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Review

Mechanisms of Parkinson's Disease Linked to Pathological α-Synuclein: New Targets for Drug Discovery

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Classic Parkinson's disease (PD) is characterized by fibrillar α-synuclein inclusions known as Lewy bodies in the substantia nigra, which are associated with nigrostriatal degeneration. However, α-synuclein pathologies accumulate throughout the CNS in areas that also undergo progressive neurodegeneration, leading to dementia and other behavioral impairments in addition to parkinsonism. Although mutations in the α-synuclein gene only cause Lewy body PD in rare families, and although there are multiple other, albeit rare, genetic causes of familial parkinsonism, sporadic Lewy body PD is the most common movement disorder, and insights into mechanisms underlying α-synuclein-mediated neurodegeneration provide novel targets for the discovery of disease-modifying therapies for PD and related neurodegenerative α-synucleinopathies.

Introduction

Long before the description of Parkinson's disease (PD) by James Parkinson nearly 200 years ago, there were accounts of a clinical syndrome consisting of tremor and akinesia described in Indian medical (*Ayurvedic*) texts written >3000 years ago (Manyam, 1990; Katzenschlager et al., 2004). Remarkably, this syndrome, termed *kampavata* in Sanskrit, was treated with natural products from *Mucuna pruriens*, a plant now known to contain levodopa (L-dopa), the symptomatic drug intervention that revolutionized PD therapy in the mid-20th Century. However, our understanding of mechanisms underlying the onset and progression of PD is undergoing a second, more profound revolution initiated by the identification of mutations in the gene encoding α-synuclein (*SNCA*) and the demonstration that α-synuclein is the principal component of filamentous Lewy bodies (Polymeropoulos et al., 1997; Spillantini et al., 1997). Indeed, these and subsequent insights into the normal biology and function of α-synuclein and the role of α-synuclein pathologies in PD are reorienting the design of drug discovery efforts to focus on targets related to α-synuclein misfolding and fibrillization into filamentous Lewy bodies and Lewy neurites, as well as other potential pathogenic pathways involving α-synuclein oxidation/nitration and degradation (Cookson, 2005; Feany, 2004; Forman et al., 2005; Giasson et al., 2004b; Savitt et al., 2006). Thus, clarity about the relationship of α-synuclein-mediated neurodegenerative mechanisms to PD will have a powerful impact on the development of disease-modifying therapies for PD and related disorders characterized by α-synuclein pathologies. Here we review recent advances in understanding the role of pathologically altered forms of α-synuclein in the onset and progression of PD, as well as the implications thereof for developing novel disease-modifying therapies for PD and related neurodegenerative disorders that are characterized neuropathologically by abundant accumulations of α-synuclein inclusions throughout the central nervous system (CNS). (Note that due to the explosive growth in new information on the genetic and molecular pathology of PD, only limited recent primary publications are listed, and earlier literature is available in reviews cited here.)

α-Synuclein and Lewy Body Parkinson's Disease

The most common neurodegenerative movement disorder is Lewy body PD, which is know to increase progressively with advancing age such that it affects about 1% of individuals who are 65 years of age, but 5% or more of those individuals 85 years of age and older. Lewy body PD is characterized clinically by L-dopa-responsive motor impairments that include (1) bradykinesia, (2) increased muscle tone, (3) resting tremor, and (4) abnormal postural righting reflexes, although it is well known that the clinical phenotype of Lewy body PD overlaps with other new and previously described forms of parkinsonism (Forman et al., 2005; Giasson et al., 2004b; Savitt et al., 2006). Although there was considerable skepticism about the existence of a genetic etiology of PD throughout the 20th Century, these doubts were dispelled following the report of the first pathogenic *SNCA* mutation in familial PD in 1997. Since then, over a dozen additional genes and genetic loci have been implicated as causes of Lewy body PD or other familial Parkinson syndromes. Thus, as reviewed elsewhere (Feany, 2004; Forman et al., 2005; Giasson et al., 2004b; Savitt et al., 2006), there are multiple, seemingly unrelated genetic causes of nigrostriatal degeneration that manifest as parkinsonism, with and without Lewy bodies. And, while familial PD caused by α-synuclein mutations is extremely rare, PD characterized by α-synuclein containing Lewy bodies accounts for >90% of sporadic Parkinsonian disorders (Feany, 2004; Forman et al., 2005; Savitt et al., 2006).

