

Roles of α -synuclein in gastrointestinal microbiome dysbiosis-related Parkinson's disease progression (Review)

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Abstract. Parkinson's disease (PD) is the second most common neurodegenerative disease amongst the middle-aged and elderly populations. Several studies have confirmed that the microbiota-gut-brain axis (MGBA) serves a key role in the pathogenesis of PD. Changes to the gastrointestinal microbiome (GM) cause misfolding and abnormal aggregation of α -synuclein (α -syn) in the intestine. Abnormal α -syn is not eliminated via physiological mechanisms and is transported into the central nervous system (CNS) via the vagus nerve. The abnormal levels of α -syn aggregate in the substantia nigra pars compacta, not only leading to the formation of eosinophilic Lewis Bodies in the cytoplasm and mitochondrial dysfunction in dopaminergic (DA) neurons, but also leading to the stimulation of an inflammatory response in the microglia. These pathological changes result in an increase in oxidative stress (OS), which triggers nerve cell apoptosis, a characteristic of PD. This increase in OS further oxidizes and intensifies abnormal aggregation of α -syn, eventually forming a positive feedback loop. The present review discusses the abnormal accumulation of α -syn in the intestine caused by the GM changes and the increased levels of α -syn transport to the CNS via the MGBA, resulting in the loss of DA neurons and an increase in the inflammatory response of microglial cells in the brain of patients with PD. In addition, relevant clinical therapeutic strategies for improving the GM and reducing α -syn accumulation to relieve the symptoms and progression of PD are described.

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1. Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after Alzheimer's disease (AD). With the increase in the proportion of the aging population, the incidence of PD is increasing (1). The prevalence of PD is 0.3% in the general population, and as high as 1% in the elderly over the age of 60 years, and 3% in individuals >80 years in industrialized countries (2). A major characteristic of PD is the accumulation of the misfolded α -synuclein (α -syn) protein in cerebral nerve cells, eventually leading to the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) or the death of nerve tissue (3). This results in large areas of dead brain tissue, and the promotion of the formation of eosinophilic inclusion bodies, such as Lewis bodies (LBs) and Lewy neuritis (LN), in the cytoplasm (4). With the death of neurons in the brain, the clinical manifestations of PD comprise a static tremor, muscle rigidity, bradykinesia, abnormal posture gait and a series of non-motor symptoms, such as olfactory disorders, anxiety and depression, cognitive decline, sleep disorders, autonomic dysfunction, fatigue and pain (5,6). Several studies have demonstrated that mitochondrial dysfunction and oxidative stress (OS) serve a key role in the pathogenesis of PD, as they cause a loss of DA neurons (7-10). Several genes and signaling pathways are involved in the initiation and development of PD. For example, the familial autosomal recessive genes PTEN induced kinase 1 (PINK1)/parkin and the autosomal dominant mutations of Leucine-rich repeat kinase 2 (LRRK2) regulate mitochondrial dysfunction and PD pathogenesis (11,12). Furthermore, it has been reported that a mutated LRRK2

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gene can induce PD pathogenesis, which is dependent on the PINK1/parkin pathway via independent mechanisms (13). In addition, several genes, such as GTP cyclohydrolase 1 (GCH1) (14), coiled-coil-helix-coiled-coil-helix domain containing 2 (CHCHD2/PARK22) (15) and VPS35 retromer complex component (VPS35/PARK17) (16) are involved in the induction of mitochondrial dysfunction of patients with PD. Environmental factors are also a pathogenic factor of PD. For example, the occupational and environmental exposure to pesticides and cytokine pathways (17), the influence of genetic polymorphisms on pesticides (18) and the dysregulation of the microRNA (miRNA/miR) network caused by pesticide exposure (19), all serve an important role in the pathophysiology of PD via neurodegeneration of SNpc DA neurons, mitochondrial dysfunction or oxidative damage (20). Some of the related genes, such as miRNAs, may serve as novel non-invasive early biomarkers for the prediction and prognosis of PD (21). It has also been demonstrated that exposure to polystyrene microplastics can induce intestinal injury and neurodegeneration through increased production of reactive oxygen species (ROS) in *Caenorhabditis elegans* (*C. elegans*) (22). Recent reports have reported that coronavirus 2019 (COVID-19) colonizes in the gut and the central nervous system (CNS), where it triggers neuroinflammation and neurodegenerative processes, suggesting that patients infected with COVID-19 may be susceptible to certain neurodegenerative disorders, such as PD (23,24). However, the molecular mechanism underlying the development and progression of PD requires further investigation.

Gastrointestinal dysfunction, an important non-motor symptom of PD, not only has a high incidence, but also appears several years prior to the characteristic motor symptoms (25). Currently, the influence of intestinal microbiota on PD has been studied extensively by scientists, and has been termed the microbiota-gut-brain axis (MGBA) (26). Numerous studies have demonstrated that abnormal intestinal microbiotas are not only closely associated with gastrointestinal dysfunction in patients with PD but may also be an important mechanism underlying the pathological process of PD (27,28). Recently, it has been reported that dysfunction of gastrointestinal microbiomes (GM) occurs earlier than/or at the same time as PD, and the pathology of PD is closely associated with the changes of the GM (29). Nielsen *et al* (30) demonstrated that individuals infected with *Helicobacter pylori* are more likely to induce the development of PD. Devos *et al* (31) reported that most patients with PD have colitis, which can enhance the peripheral and brain inflammatory response, and promote the pathogenesis of PD.

