REVIEW ARTICLE

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Structure, Distribution, and Genetic Profile of α-Synuclein and Their Potential Clinical Application in Parkinson's Disease

Xiaoli Si,* Jiali Pu,* Baorong Zhang

Department of Neurology, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

ABSTRACT

Parkinson's disease (PD), the second most common neurodegenerative disorder after Alzheimer's disease, is characterized by the loss of nigral dopaminergic neurons. PD leads to a series of clinical symptoms, including motor and non-motor disturbances. α-synuclein, the major component of Lewy bodies, is a hallmark lesion in PD. In this review, we concentrate on presenting the latest research on the structure, distribution, and function of α-synuclein, and its interactions with PD. We also summarize the clinic applications of α-synuclein, which suggest its use as a biomarker, and the latest progress in α-synuclein therapy.

Key Words
Parkinson's disease; alpha-synuclein; pathogenic mechanism; biomarker; therapeutic strategy.

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Corresponding author: Baorong Zhang, MD, Department of Neurology, Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, China
Tel: +86-571-87784752 **Fax:** +86-571-87784752 **E-mail:** brzhang@zju.edu.cn

*These authors contributed equally to this work.

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INTRODUCTION

Parkinson's disease (PD), with an incidence ranging from approximately 1% in people over 65 years old to 4% in people over 86 years old, is the second most common neurodegenerative disorder after Alzheimer's disease (AD).[1] Dopaminergic neuron loss in the substantia nigra (SN) gradually results in PD, with the presence of α-synuclein aggregations called Lewy bodies (LBs) in the living neurons. The clinical manifestations of PD include motor symptoms, such as resting tremor, muscular rigidity, bradykinesia, and postural instability,[2] as well as non-motor features such as autonomic dysfunctions, sensory abnormalities, sleep disorders, depression, and dementia.[3] Only 5–10% of PD patients are known to have monogenic forms of PD. The majority of patients are sporadic, which might be caused by a complex interaction of genetic variants with environmental risk factors.[4] Decades of research have proven the important role of α-synuclein in the pathogenesis of PD. There is increasing research interest in identifying causative mutations of *SNCA* (the gene encoding α-synuclein), as well as their mechanism, using cellular and animal models. To date, some hypotheses have been proposed concerning the involvement of α-synuclein in the pathogenesis of PD, such as aggregation, mitochondrial dysfunction, oxidative stress, microglial activation and inflammation, and impaired autophagy; however, the detailed mechanisms remain unclear. The function of LBs in the disease process has also proved elusive, with evidence of both neuroprotection and toxicity.[5] In this review, we summarize the current progress in the field of α-synuclein, especially its clinical application in earlier diagnosis and treatment.

THE TOXICITIES OF DIFFERENT STRUCTURES OF α-SYNUCLEIN

PD is a common neurodegenerative movement disorder caused by the loss of nigral dopaminergic neurons, with the presence of misfolded proteins in the affected brain regions. These deposits are mainly formed by amyloid fibrils of the 140-amino acid presynaptic protein α-synuclein. Currently, it is believed that α-synuclein oligomers (oAS) are the most toxic species.[6] It seems that toxicity mediated by oAS is a general phenomenon related to their structure. Understanding the structural elements of α-synuclein-induced toxicity might contribute to developing clinical applications in the diagnosis and treatment of PD and other synucleinopathies.

As the major constituent of LBs, α-synuclein plays an important role in the regulation of synaptic vesicle release and trafficking, fatty acid-binding, and neuronal survival.[7] In its native state, human α-synuclein is largely unfolded. Through a nucleation polymerization mechanism, involving major structural rearrangements, α-synuclein transforms into different, transient prefibrillar species that vary in size and morphology.[8] Despite the assistance of molecular chaperones during protein folding, protein misfolding is inevitable because of changes in the environment and various other factors, including errors in post-translational modifications, an increase in the rate of degradation, and errors in trafficking. When the misfolded protein interacts with complementary intermediates, the pathway of amyloid fibril formation from monomers to oligomers, and consequently mature fibrils, is initiated.[8]

The aggregation of soluble proteins into insoluble, β-sheet-rich amyloid fibrils with different structures can trigger different diseases. For example, intraneuronal α-synuclein aggregates are found in PD, Lewy body dementia (LBD), and multiple system atrophy (MSA), while extracellular β-amyloid deposits and intracellular phosphorylated tau proteins are found in AD. In Huntington's disease, polyglutamine-expanded huntingtin protein accumulates in intranuclear inclusion bodies or neurites.

