al. Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. Ann Clin Transl Neurol 2016;3(10):812-818.
87. Pouclet H, Lebouvier T, Coron E, Neunlist M, Derkinderen P. Lewy pathology in gastric and duodenal biopsies in Parkinson's Disease. Mov Disord 2012;27(6):708.
88. Bottner M, Zorenkov D, Hellwig I, et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. Neurobiol Dis 2012;48(3):474-480.
89. Visanji NP, Marras C, Kern DS, et al. Colonic mucosal a-synuclein lacks specificity as a biomarker for Parkinson disease. Neurology 2015;84(6):609-616.
90. Beach TG, Corbille AG, Letournel F, et al. Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects. Journal of Parkinson's disease 2016;6(4):761-770.
91. Corbille AG, Letournel F, Kordower JH, et al. Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies. Acta Neuropathol Commun 2016;4:35.
92. Lee JM, Derkinderen P, Kordower JH, et al. The Search for a Peripheral Biopsy Indicator of alpha-Synuclein Pathology for Parkinson Disease. J Neuropathol Exp Neurol 2017;76(1):2-15.
93. Visanji NP, Mollenhauer B, Beach TG, et al. The Systemic Synuclein Sampling Study: toward a biomarker for Parkinson's disease. Biomark Med 2017;11(4):359-368.
94. Beach TG, Adler CH, Lue L, et al. Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol 2009;117(6):613-634.
95. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51(6):745-752.
96. Saito Y, Ruberu NN, Sawabe M, et al. Lewy body-related alpha-synucleinopathy in aging. J Neuropathol Exp Neurol 2004;63(7): 742-749.
97. Oinas M, Paetau A, Myllykangas L, Notkola IL, Kalimo H, Polvikoski T. alpha-Synuclein pathology in the spinal cord autonomic nuclei associates with alpha-synuclein pathology in the brain: a population-based Vantaa 85+ study. Acta Neuropathol 2010;119(6):715-722.
98. Jansen WJ, Ossenkoppele R, Knol DL, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA 2015;313(19):1924-1938.
99. Jordan PH, Jr. Operations for duodenal ulcer disease. Annu Rev Med 1989;40:1-15.
100. Glatzel M, Heppner FL, Albers KM, Aguzzi A. Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion. Neuron 2001;31(1):25-34.
101. Pan-Montojo F, Schwarz M, Winkler C, et al. Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. Scientific reports 2012;2: 898.
102. Liu B, Fang F, Pedersen NL, et al. Vagotomy and Parkinson disease: A Swedish register-based matched-cohort study. Neurology 2017.
103. Tysnes OB, Kenborg L, Herlofson K, et al. Does vagotomy reduce the risk of Parkinson's disease? Ann Neurol 2015;78(6): 1011-1012.
104. Svensson E, Horvath-Puho E, Thomsen RW, et al. Does vagotomy reduce the risk of Parkinson's disease: The authors reply. Ann Neurol 2015;78(6):1012-1013.
105. Wu WY, Kang KH, Chen SL, et al. Hepatitis C virus infection: a risk factor for Parkinson's disease. J Viral Hepat 2015;22(10): 784-791.
106. Bu XL, Wang X, Xiang Y, et al. The association between infectious burden and Parkinson's disease: A case-control study. Parkinsonism Relat Disord 2015;21(8):877-881.
107. Nielsen HH, Qiu J, Friis S, Wermuth L, Ritz B. Treatment for Helicobacter pylori infection and risk of Parkinson's disease in Denmark. Eur J Neurol 2012;19(6):864-869.
108. Tsai HH, Liou HH, Muo CH, Lee CZ, Yen RF, Kao CH. Hepatitis C virus infection as a risk factor for Parkinson disease: A nationwide cohort study. Neurology 2016;86(9):840-846.
109. Harris MA, Tsui JK, Marion SA, Shen H, Teschke K. Association of Parkinson's disease with infections and occupational exposure to possible vectors. Mov Disord 2012;27(9):1111-1117.
110. Svensson E, Horvath-Puho E, Stokholm MG, Sorensen HT, Henderson VW, Borghammer P. Appendectomy and risk of Parkinson's disease: A nationwide cohort study with more than 10 years of follow-up. Mov Disord 2016.
111. Marras C, Lang AE, Austin PC, Lau C, Urbach DR. Appendectomy in mid and later life and risk of Parkinson's disease: A population-based study. Mov Disord 2016;31(8):1243-1247.
112. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. Autonomic neuroscience : basic & clinical 2014;182:65-69.
113. Braak H, Del Tredici K. Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. Journal of Parkinson's disease 2017;7(s1):S73-S87.
114. Rey NL, Steiner JA, Maroof N, et al. Widespread transneuronal propagation of alpha-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. J Exp Med 2016;213(9): 1759-1778.
115. Funabe S, Takao M, Saito Y, et al. Neuropathologic analysis of Lewy-related alpha-synucleinopathy in olfactory mucosa. Neuropathology 2013;33(1):47-58.
116. Rey NL, Wesson DW, Brundin P. The olfactory bulb as the entry site for prion-like propagation in neurodegenerative diseases. Neurobiol Dis 2016.
117. Sikorska B, Knight R, Ironside JW, Liberski PP. Creutzfeldt-Jakob disease. Adv Exp Med Biol 2012;724:76-90.