α -syn consists of 140 amino acids and the gene encoding it, synuclein α (SNCA), is comprised of five exons and is located at chromosome 4q21.3-q22 (32). It is widely expressed in the CNS, mainly in the presynaptic terminals, and is involved in the regulation of neurotransmission and synaptic homeostasis (33). The α -syn family of proteins contains three members, synapsin-I, synapsin-II and synapsin-III (34). According to the C-terminal splicing structure, α -syn can be divided into α and β subtypes (34). Currently, five synapsin proteins (synapsin-I α , synapsin-I β , synapsin-II α , synapsin-II β and synapsin-III α) have been detected (35). A pathological characteristic of PD is the accumulation of the misfolded α -syn protein involved

in slow and progressive degeneration of DA neurons in the SNpc (36). α -syn exhibits characteristics of prion-like protein during PD pathogenesis; the misfolded α -syn is an 'infectious' protein spreading pathology into the CNS via the vagus nerve (VN) by forming a template that seeds misfolding for nearby α -syn proteins, turning the endogenous physiological protein into a pathogenic protein (37). Previous studies have demonstrated that neurons can absorb α -syn *in vitro* and *in vivo*, and α -syn can also transmit between two neurons to neighboring neurons via endocytosis (38,39). However, further studies are required to determine the specific mechanisms. In addition, studies have reported that an abnormal GM can enhance the levels of inflammation via the induction of immune responses, leading to the misfolding of α -syn in patients with PD (40,41). The dysfunction of the enteric nervous system (ENS), and the accumulation of anti- α -syn immune response proteins were detected in the ENS ganglia in mice with α -syn mutations (either A53T or A30P from insertions of an entire human SNCA gene) when they were 3 months old (42). Braak *et al* (43) demonstrated that PD is initiated by unknown pathogens that traverse the gastric epithelial lining and lead to the formation of misfolded α -syn in nerve cells of the submucosal plexus. The pathological formation of α -syn is retrogradely propagated along the axonal and transneuronal axis along with the VN to reach the CNS (37). Braak *et al* (43) also reported that the accumulation of misfolded α -syn in the peripheral nervous system (PNS) occurred earlier than that in the CNS of patients with PD. Several studies have speculated that the initiation of PD originates from the PNS, and is retrogradely transported towards the CNS via the PNS (44,45). These results suggest that the abnormal GM can affect the development of PD by inducing α -syn misfolding, abnormal aggregation and transmission from VN to CNS.

Several studies have reported that GM serves an important role in the development of PD by regulating misfolded and abnormal aggregation of α -syn and the MGBA (46,47). The present review focuses on the association between GM, α -syn and MGBA in patients with PD, with an emphasis on the mechanisms of the GM and its role in the pathogenesis of PD (Fig. 1). In addition, adaptable novel potential treatment strategies are discussed. The present review offers an insight into the role of α -syn and MGBA in the pathological progression of PD, and highlights the potential of α -syn and MGBA as relevant drug targets, as well as discussing potential therapeutic candidates.

2. An aberrant GM affects α -syn accumulation in the intestines of patients with PD

Numerous studies have confirmed that the GM regulates the autonomic nervous system and CNS via the immune system, neuroendocrine system, direct neural conduction and the interaction of the MGBA (48-50). Changes in GM are associated with the pathological process of PD (51).

Changes of the GM in patients with PD. Changes to the GM have been widely reported amongst patients with PD. Scheperjans *et al* (48) reported that ~77.6% of patients with PD exhibited a reduction of *Prevotellaceae* (family), and a notable increase in several pathogenic Gram-negative bacteria, such as