The α-synuclein aggregations found in PD, LBD, and MSA also have different structures, which led to the hypothesis that different variants of α-synuclein account for different neurotoxic phenotypes within synucleinopathies. Recently, researchers from Belgium injected oligomers, ribbons, and fibrils of α-synuclein into rat brains separately. They found that each assembly could affect neuron transmission after acute exposure, but only fibrillar α-synuclein had a lasting toxic effect, whereas ribbon-like α-synuclein resulted in more Lewy body inclusions.[9] This discovery contradicts the widely-accepted hypothesis that oligomers are the most neurotoxic entities in PD.[10] Therefore, although all α-synuclein assemblies might have a role during pathogenesis, fibrillar α-synuclein might have the largest neurotoxic potential over the long-term.

Recently, chronic neuroinflammation has been accepted widely as another molecular mechanism of PD. Mounting evidence supports the view that inflammation plays an important role in mediating the processing of cytokines into mature forms. The best-known cytokine is IL-1β, a pro-inflammatory cytokine critical for initiating the immune response. α-synuclein was proven to activate microglia and to induce IL-1β secretion. Recently, some researchers focused on the ability of different α-synuclein assemblies to induce inflammation. A study conducted in England showed that only fibrillar α-synuclein triggers inflammation by activating Toll-like receptor 2 and the nucleotide oligomerization domain-like receptor pyrin domain containing 3 inflammasome.[11] This finding contrasts with the idea that α-synuclein oligomers are the primary neurotoxic species in PD. From this study, we suggest that the ability of amyloids to induce inflammatory responses relies on α-synuclein in its fibrillar state, and fibrillar α-synuclein might be the primary cytotoxic agent in the pathogenesis of PD.

Above all, we believe that fibrillar α-synuclein may be more toxic than the α-synuclein oligomer in PD. The discoveries of α-synuclein in different assemblies led to the hypothesis that structural elements could account for the different clinicopathological traits of synucleinopathies. Therefore, future research should develop therapeutic strategies targeted toward fibrillar α-synuclein.

DISTRIBUTION OF α-SYNUCLEIN PATHOLOGY

Intrinsic, unfolded α-synuclein is mainly located in human mature neurons, although it also exists in erythrocytes and platelets. Intrinsic α-synuclein plays an important role in neurotransmission, maintains synaptic homeostasis and is present in presynaptic nerve terminals.[12] Recently, studies showed that Lewy pathology in neurons can graft across the brain and α-synuclein aggregates might be transported from neuron-to-neuron in a prion-like way.[13] By fluorescence labeling of α-synuclein, Braak and Del Tredici[14] proposed that certain brain regions, besides the SN, such as the olfactory bulb, dorsal nucleus of the vagus nerve, and in later stages, the amygdala, hippocampus, and neocortex, were vulnerable to the progression of sporadic PD. Research conducted in Japan suggested that different brain regions express α-synuclein in different ways. α-synuclein was observed in early-affected PD regions in both excitatory and inhibitory synapses; however, it was only observed in excitatory synapses at later stages, such as in the cerebral cortex, hippocampus, and thalamus, although some was detected in inhibitory synapses. This Lewy pathology seems to spread throughout the brain as the disease progresses.[15] These studies might provide a basis for investigating the neural connections within vulnerable regions.

Recently, researchers from the USA injected radiolabeled α-synuclein into mouse brains via an intracerebroventricular route and discovered that α-synuclein in cerebrospinal fluid (CSF) can be released into blood, with a small fraction being contained in exosomes that are specific to the central nervous system (CNS).[16] According to this study, we speculated that the exosomes, 40–100 nm membrane vesicles of endocytic origin, might mediate toxic α-synuclein propagation between neurons. This study revealed that α-synuclein could be transported from CSF to blood *in vivo* by intracerebroventricular injection for the first time. In a future study, we may evaluate plasma neuronal derived exosomes to assist with the prediction of the stage of PD and other synucleinopathies. In another study, researchers from Belgium found that α-synuclein can cross the blood-brain barrier and spread to the CNS after intravenous injection. Furthermore, by tracking α-synuclein assembly, they found that misfolded α-synuclein always occurs in defined brain areas in the early stages and then diffuses in the later stages; in this process, α-synuclein oligomers diffused the fastest.[9] Perhaps the blood α-synuclein could enter the CNS in exosomes through a similar mechanism. Further investigations are needed to understand the methods and routes by which α-synuclein spreads in association with pathology.