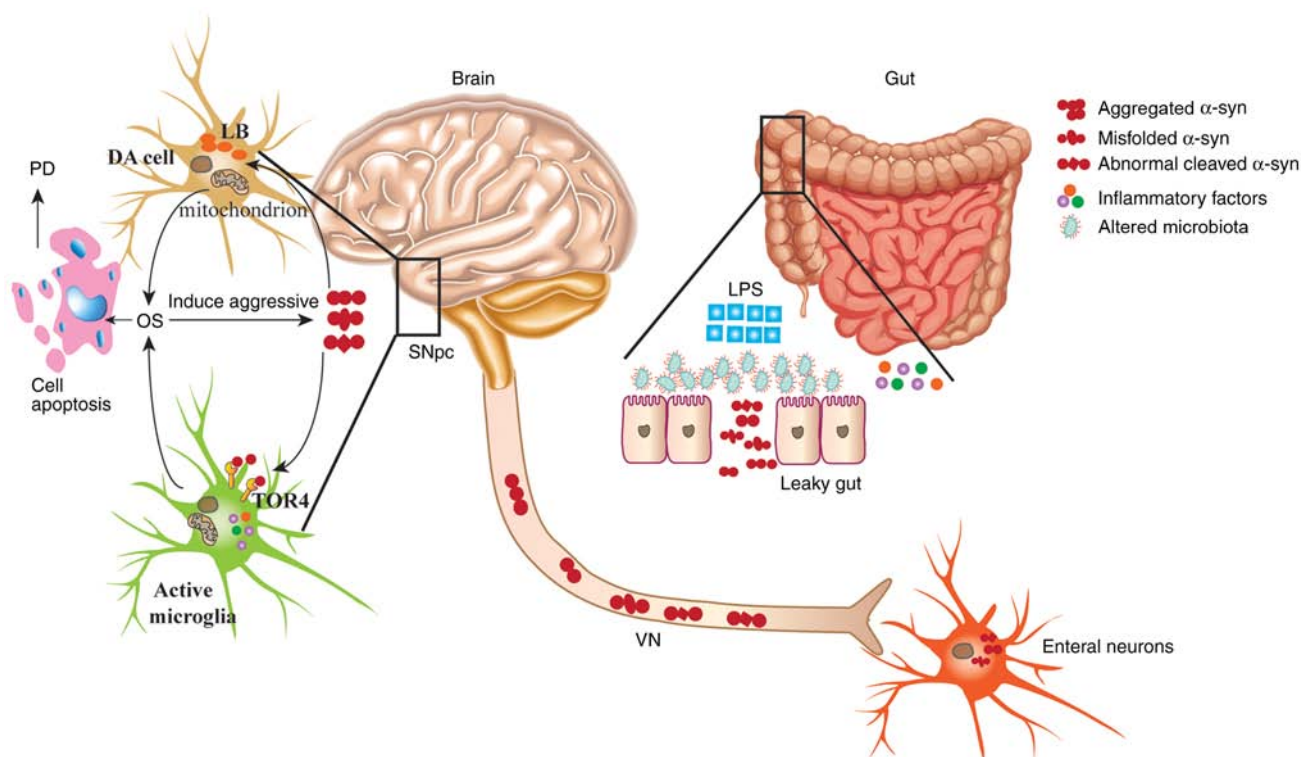


Figure 1. Schematic representation of dysregulation of the MGBA pathways implicated in the pathogenesis of PD. The aberrant GM or their products, such as LPS and the inflammatory factors involved in intestinal mucosal inflammation, reactive oxygen/nitrogen species and disruption of intestinal barrier integrity, induce misfolding of α -syn, resulting in abnormal aggregation and the formation of truncated fragments in the ENS of patients with PD. The misfolded, abnormal aggregation and truncated fragments of α -syn are transported from the ENS to the CNS via projections of the VN, as well as autonomic enteric fibers at a speed of 5-10 mm/day. Once abnormal α -syn from the ENS reaches and deposits into the CNS, it subsequently spreads in the CNS through the brainstem, midbrain, basal forebrain and finally reaches the cortical areas via a mechanism similar to that of prion-like protein. Due to an increase in α -syn aggregation in the SNpc of CNS, eosinophilic LBs are formed in the cytoplasm and mitochondrial dysfunction occurs in the DA neurons, resulting in their degradation, which stimulates an inflammatory response of the microglia. These pathological changes result in the production of OS in the brain, which triggers cell apoptosis, and thus is involved in the initiation and progression of PD. The increase in OS further oxidizes and intensifies abnormal aggregation of α -syn in the brain, eventually forming a positive feedback loop. MGBA, microbiota-gut-brain axis; PD, Parkinson's disease; GM, gut microbiota; LPS, lipopolysaccharide; α -syn, α -synuclein; ENS, enteric nervous system; CNS, central nervous system; VN, vagus nerve; SNpc, substantia nigra pars compacta; LB, Lewy Body; DA, dopaminergic; OS, oxidative stress.

Enterobacteriaceae (family), *Verrucomicrobiaceae* (family) and *Escherichia coli* (species) from their feces. Gerhardt and Mohajeri (52) demonstrated that there was a significant decrease of *Prevotellaceae* (family), *Prevotella* (genus) and *Prevotella Copri* (species) in the intestines of patients with PD. Lubomski *et al* (53) reported a notable alteration in the microbial population of *Firmicutes* in the GM of patients with PD compared with healthy individuals. The quantity of *Lactobacillaceae* (family) and *Lactobacillus* (genus) increased, whereas the abundance of *Lachnospiraceae* (family), such as *Ruminococcus* (genus), *Blautia* (genus), *Dorea* (genus), *Roseburia* (genus) and *Faecalibacterium* (genus) decreased. According to these results, the researchers hypothesized that the types of pro-inflammatory bacteria in the intestines of patients with PD significantly increased, whereas the types of probiotic bacteria decreased. Keshavarzian *et al* (54) further confirmed this hypothesis, demonstrating that the proportion of pro-inflammatory bacteria in the intestines of patients with PD increased by studying the mucosal-associated bacteria and fecal microbiota. The results demonstrated that the diversity of fecal microbiota communities was not significantly altered between PD and healthy control (HC) individuals, but the α -diversity at the phylum level and the richness of the genus

level were significantly different. Specifically, at the phylum level, the abundance of *Bacteroidetes*, *Proteobacteria* and *Verrucomicrobia* in the fecal microbiota of the PD group significantly increased, whereas the presence of *Firmicutes* in the fecal microbiota of HC was higher in the HC group. Similar results were obtained by assessing the fecal samples of the PD group compared with the HC group at the genus level, in which the abundance of pro-inflammatory bacteria, such as the *Akkermansia*, *Oscillospira* and *Bacteroides* were increased, and the abundance of butyrate-producing bacteria that produce anti-inflammatory short chain fatty acids (SCFAs), such as *Blautia*, *Coprococcus* and *Roseburia* were significantly reduced in the fecal samples of the PD group. The abundance of *Coprobacillaceae* (family), *Dorea* (genus) and anti-inflammatory *Faecalibacterium* (genus) were rich in the mucosal-associated bacterial populations of the HC group, whereas the *Oxalobac-teraceae* (family) and *Ralstonia* (genus) were richer in the PD group (55). Li *et al* (56) reported that the composition of GM was slightly altered between the healthy and Han Chinese patients with PD using next generation sequencing to analyze the feces. Consistent with other studies, the abundance of *Bacteroides* (genus) and *Prevotellaceae* (family) significantly increased in

Table I. Studies evaluating the changes in the gut microbiota in patients with PD.

| Changes in the GM in patients with PD | ↓/↑ | (Refs.) |
|--|-----|---------|
| Changes at the phylum level | | |
| <i>Bacteroidetes</i> ; <i>Proteobacteria</i> ; <i>Verrucomicrobia</i> | ↑ | (54) |
| Changes at the family level | | |
| <i>Prevotellaceae</i> | ↓ | (48,52) |
| <i>Enterobacteriaceae</i> , <i>Verrucomicrobiaceae</i> | ↑ | (48,56) |
| <i>Lactobacillaceae</i> | ↑ | (53) |
| <i>Lachnospiraceae</i> | ↓ | (53) |
| <i>Coprobacillaceae</i> , <i>Oxalobac-teraceae</i> | ↑ | (55) |
| <i>Ruminococcaceae</i> , <i>Porphyromonaceae</i> | ↑ | (56) |
| <i>Hydrogenoanaerobacterium</i> , <i>Lachnospiraceae NK4A</i> | ↑ | (56) |
| Changes at the genus level | | |
| <i>Prevotella</i> | ↓ | (52) |
| <i>Lactobacillus</i> | ↑ | (53) |
| <i>Ruminococcus</i> , <i>Blautia</i> , <i>Dorea</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> | ↓ | (53,55) |
| <i>Akkermansia</i> , <i>Oscillospira</i> , <i>Bacteroides</i> , <i>Ralstonia</i> | ↑ | (55) |
| <i>Coprococcus</i> | ↓ | (55) |
| Changed in species level | | |
| <i>Escherichia coli</i> | ↑ | (48) |
| <i>Prevotella copri</i> | ↓ | (52) |

↓, significantly reduced in patients with PD; ↑, significantly increased in patients with PD; PD, Parkinson's disease.

the healthy Han Chinese individuals, whereas the abundance of *Ruminococcaceae* (family), *Verrucomicrobiaceae* (family), *Porphyromonaceae* (family), *Hydrogenoanaerobacterium* (family) and *Lachnospiraceae NK4A* (family) increased significantly in the Han Chinese patients with PD. In addition, these studies failed to demonstrate an increase in the abundance of *Bifidobacterium* (family) and *Enterobacteriaceae* (family) in the feces of Han Chinese patients with PD, an inconsistent result compared with the study of Caucasian patients with PD (57-59). In summary, numerous changes to the GM are involved in the pathological process of PD (Table I).