GENETIC BACKGROUND AND CURRENT PROGRESS OF α-SYNUCLEIN RESEARCH

Nineteen years ago, researchers reported a pattern of familial aggregation for PD and found the first PD mutation (*A53T*, in *SNCA*) in a large Italian pedigree. This mutation encodes a presynaptic protein involved in the modulation of neurotransmitter release,

endoplasmic reticulum (ER)/Golgi trafficking, or is loosely associated with synaptic vesicles in the Italian family and in three unrelated families of Greek origin with an autosomal dominant inheritance for PD. This finding of a specific molecular alteration associated with PD promoted our understanding of the pathophysiology of the disease.[17] Later, several genes had been reported to be involved in the pathogenesis of PD, such as *SNCA*, *LRRK2*, *VPS35*, *PARK2*, *PINK1*, and *DJ-1*.[18] In addition to *A53T*, four other point mutations have been discovered in *SNCA*–*A30P*, *E46K*, *G51D*, and *H50Q*–that are associated with an autosomal inheritance of PD, with the last two being newly discovered.[19]

Missense or multiplication (duplicated and triplicated) mutations in *SNCA* contribute to the development of intracellular inclusion bodies and play an important role in PD pathology. α-synuclein was defined as the core protein component of LBs in the late 1990s. In addition, strong evidence has been presented for *SNCA* being the gene responsible for sporadic PD.[20]

Patients with different mutations in *SNCA* display different clinical features, ranging from classical PD symptoms (resting tremor, muscle rigidity, bradykinesia, and postural instability) to sensitivity to levodopa therapy and atypical symptoms.[21] For example, PD patients carrying the *A53T* mutation usually have an earlier onset, faster disease progression, lower prevalence of tremor, and a higher prevalence of dementia, bad moods, and autonomic disturbances compared with sporadic PD patients. Patients with the *A30P* mutation seems to present later after disease onset and have milder clinical features, whereas carriers of the *E46K* mutation usually show more severe symptoms with early onset of dementia, which is similar to LBD.[22] Mutation of *G51D* was reported recently and presents similar clinical symptoms as *A53T*. It has been detected in PD patients from French, British, and Japanese families while being absent in control subjects. Another new point mutation, *H50Q*, mainly occurs in PD patients with a positive family history and dementia but also appeared in one patient with later onset sporadic PD (Table 1).[23]

To summarize the latest research, not only α-synuclein expression but also gene expression dosage is related to the clinical features of familiar PD, because genomic triplications of α-synuclein are associated with an earlier occurrence of the disease, faster progression, more common dementia, and a shorter lifespan compared with patients carrying duplicated α-synuclein.[24-26] Thus, the clinical phenotype of familiar PD appears to be gene dose-dependent. Recently, genome-wide association studies (GWASs) have demonstrated that mutated and multiplicated *SNCA* can also increase the risk of sporadic PD (Table 1); however, further investigations are necessary to better understand the role of *SNCA* in sporadic PD. Recently, researchers from China suggested that

Table 1. Overview of different *SNCA* mutations and clinical features

Variable	*SNCA* mutation						
	p.A53T	p.A30P	p.E46K	*G51D*	*H50Q*	Duplication	Triplication
References	17, 28–34	34, 35	22	36, 37	23, 36, 38	39–42	25, 43, 44
Mean age of onset	47 yr	60 yr	60 yr	40 yr	58 yr	50 yr	40 yr
Mean time from motor onset to death	8 yr	4 yr in 1 individual, others alive	10 yr	8 yr	8 yr	15 yr	7 yr
Motor features*							
Tremor	↓*	↓	↑	↓	-	-	↑
Rigidity	-*	↓	↑	-	-	-	↑
Bradykinesia	-	↓	↑	-	-	-	↑
Postural instability	-	↓	↑	-	-	-	↑
Non-motor features*							
Dementia	↑*	-	↑	↑	↑	-	↑
Depression	↑	-	-	↑	-	-	-
Autonomic disturbances	↑	-	-	↑	-	-	-
Sensitivity to levodopa therapy	+*	?	With hallucinations and fluctuation of consciousness at therapeutic doses of L-dopa	+, induce involuntary movement	+	+	-