Due to changes in the gut microbiota composition that occur during the course of PD, several studies have investigated fecal microbiota transplant (FMT), a novel therapeutic method, which involves the transfer of gut microbiota from a healthy individual to another via oral administration of fecal material in rodents, or via medication that alters the GM or gastrointestinal endoscopy in humans, as a method to improve the symptoms of constipation in patients with PD (60,61). Tan *et al* (62) reported that the FMT procedure is an effective treatment in 65.6% of patients with PD, and that several patients exhibited an increase in spontaneous bowel movements by 1-2 times per week in the process of FMT treatment. In a PD mouse model, FMT is necessary for the neuroprotective effects of osteocalcin (63). In addition, FMT alleviates dyskinesia and neurodegeneration of striatal DA, reduces neuroinflammation and activates microglia and astrocytes in the brain of PD mice. Furthermore, FMT can also increase the levels of 5-hydroxytryptophan, decrease fecal SCFAs and improve gut microbial dysbiosis in the intestinal tracts of PD mice (64).

Influence of GM alterations on α -syn. The barrier functions of the gut epithelium serve an important role during host-microbiome interactions, and the disruption of this barrier can lead to intestinal inflammation, production of reactive oxygen/nitrogen species in the gut, and a shift in microbial composition towards pro-inflammatory bacteria (65). Several studies have demonstrated that one of the potential mechanisms by which α -syn enters into the mucosal neuronal tissue is the generation of OS and the disruption of intestinal barrier integrity via aberrant changes to the GM in patients with PD (66,67). Given the effect of aberrant GM on the gastrointestinal barrier, the resultant translocation of bacteria or their products, such as lipopolysaccharide (LPS) can increase OS and intestinal inflammation, which in turn increases the mucosal intestinal permeability, also known as leaky gut, and increases the ability of α -syn to communicate with the ENS (53). Immunohistochemical staining of postmortem analyses of intestines in patients with PD exhibited an inevitable association between impaired intestinal barrier integrity, the increase in the intestinal bacterial flora, the high levels of expression of inflammatory genes and the abnormal accumulation of α -syn in the ENS (68-70). Other studies have reported that the volatile SCFA, particularly butyrate, serves a vital role in maintaining intestinal barrier integrity, and a lack of SCFA can increase intestinal permeability, which has been confirmed in studies of patients with inflammatory bowel disease (71,72). Further studies on the GM have reported that *Prevotellaceae* is involved in the formation of intestinal mucins and the production of SCFA through fiber fermentation in the sigmoid (73,74). Thus, the decrease of *Prevotellaceae* in the intestines of patients with

PD can lead to a decrease in intestinal mucus and an increase in intestinal permeability, and this serves as a prerequisite for entry of α -syn into the ENS via the intestinal barrier, and to maintain excessive α -syn expression or even promote its misfolding (75). The treatment of a colitis mouse model with butyrate can reduce the expression of TNF- α in the intestines and reduce cell shedding (76).

An aberrant GM or their products are involved in the misfolding, abnormal aggregation and presence of truncated fragments of α -syn in the ENS of patients with PD (66,69). In particular, the LPS generated by inflammatory bacteria can increase the nitration and oligomerization of α -syn by upregulating the expression of inducible nitric oxide synthase (iNOS), suggesting that LPS not only increases the inflammatory response to induce gut leakiness and the communication of α -syn, but can also accelerate the neurodegenerative process via the influence of α -syn (55). The monocyte/macrophage-related signaling pathway is involved in the aforementioned biological processes (77). The LPS generated from the gram-negative bacteria can downregulate the expression of tight junction proteins in the intestinal epithelial cells, such as occludin, and upregulate the expression of TNF- α , which activates the macrophages and promotes the expression of α -syn in a mouse model (78,79). Bhattacharyya *et al* (80) demonstrated that LPS binds to the α -helical intermediates of α -syn to form a lipid-protein complex that acts as a scaffold for growth of α -syn fibers, and rapid nucleation based on CD spectra analysis. LPS also accelerates abnormal aggregation of α -syn by increasing the production and reducing the half-life and lag time of α -syn (81). Wang *et al* (82) reported that inflammatory activators, such as LPS, aluminum potassium sulfate crystals, nigericin and vitamin K3 (menadione) can activate the inflammasome, including caspase-1, by inducing the cleavage of procaspase-1 to the active caspase-1, which is directly involved in cleaving and inducing the aggregation of α -syn. Furthermore, the 10 or 30-residues of α -syn N-terminal truncations alter the conformation of fibril, thus contributing to a reduction in its stability and induce their compatibility with normal α -syn. The 20-residues of α -syn C-terminal truncations result in it exhibiting unique prion-like properties (83). The prion conformation of α -syn interferes with the lysosomal and proteasomal degradation processes, and ultimately promotes aberrant accumulation (84).

3. α -syn accumulation contributes to neurodegeneration in the CNS of patients with PD

The primary pathological characteristic of PD is the abnormal aggregation of α -syn in the SNpc (85). Due to its prion-like protein activation mechanism, α -syn can misfold to cause self-abnormal aggregation, and can also transmit signals amongst nervous cells, and spread throughout the nervous system (86). Several studies have indicated that the ENS may be the channel by which α -syn is transferred from the PNS to the CNS (41,87).

Abnormal α -syn in the intestines of patients with PD enters the CNS via the MGBA. Misfolded α -syn can transfer from affected to unaffected cells and serve as a template for pathological aggregation of α -syn in neuronal cells (88-91). It

has been speculated that α -syn may be transmitted to the dorsal nucleus of the VN via VN fibers at a speed of 5-10 mm/day, and it accumulated in the neurons to trigger cell apoptosis after α -syn was injected into the intestinal wall of rats (92). Kim *et al* (93) injected 25 μ g misfolded α -syn, preformed fibrils (PFF), into the pyloric stomach and upper duodenum (UD) of mice, and detected the distribution of PFF in the brain tissues after 1, 3, 7 and 10 months. The results demonstrated that PFF aggregated in the stomach and spread into the VN 1 month after injection. In the following 3-10 months, the distribution of PFF was detected consecutively in the medulla oblongata, locus coeruleus in the pons, SNc in the ventral mesencephalon brain (VMB), prefrontal cortex and olfactory bulb. The number of DA neurons in the SNpc of mice significantly decreased, and this was accompanied by a decrease in forelimb strength, grip strength, the hindlimb strength, and muscle and motor coordination ability of mice based on a rotarod test 7 months after injection. However, PFF was not detected in the aforementioned areas of the brain in the mice whose VN fibers near UD and neck were cut by truncal vagotomy 7 months after injection with the same dosage of PFF. The death of neuronal cells and the production of LBs were not detected. Braak *et al* (94) demonstrated that several misfolded α -syn proteins had accumulated in a portion of the ENS that regulated intestinal function and connected the gut to the brain of patients with PD. They speculated that the ENS may be the α -syn accumulation starting point, and α -syn was transported from the VN to the VMB, where the SNpc killed DA cells selectively, resulting in PD.

Abnormal α -syn causes the death of DA neurons in patients with PD via OS. The abnormal α -syn in DA cells transported from MGBA often involves mitochondrial dysfunction and the generation of OS, which serve important roles in the development of PD (95,96). It has been reported that overexpression of α -syn in GT1-7 cells increases mitochondrial volume and abnormal vacuolated cristae (97). Hu *et al* (98) demonstrated that in the α -syn-overexpressing SH-SY5Y cells, α -syn can interact with ATP-dependent Clp protease (ClpP) to inhibit the activity of ClpP, leading to mitochondrial oxidative damage and neurotoxicity. Chong *et al* (99) performed experiments using an N27 immortalized rat mesencephalic dopaminergic neuronal model of PD cell lines that stably expressed wild-type human α -syn, and hypothesized that the expression levels of caspase-3 and 9 increased as the OS increased, and the expression of Akt gradually decreased, resulting in cells undergoing mitochondrial dependent apoptosis. This suggests that α -syn promotes apoptosis by increasing OS. Dryanovski *et al* (100) and Tapias *et al* (101) reported that the increase in levels of α -syn in DA cells promotes the generation of OS. Musgrove *et al* (102) detected large quantities of nitrated α -syn in the brain tissues of patients with PD after death using an oxidized modified antibody. The study of SH-SY5Y human neuroblastoma cells stably expressing the C-terminal half of Venus YFP-tagged α -syn or the N-terminal half of Venus YFP-tagged α -syn demonstrated that the cells treated with 25 μ M paraquat exhibited a stronger fluorescent signal due to the increase in OS compared with the control groups. However, after co-culturing with 25 μ M paraquat and oxidized modified inhibitor, the intensity of the fluorescent signal in

the experimental group decreased compared with the control group. Therefore, it was hypothesized that the increased OS was the result of increased spreading of α -syn from the dorsal motor nucleus of the VN to the rostral brain regions, resulting in enhanced cell-to-cell α -syn transmission in patients with PD. Martin *et al* (103) and Stichel *et al* (104) reported that LBs were formed in transgenic mice that expressed the human mutational α -syn. Furthermore, the activity of neuronal mitochondrial complexes I and IV significantly reduced. Conversely, inhibiting the activity of mitochondrial complex I promoted the accumulation of α -syn in mice brains, suggesting that increased OS resulted in increased levels of oxidatively modified forms of α -syn, resulting in increased α -syn aggregation and thus, cell-to-cell transmission (105). Thus, an increase in OS and the aggregation of α -syn forms a positive feedback loop throughout the progression of PD (95).

Abnormal α -syn induces neuroinflammation in patients with PD via activation of microglia. In the brain, microglia act as innate immune cells and serve a vital role in the pathogenesis of PD (106). During the early stages of PD, microglia are activated and are positive for human leukocyte antigen-DR (HLA-DR) (107). Microglia maintain continuous activation through the development of the disease (108). The activated microglia release several pro-inflammatory factors that target the blood vessel endothelial cells of the blood-brain barrier (BBB) and promote the expression of adhesion molecules on their surface (109). The adhesion molecules induce T cells and monocytes to enter the brain through the BBB, which further release more pro-inflammatory factors, resulting in neuroinflammation and neuronal apoptosis (110). α -syn is involved in the activity of microglial cells in patients with PD (111). Studies on brain tissue samples of patients with PD revealed high expression of α -syn in neurons, and the microglia were HLA-DR positive (112,113). The microglial cells treated with different (from low to high) concentrations of α -syn can increase the pro-inflammatory effect of microglial cells, and the mRNA expression levels of TNF- α , interleukin (IL)-1 β , cyclooxygenase-2 and iNOS levels also increase, which causes apoptosis of nerve cells (114). In the brain, toll-like receptor 4 (TLR4) is primarily expressed by microglial cells, and a small amount of TLR4 is expressed by astrocytes, oligodendrocytes and neurons (115,116). TLR4 can induce the activity of microglial cells, and subsequently induce the secretion of inflammatory chemokines and cytokines (117,118). Fellner *et al* (119) demonstrated that α -syn can activate microglial cells by targeting TLR4, and inducing the production of nuclear factor- κ B (NF- κ B) and the secretion of cytokines by treating TLR4-deficient and wild-type mice with different forms of α -syn (full length soluble, fibrillized and C-terminally truncated). Further research demonstrated that the C-terminally truncated α -syn was the most effective inducer of TLR4-dependent microglial activity (120). Choi *et al* (121) indicated that α -syn also reduces cell autophagy by decreasing lysosomal and proteasomal degradation, which further results in increased expression of TLR4 and further strengthened TLR4-dependent microglial activity. Thus, α -syn is considered an inducer of microglial activation in patients with PD. Notably, several studies have reported that NF- κ B, matrix metalloproteinase and protease activated receptor 1 are also

involved in microglial activity via monomers, polymers and nitration of α -syn (122,123), which further induces the production of excessive ROS in microglia, and thus, the death of DA neurons (124).

Activated microglia cells inhibit the activity of nuclear factor E2-related factor 2 (Nrf2), an antioxidant transcription factor associated with the anti-inflammatory capacity of microglia, via oxidative modification (125). The expression levels of TNF- α , IL-6, IL-1 β and iNOS are significantly upregulated in Nrf2-deficient microglia (126). Shavali *et al* (127) reported that activated microglia further promote nitric oxide, thereby inducing the continuous spread of α -syn to the adjacent neurons and resulting in neuronal cell death. It has been demonstrated that TNF- α produced by α -syn-activated microglia can result in impaired function of mitochondrial complex I and the generation of OS; in turn, the OS promotes further aggregation of α -syn in neuronal cells (128). Subsequently, the aggregated α -syn increases OS further, forming a positive feedback loop between neuroinflammation, OS and the aggregation and spread of α -syn in patients with PD (129).