This table was assembled using updated genetic insights of different *SNCA* mutations according to the reference by Kasten and Klein.[21] *different *SNCA* mutations' motor/non-motor features compared with typical Parkinson's disease symptoms. ↓: means moderate, -: means no difference/not sensitive, ↑: means severe, +: means sensitive, ?: means unknown.

SNCA is associated with motor progression, while *MAPT* (encoding microtubule-associated protein tau) variants are associated with clinical severity, leading to a hypothesis that if different genes are mutated, then different point mutations in each gene could result in different types of PD progression.[27] This hypothesis may have an application in clinical traits, and the gene mutations might become biomarkers for PD in the future. In conclusion, the differences between mutations and mutation types are considerable (Table 1), and the detection of novel mutations (types) of *SNCA* is justified both in clinical diagnosis and research. This could allow clinicians to predict those patients who are at risk for specific motor or non-motor features, such as autonomic dysfunctions. For a successful study, large amounts of clinical data will be needed, and the information obtained from the majority of GWASs will be insufficient.

THE CLINICAL APPLICATION OF α-SYNUCLEIN

Diagnosis of PD depends mainly on clinical symptoms and physical examination. However, before the appearance of classical symptoms, at least 60% of nigral dopaminergic neurons have already degenerated.[45] There is no biomarker available to reveal neurodegeneration before the appearance of clinical symptoms; therefore, misdiagnosis is common, occurring in up to 20% of cases, particularly in the early stage of the disease.[46] Here we summarize 11 studies of α-synuclein in body tissues which targeting α-synuclein as a biomarker for the diagnosis of PD (Table 2).

The Parkinson's Progression Markers Initiative study found that the α-synuclein level in CSF was lower in PD compared with that in healthy controls. Moreover, the α-synuclein level was significantly lower in PD patients with the non-tremor-dominant type than in those with the tremor-dominant type.[47] Thus, CSF α-synuclein could be a potential biomarker to predict the progression of PD from its early stage. In the study mentioned above, by injecting radiolabeled α-synuclein intracerebroventricularly into the mouse brain, researchers from the USA found that a small portion of α-synuclein specific to the CSF was transported to blood via exosomes. The levels of plasma exosomal α-synuclein were higher in PD patients. By contrast, the CSF α-synuclein levels were lower in PD patients when compared with controls, which suggested an increased secretion of this protein into the blood. Furthermore, researchers observed a significant correlation between plasma exosomal α-synuclein and disease severity.[16] Thus, we could hypothesize that the CSF exosomes might serve as carriers for interneuronal transmission of a pathogenic species of α-synuclein that could initiate oligomerization of soluble α-synuclein in target cells, there-

Table 2. Overview of previous studies targeting α-synuclein as a biomarker for the diagnosis of PD

Ref (year)	Specimen	Methods	PD α-synuclein positive cases/total	Sensitivity (%) PD vs. controls	Specificity (%) PD vs. controls
Iwanaga et al. (1999)[60]	Cardiac plexus, sympathetic ganglia	IHC	9/11	88	100
Duda et al. (1999)[61]	Olfactory mucosa	IHC	5/5	100	0
Michell et al. (2005)[62]	Skin	IHC	3/16	19	80
Braak et al. (2006)[63]	Gastric myenteric plexus	IHC	3/3	100	100
El-Agnaf et al. (2006)[64]	Plasma	ELISA	Higher in PD	53	85
Beach et al. (2010)[65]	Sections of SpCd, Sym, Vagus, GI, and Endo*	IHC	55/58	95	91
Shannon et al. (2012)[66]	Distal sigmoid colon	IHC	9/10	90	100
Mollenhauer et al. (2013)[67]	CSF	ELISA	Lower in PD	NA	NA
Besong-Agbo et al. (2013)[68]	Serum	ELISA	Lower in PD	NA	NA
Woulfe et al. (2015)[69]	Distal sigmoid colon and rectum	Paraffin-embedded tissue blot and IHC	By the method applied, the feature of colonic mucosal α-synuclein staining cannot distinguish PD from controls	NA	NA
Klettner et al. (2016)[59]	Crystalline lens	WB	2/5	NA	NA

This table was assembled using previous studies targeting a-synuclein as biomarker for the diagnosis of PD according to Malek et al.[58] IHC: immunohistochemistry, ELISA: enzyme linked immunosorbent assay, NA: not available, SpCd: spinal cord, Sym: sympathetic ganglia, Vagus: vagus nerve, GI: gastrointestinal system, Endo: endocrine system, CSF: cerebrospinal, PD: Parkinson's disease, WB: western blot.

by inducing disease pathology. Researchers showed that not only α-synuclein but also changes in the CSF levels of neurofilament light, YKL-40, tau, phosphorylated tau, and β-amyloid correlated with motor progression and cognitive decline in patients with PD.[48] Thus, we could combine α-synuclein with other CSF biomarkers to improve the accuracy of diagnosis and prognosis.

Although diagnosis of PD primarily depends on clinical symptoms and physical examination, cognitive impairment is a common and important feature of PD, especially in the early stage. *SNCA* was the first gene implicated in the pathogenesis of PD and it remains the primary risk factor for idiopathic PD worldwide. However, the role of α-synuclein in cognitive impairment is not completely clear. To examine the relationship between CSF markers (α-synuclein) and cognition, a large, multicenter cohort study including 414 early, untreated PD [34% with mild cognitive impairment (MCI)] and 189 healthy controls was conducted in Norway. The researchers found that in the PD group, lower α-synuclein was associated with reduced performance on the executive-attention and composite cognition. The data also revealed that abeta[42] was significantly decreased in PD patients with MCI compared to PD patients without MCI and controls. This study suggested that α-synuclein pathology contributes to early cognitive impairment in PD, particularly executive-attentional dysfunction. CSF α-synuclein, as a biomarker, could assist in diagnosis and in the development of new cognition-enhancing treatments.[49] Cognitive impairment is often the only symptom that presents at the early stage of PD; therefore, it is difficult to discriminate among PD, PD with dementia (PDD), and dementia with Lewy bodies (DLB). These disorders are similar in their motor and cognitive dysfunctions and are characterized by Lewy body pathology. Recently, researchers used targeted high-throughput sequencing to characterize the 135 kb *SNCA* locus in a large multinational cohort of patients with PD, PDD, DLB, and healthy controls. Their data showed that PD, PDD, and DLB, rather than a disease continuum, have distinct genetic etiologies despite being on one genomic locus. Their analysis of 43 tagging single nucleotide polymorphisms across the *SNCA* locus showed two distinct association profiles for symptoms of parkinsonism and/or dementia, respectively, toward the 3' or the 5' end of the *SNCA* gene. In addition, they defined a specific haplotype of intron 4 that is associated directly with PDD. Such genetic predictors of cognitive decline might serve as diagnosis biomarkers for these disorders and will assist with treatment.[50] Longitudinal studies that investigate *SNCA* variability and the progression of motor and non-motor features are required.

However, the procedure for collecting CSF is invasive and is not suitable or necessary in most cases, leading to development of alternative methods to test α-synuclein.[51] Recently, several studies showed that α-synuclein aggregation could be found outside the CNS, especially in the enteric nervous system of the gastrointestinal (GI) tract. Although previous studies with small cohorts revealed high sensitivity and specificity rates of enteric neuronal α-synuclein in PD patients,[52,53] this was not sustained in large-scale studies that showed that enteric α-synuclein could also be found in healthy individuals.[54] Researchers from South Korea detected the presence of α-synuclein immunoreactivity in gastric and colonic mucosa in a similar manner in patients with PD, MSA, and controls, which suggested that enteric mucosal α-synuclein would play a limited role as a biomarker in PD diagnosis.[55] Longitudinal studies are needed to identify more sensitive and specific biopsy samples and biomarkers to define reliably the presence of pathological α-synuclein. Advanced alternative detection methods and multicenter efforts, including software-based image analysis, are also warranted.