4. Novel treatments based on the MGBA and α -syn for PD

The current treatments available for patients with PD not only have no effect on its relentless progression but may also induce several side effects. There are no effective therapeutic strategies that target the MGBA to slow or halt the neurodegenerative process, or reduce motor and non-motor symptoms. Nutrition based probiotics or prebiotics-based interventions inhibit neuroinflammation and ameliorate the diffusion of α -syn in the MGBA, which offer a novel therapeutic strategy for the treatment of PD and overcome the disadvantages of current therapies (130). An overview of the studies summarizing the novel treatments based on MGBA and α -syn for the management of PD is presented in Fig. 2.

Current clinical treatments for PD. The death of cerebral DA neurons in patients with PD leads to a decrease in secretion of dopamine and other neurotransmitters, resulting in the motor symptoms. Currently, pharmacological therapeutic strategies primarily compensate for the loss of dopamine and other neurotransmitters to alleviate motor symptoms temporarily. The drugs often include dopamine receptor activators and/or the dopamine precursor L-3, 4-dihydrophenylalanine (levodopa). However, levodopa not only relieves the neurodegenerative processes, but also causes several effects, including nausea, emesis, abnormal involuntary movement of head, face, tongue, upper limbs, depression and dysuria. Conversely, levodopa administered orally provides a favorable gastrointestinal environment required for the drug absorption (131). However, most patients with PD also exhibit gastrointestinal disorders, resulting in poor drug absorption (132). Several drug trials on healthy volunteers had demonstrated that levodopa can delay the time of gastric emptying, and long-term use of levodopa can exacerbate the gastrointestinal dysfunction in patients with PD (133). Further studies have reported that long-term use of levodopa may lead to fluctuation of symptoms and resistance to levodopa (134,135). Tyrosine decarboxylase (TDC), which is involved in the transformation of levodopa

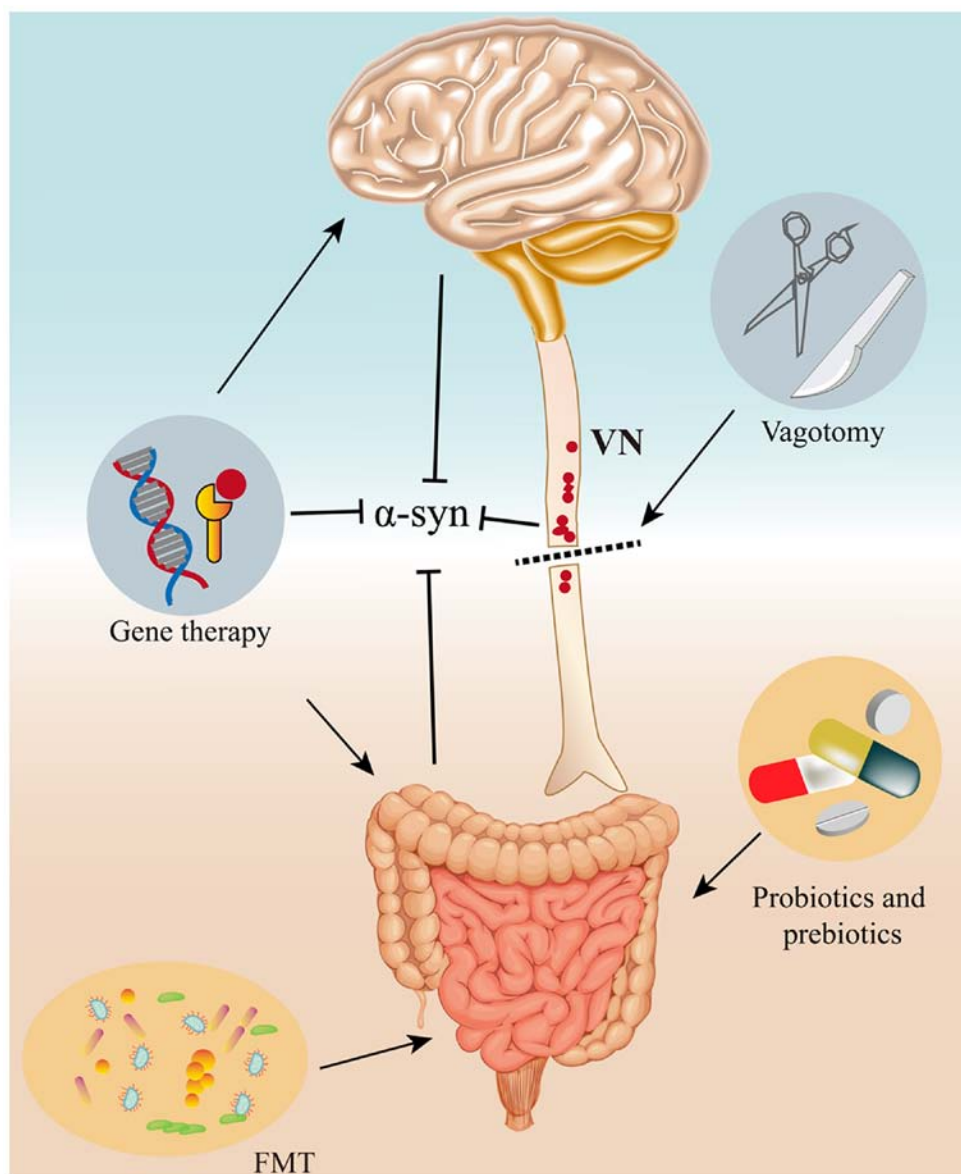


Figure 2. Novel treatments based on MGBA and α -syn in the pathogenesis of PD, including improving GM dysbiosis using probiotics and prebiotics, inhibiting the expression and abnormal aggregation of α -syn using RNAi or other gene modification techniques, and FMT that involves the transfer of intestinal microbiota from one healthy individual to another, or vagotomy. All of these treatments are known to modulate α -syn pathophysiology and prevent the neuropathological and neurobehavioral deficits induced by the transmission of pathological α -syn via the MGBA. MGBA, microbiota-gut-brain axis; PD, Parkinson's disease; GM, gut microbiota; RNAi, RNA interference; FMT, fecal microbiota transplant; α -syn, α -synuclein; VN, vagus nerve.

to dopamine and prevents the uptake of levodopa in the small intestine of patients with PD, is specially coded in the genome of *Lactobacillus* and *Enterococcus* in the intestine (136). The content of TDC is negatively correlated with the dosage of levodopa in the feces of patients, which may be used as a biomarker to evaluate the therapeutic effect of levodopa (137). The combination of a TDC inhibitor and levodopa can reduce the dosage and the dependence on levodopa for more effective treatment of PD (138).