α-synuclein has been detected not only in the GI tract but also in PD patients' serum, saliva, submandibular gland,[56] skin,[57] olfactory mucosa and urine.[58] Interestingly, a recent study suggested that crystallins prevent protein (α-synuclein) aggregation in a physiological state, but that they might lose this ability in cataract disease because the prevalence of cataracts is higher in PD patients. During *in vivo* access to lens tissue during standard cataract surgery, researchers found higher levels of α-synuclein in the residual lens fragments in the supernatant of the PD group than in that of the controls.[59] To the best of our knowledge, this is the first study to investigate the cataract lenses of patients with PD *in vivo*. Although this study did not reveal an accurate biomarker, there are indications of differences between the lenses of the PD group and those of the controls. We encourage further research of lenses

and other human tissues in PD patients that could contribute to the ongoing search for PD biomarkers (such as α-synuclein) for early diagnosis and prognosis, which will lead to a better understanding of the mechanism of PD.

α-SYNUCLEIN, A PROMISING APPROACH FOR NEW THERAPIES FOR PARKINSON'S DISEASE

Currently there is no disease-modifying treatment for most neurodegenerative disorders, particularly PD, which is one of the most challenging neurodegenerative disorders to treat. Currently, despite limitations such as a large variety of motor and non-motor symptoms and drug complications, medical therapies, especially levodopa, remain the most effective treatment of PD.[70] However, medical or surgical treatments, such as deep brain stimulation, that focus on managing the disease symptoms cannot delay or recover the progressive loss of dopamine (DA) neurons.[71,72] In pioneering work, researchers induced stem cells such as embryonic stem cells, induced pluripotent stem cells, and neural stem cells into DA neurons in animal models. However, further studies are needed to better understand the cell sources, mechanisms, and appropriate methods of transplantation.[73] Here, we will introduce some updates for new discoveries and novel therapeutic strategies based on the pathogenic mechanism of PD.

PD involves several pathogenic pathways, including the lysosome-autophagy pathway. Numerically, approximately 5–10% of PD patients carry mutations in the glucocerebrosidase gene (*GBA*), which might increase the risk of developing PD by approximately 20 times.[74] Glucocerebrosidase is a lysosomal enzyme that metabolizes glucocerebroside to glucose and ceramide.[75] *GBA* mutations lead to reduced enzyme activity and the mutant protein might be trapped in the ER, leading to an unfolded protein response and ER-associated degradation and stress. Recently, an interaction between glucocerebrosidase and α-synuclein levels was reported. This interaction could be exploited to elevate glucocerebrosidase enzyme activities and therefore reduce α-synuclein levels to modify the course of PD.[76] This approach offers the potential to modify glucocerebrosidase activity or substrate accumulation as an alternative neuroprotective strategy in the future.

Currently, increasing numbers of researchers are focusing on the proteotoxic mechanisms underlying α-synuclein's effects, exploring not only neuroprotective approaches but also restorative strategies.[77] Immunotherapy using antibodies targeting α-synuclein in animal and cellular models has been shown to be effective in alleviating pathological, motor, and non-motor symptoms. Indeed, not only the active immunization model, in which a transgenic mouse model for synucleinopathies is immunized with recombinant α-synuclein, but also the passive immunization mouse model, in which a monoclonal antibody has been built that can generate antibodies that target α-synuclein, can achieve palliation or cure PD.[78,79] The mechanisms underlying such immunotherapy are as follows: the antibodies aid the clearance of extracellular α-synuclein and the block cell-to-cell transmission of the protein. However, these methods have some limitations: first, immunotherapy should not interfere with the normal physiological function of α-synuclein. This could be solved by generating a conformation-specific antibody that does not bind normal α-synuclein. Second, how are the antibodies transmitted to the specific regions of brain parenchyma? Perhaps the antibodies could be engineered artificially such they could pass the blood-brain barrier. Finally, to ensure the safety and feasibility of the humanized antibodies, immunotherapy must be tested in a non-human primate first.[80] Moreover, it might be possible to transfer protein aggregation immunotherapy to other proteinopathies, such as Alzheimer's, Huntington's, and prion diseases. To date, there have been three clinical trials on passive immunotherapy against α-synuclein. One test was performed by Prothena Biosciences and their colleagues. Forty healthy volunteers in different groups received intravenous infusions of PRX002 at doses of 0.3, 1.0, 3.0, 10, or 30 mg/kg. Significantly, all doses decreased the free α-synuclein in plasma without any serious side effects. Undoubtedly, this research provided an important insight into antibody therapy and might imply that almost all serum α-synuclein could be bound without adverse reactions. However, we should not lose sight of the fact that the antibody concentration in the brain is far lower than that in serum; therefore, if we could engineer a specific epitope or isoform of α-synuclein to guide them to the target brain region, this might provide a platform to realize the thera-

peutic effect of immunotherapy against α-synuclein. Recently, researchers from the United States generated a new antibody for an α-synuclein peptide and tested it in a rat model of PD to verify its effectiveness. The researchers injected an adeno-associated virus (AAV)-α-synuclein vector into the right SN of 344 Fisher rats, while control rats were injected with an AAV vector expressing green fluorescent protein. After one week, the researchers found that the α-synuclein peptide antibodies (AB1 and AB2) reduce the loss of α-synuclein-induced dopaminergic cells significantly. Antibody-treated rats had lower α-synuclein levels in the ipsilateral SN and revealed improved behavioral deficits. These data suggested that the α-synuclein peptide antibody, which was raised against the N-terminal region of the protein, could reduce dopaminergic neuron loss and behavioral deficits.[81] Moreover, the combined application of drug combinations and/or multi-target drugs might produce significant and long-lasting results compared with monotherapies. For example, treatment including immunotherapy against α-synuclein to reduce α-synuclein accumulation and cell-to-cell transfer combined with drugs that reduce neuroinflammation could have synergistic outcomes.[82] Therefore, such comprehensive treatment might be an efficient therapeutic strategy to halt the progression of PD.

High iron levels have been found in the SN of PD patients; therefore, there is significant interest in the idea of whether α-synuclein interacts with iron and whether iron overload could aggravate mutant α-synuclein toxicity. A study conducted in France suggested that increased intracellular α-synuclein concentrations could facilitate the accumulation of intracellular iron in neurons exposed to excess iron.[83] This in turn might promote the oligomerization and aggregation of α-synuclein. These results suggested that iron treatments, such as iron chelation or attenuation of its uptake into neurons, could be a future therapeutic option to treat PD.[83] Furthermore, researchers from Hong Kong investigated *Drosophila melanogaster* strains that overexpress A53T, A30P, and wild-type (WT) *SNCA* together with iron treatment. They found that iron treatment could induce a more distinctive motor decline in flies expressing the A53T mutant than in those expressing the A30P mutant but had little effect in the WT, which was the same order as the intrinsic aggregation order of these mutated proteins.[84] Contrary to previous findings, iron treatment did not cause any loss of dopaminergic neurons,[85] which suggested that iron overload might increase the binding between iron and α-synuclein and strengthen the aggregation of α-synuclein. This might abrogate the proteotoxic mechanism induced by α-synuclein.

The pathology of PD involves not only the CNS dopaminergic system but also other systems, including the cholinergic system and serotonergic system, which indicates that therapeutic strategies should not just focus on the dopaminergic nervous pathway. Thus, although studies concerning therapy for PD are in sight, continued efforts are required to develop new therapeutic strategies to treat, or even prevent, the occurrence and development of PD.

CONCLUSION

Studies over the last two decades have validated the important role of α-synuclein in the pathogenesis of PD, although our understanding of the relationship between α-synuclein and PD is incomplete. Recent advances have revealed the structure and distribution of α-synuclein and its mechanisms of toxicity in PD. Recently, point mutations in *SNCA* have been identified that correlate with the clinical situation. However, PD remains one of the most challenging disorders to diagnose and treat because it affects multiple organ systems and requires treatment of both motor and non-motor symptoms. In the future, it will be crucial to determine if α-synuclein represents an early stage and disease-specific diagnostic biomarker and whether it represents a promising approach for treatment.

Conflicts of Interest

The authors have no financial conflicts of interest.

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