Improving GM dysbiosis using probiotics and prebiotics in patients with PD. The abundance of *Prevotellaceae* was shown to be significantly reduced, the levels of SCFAs were decreased in the feces, whereas the content of pro-inflammatory bacteria, such as *Proteus*, *Pseudomonas* and *Enterobacteriaceae* were increased in patients with PD (6). A study of a nude mouse

was demonstrated to exhibit motor symptoms and loss of DA neurons following transplant with intestinal microflora from PD mice (139). However, the transplantation of probiotics can significantly maintain intestinal microbial homeostasis, increase the levels of SCFAs in feces, decrease the activation of microglia and increase the activity of DA neurons in the brains of PD mice (64). Currently, the most common probiotics are *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and yeast (140). Several studies have reported that probiotics can enhance intestinal epithelial integrity and stimulate the homeostasis of the intestinal mucosal immune system (40,141). In addition, probiotics can regulate the immune response, inhibit the growth of pro-inflammatory bacteria and increase the levels of SCFAs in the intestines of patients with PD by regulating the MGBA, reducing the production and aggregation of prion-like α -syn (142,143). Goya *et al* (144)

reported that the probiotic, *Bacillus subtilis* can reduce α -syn aggregation and clear previously aggregated α -syn via biofilm formation in a *C. elegans* model of PD. Currently, the most effective means of transplanting probiotics is FMT, which contributes to the reconstruction of a healthy intestinal microflora by transplanting the healthy intestinal microflora into the gastrointestinal tract to effectively improve the symptoms of patients with PD (145). This treatment method has achieved positive effects in clinical trials of PD, autism and chronic fatigue syndrome (146). The supplementation of probiotics or FMT not only improves gastrointestinal function, but also enhances the intestinal absorption capacity of levodopa (147). Although most of the animal model studies demonstrated that FMT is an effective treatment for PD (148,149), further studies are required to determine the molecular mechanism by which FMT exerts its beneficial effects before it can be recommended for use in humans. Optimistically, FMT is gradually being clinically used as a medicinal therapy in humans for the treatment of PD (149). This may effectively reconstitute the major commensal bacterial populations and re-establish the diversity and composition of the GM in patients with PD. Thus, this practice may become commonplace in the near future.

Prebiotics, such as Galacto-Oligosaccharides and Fructo-Oligosaccharide, are non-digestible oligosaccharides, primarily synthesized from lactose or fructose (150). Prebiotics selectively activate certain probiotics in the gut, such as *Bifidobacteria* (151), promote its metabolism and produce SCFAs to maintain the integrity of the intestinal epithelia and regulate the mucosal immune response (152). Savignac *et al* (153) demonstrated that prebiotics can increase the expression levels of brain derived neurotrophic factor, which serves a protective role in neurons in the dentate gyrus of the hippocampus of rats. Taken together, prebiotics can selectively reduce intestinal permeability and inflammation via activation of the metabolism of probiotics. Thus, it is hypothesized that the combination of FMT, prebiotics and traditional drugs may both improve intestinal dysfunction and protect neurons, as well as effectively reducing the dosage of levodopa or other drugs to reduce drug dependence (154).

Inhibiting the expression and abnormal aggregation of α -syn in patients with PD. Given that α -syn serves a key role in the development of PD, an increasing number of studies have demonstrated that the inhibition of α -syn expression and abnormal aggregation is an important method for the treatment of PD (155). Preclinical gene therapy using RNA interference (RNAi) targeting α -syn mRNA via gene-silencing is being assessed, and the results have indicated that it can effectively inhibit the expression of α -syn in fibroblasts (156). In addition, miRNAs can downregulate the translation of target mRNAs as post-transcriptional regulators, through binding to the 3'-untranslated region (UTR) complementary sequences (157), which can be used as another means of gene therapy for the treatment of PD. Junn *et al* (158) reported that miR-7 can bind to the 3'-UTR of α -syn mRNA complementary sequences to inhibit the expression of α -syn, effectively relieving its inhibitory effect on the proteasome, and thus promoting the degradation of α -syn. Masliah *et al* (159) demonstrated that PD transgenic mice immunized with full length human α -syn

protein exhibit decreased accumulation of the misfolded α -syn in neuronal cell bodies and synapses, resulting in reduced PD symptoms, and the mice that produced high affinity antibodies exhibited a greater capacity to reduce α -syn expression compared with the mice that produced lower affinity and/or lower titers of anti- α -syn antibodies. Ghochikyan *et al* (160) also indicated that a PD mouse model immunized with full length human α -syn protein can produce high affinity antibodies, which can reduce the accumulation of the aggregated forms of α -syn in neuronal cells to relieve neurodegeneration. Other studies indicated that monoclonal antibodies that target α -syn can inhibit the expression and aggregation of α -syn in a PD mouse model, as well as in patients (161,162). Upregulation of α -syn in the extracellular matrix can be recognized by specific antibodies and cleared by the macrophages through phagocytosis (163,164). In addition, α -syn specific antibodies can reduce the activation of CD4⁺ T cells by clearing cytokines, such as IL-2 and TNF- β , which are released by the activated CD4⁺ T cell-mediated neurodegeneration (165). A study demonstrated that monoclonal antibodies against α -syn can decrease LB/LN formation to reduce the loss of primary cultured neuronal cells by preventing the uptake of α -syn-PFF (166). Due to the α -syn form, the oligomers and fibrils, as well as the post-translational modifications of α -syn in patients with PD, such as acetylation, phosphorylation and truncation, several studies have demonstrated that D10, a single chain antibody of α -syn, had the highest affinity with α -syn; the single chain antibody D5 can effectively target the oligomer of α -syn; the single chain antibody syn-O1, -O2 and -O4 can effectively reduce neuroinflammation and the loss of neurons by specifically targeting the oligomer of α -syn in the CA3 area of the hippocampus; the single chain antibody syn-10h targets α -syn trimers, and antibodies syn-F1 and F2 prevent neuronal loss and facilitated amelioration in behavioral defects by specifically recognizing and clearing α -syn fibers (167,168). In addition, a specific antibody, LS4-2G12, which recognizes phospho- α -syn (ser129), is highly sensitive to the aggregation in the tissues of patients with PD, and protects neurons from damage mediated by the immune system (169,170). TLR4 inhibitors can be used as a potential drug for the treatment of PD (171). The inhibitor of TLR4 can effectively reduce intestinal inflammation and permeability in patients with PD, which can be used as a competitive inhibitor of mutant α -syn in the brain of patients with PD to inhibit the activation of microglia (172). Taken together, the use of small molecules or antibodies to reduce the toxicity of α -syn may serve as an effective method to inhibit the pathogenesis and development of PD (173).

The failure to eliminate α -syn is also an important cause of PD, and autophagy serves a vital role in the elimination of abnormally aggregated α -syn (174). mTOR complexes are widely involved in regulating apoptosis and autophagy (175). MSDC-0160, a mitochondrial pyruvate carrier inhibitor, enhances autophagy by inhibiting the activation of mTOR in cells (176). It has been reported that MSDC-0160 can effectively protect DA neurons in an MPTP-induced mouse model, and decreases α -syn-induced neural toxicity by increasing autophagy in a *C. elegans* model of PD (177). In nerve cells, the expression of Beclin 1 can reduce apoptosis and enhance autophagy (178). Spencer *et al* (179) overexpressed Beclin 1

in a PD mouse model using lentivirus to activate autophagy and reduce the accumulation of α -syn; however, the study only assessed this method in a mouse model. The levels of activated C-Abl, a tyrosine kinase, in the brain of patients with PD increased due to α -syn aggregation (180). Nilotinib, an anti-tumor drug, can promote intracellular autophagy by inhibiting the PI3k/Akt/mTOR signaling pathway to inhibit C-Abl (181). A recent non-placebo-controlled study reported that the lower dose of Nilotinib is effective and safe for the treatment of PD by effectively inhibiting α -syn aggregation (182). Taken together, these findings suggest that enhancing autophagy and inducing the degradation of α -syn are potential therapeutic strategies for the treatment of PD.

5. Conclusion

The intestinal microbiota contributes to the pathogenesis of PD, which has changed the previous view that the etiology of PD is concentrated on the brain. Misfolded and abnormally aggregated α -syn in the intestines of patients with PD is transported from the intestines to the brain via the MGBA. The spreading and abnormal aggregation of α -syn in the brain results in the formation of LBs in DA neurons, the activation of microglia, the production of inflammatory factors, an increase in OS, and ultimately apoptosis. MGBA not only serves an important role in the stability of the digestive system, but also serves a key role in the pathogenesis of PD. MGBA abnormalities may be one of the causes of PD. Currently, several novel clinical therapeutic strategies that target the MGBA to slow or halt the neurodegenerative process or reduce the motor and non-motor symptoms, such as food-based therapies, vagotomy, inhibiting the expression and abnormal aggregation of α -syn in patients with PD using RNAi or other gene modification measures, as well as FMT have been suggested. In addition, several genes serve prominent roles between MGBA and the progression of PD, such as the aforementioned PINK1/parkin pathway, LRRK2, TLRs and the PI3k/Akt/mTOR pathway. These genes/pathways may serve as potential therapeutic targets for PD clinical therapy via modulation of the MGBA in the near future. Thus, an improved understanding of the molecular mechanisms that are involved between the gut microbiota, MGBA and the brain in patients with PD may assist in the development of novel therapeutic strategies for the treatment of PD.

Although several changes in the taxa of the GM have been found in patients with PD, several questions regarding the association between the MGBA and PD remain unanswered. For example, similar changes to the taxa of the GM have been found in other diseases, such as decreased levels of *prevotella* in type I diabetes and an increased abundance of *Lactobacillus* in type II diabetes (183,184). Thus, identifying specific PD-associated bacterial taxa as a method to diagnose PD may not be viable. In the future, studies should concentrate on confirming the changes to the bacterial taxa in the intestinal tract that may serve as biomarkers for diagnosis of PD. Furthermore, although several studies have attempted to understand the association between the changes in the GM and the pathogenesis of PD, specific molecular mechanisms regarding the associations between the constituents of the intestinal bacterial taxa and PD remain to be determined.

Notably, whether the changes in the bacterial taxa are the cause or consequence of PD should be addressed.

Despite recognizing the pathogeny of PD and the role of abnormal aggregation of α -syn, Horsager *et al* (185) reported that PD can be separated into two hypotypes based on the initial position of the pathological α -syn aggregation. The initial site of the build-up of α -syn aggregates has not been determined, thus, further studies are required to determine the starting position of α -syn polymers and the associated mechanisms. In addition, in the gastrointestinal tract, why α -syn tends to oligomerization or polymer and its functional roles remain unclear. Barbut *et al* (186) reported that monomeric α -syn did not exhibit the ability of antimicrobial infection, but aggregated α -syn exhibited a property of antimicrobial peptides (AMPs), which is concentration-dependent aggregation in the gastrointestinal tract of patients with PD. Briefly, the 'intrinsically disordered' α -syn assumes no specific structure in the aqueous solvent. However, α -syn exhibits a net of cationic charge by the N-terminal (~60 amino acid residue) in the presence of lipid membranes, and interacts with the phospholipids of lipid membranes that contain an abundance of negatively charged headgroups, such as the phosphatidyl serine. Subsequently, the α -syn is pulled toward the lipid membranes electrostatically, which increases the concentration of α -syn on the lipid membrane. The bound α -syn molecules are closer to one another and begin to aggregate, exhibiting the characteristic of concentration-dependent aggregation, which has been described for numerous AMPs (187,188). Despite the understanding of the process of α -syn aggregation, the downstream targeted molecules of aggregated α -syn in the lipid membrane remain unclear. Goya *et al* (144) demonstrated that a probiotic strain, *B. subtilis* PXN21, can effectively clear aggregated α -syn. However, whether probiotic can remove α -syn that has bound to the lipid membrane remains unknown. Abbott *et al* (189) reported that the unbalance of lipid metabolism is an important pathogenesis of PD. Whether the unbalance of lipid metabolism can increase an affinity between α -syn and lipid membrane requires further investigation. If such means are exposed, it can unravel a new wave of therapies that target PD from the foundational pathophysiology, rather than just suppressing the symptoms.

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QL, TW, JY and GS conceived and designed the present review. QL, TW, JW, XH, YG, YW, JY and GS performed the

literature review and acquired the relevant data. QL, TW, JY and GS drafted the initial manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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