Normal α-Synuclein and Its Putative Function

α-Synuclein is an abundant, 140 amino acid long, highly soluble neuronal cytoplasmic protein that is, for the most part, unstructured in aqueous solution. It is predominantly localized to presynaptic terminals in the CNS, where it is loosely associated with synaptic vesicles (Cookson, 2005; Giasson et al., 2004b; Savitt et al., 2006). While the exact functions of normal α-synuclein remain to be fully elucidated, studies in songbirds suggest it may play a role in synaptic plasticity, and data obtained from studies of α-synuclein knockout mice, though controversial, indicate that this synaptic protein may be involved in activity-dependent negative

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regulation of dopamine neurotransmission or regulation of synaptic vesicle pools. Furthermore, a recent genetic study in mice suggests that α-synuclein may function as a cochaperone with cystein-string protein α in protecting nerve terminals against injury (Chandra et al., 2005). Finally, other recent studies suggest that α-synuclein may be involved in the trafficking of cargoes within the endoplasmic reticulum/Golgi network since one of the most profound initial consequences of overexpressing α-synuclein in yeasts appears to be a disruption of this trafficking (Cooper, et al., 2006).

α-Synuclein is a member of a family of three synaptic proteins that include α-synuclein, β-synuclein, and γ-synuclein. While the members of the synuclein family share considerable sequence homology, α-synuclein is unique in that it contains a highly amyloidogenic domain within its midregion that, by itself, fibrillizes more readily than the holoprotein (Giasson et al., 2004b). Indeed, this may explain why α-synuclein is the only member of the synuclein family to form fibrillar structures in vitro. Of the other synuclein family members, β-synuclein is the most closely related to α-synuclien, and it also shows a highly overlapping subcellular pattern of expression within the CNS, localizing to presynaptic nerve terminals. Although the functions of the other members of the synuclein protein family are equally enigmatic, there is evidence that the expression of β-synuclein may regulate the levels or metabolism of α-synuclein, since β-synuclein inhibits α-synuclein aggregation in transgenic mice (Hashimoto et al., 2001). Furthermore, more recent studies indicate that increased expression of β-synuclein can decrease the levels of α-synuclein by mechanisms that do not appear to affect α-synuclein mRNA levels (Fan et al., 2006), but it is likely that the functions of β-synuclein extend beyond regulating the expression of α-synuclein.

α-Synuclein Gene Mutations and Pathologies in Lewy Body Parkinson's Disease

Two seminal findings clearly linked α-synuclein to PD: (1) the discovery that point mutations in *SNCA* are pathogenic for familial forms of PD (Polymeropoulos et al., 1997); and (2) the demonstration that α-synuclein is the major component of Lewy bodies and Lewy neurites in idiopathic or sporadic PD (Spillantini et al., 1997).

Three missense mutations in the *SNCA* gene, as well as duplication and triplication of the locus containing the *SNCA* gene, have been identified in familial forms of PD (Forman et al., 2005; Savitt et al., 2006). However, familial PD caused by α-synuclein missense mutations is extremely rare, with the A53T mutation originating from a single common founder, the A30P mutation from a separate family, and the E46K mutation from yet another family. Several clinical features may distinguish patients with the A53T mutation from those with idiopathic PD, including slightly earlier onset, more rapid disease progression, lower prevalence of tremor, and noncardinal features such as dementia and myoclonus earlier in the disease course. By contrast, patients with the A30P α-synuclein gene mutation clinically resemble idiopathic PD. Although no neuropathology reports on patients with the A30P mutation have been reported, postmortem examination of A53T brains revealed substantia nigra degeneration and an abundance of α-synuclein Lewy bodies and neurites (Kotzbauer et al., 2004). Interestingly, affected E46K family members exhibit the clinical features of dementia and visual hallucinations in addition to parkinsonism, and neuropathologically, Lewy bodies were present in neurons of the substantia nigra as well as the cortex. Thus, this appears to be a familial form of a sporadic neurodegenerative disorder known as dementia with Lewy bodies (DLB). However, neuropathologically, DLB is difficult to distinguish from PD with dementia (PDD), since the burden of Lewy bodies and neurites is similar in both, but in PDD the dementia occurs after the onset of parkinsonism, while in DLB the dementia precedes the movement disorder (McKeith et al., 2005). Finally, the finding of kindreds with duplication and triplication of the *SNCA* gene locus in autosomal dominant PD implicates gene dosage effects in the pathogenesis of PD (Singleton et al., 2003, Chartier-Harlin et al., 2004). Neuropathological analysis of patients from some of these kindreds has revealed extensive Lewy body pathology. Taken together, data showing that α-synuclein gene mutations are inherited in an autosomal dominant manner and that similar α-synuclein pathologies are found in both familial and sporadic PD firmly establish a mechanistic link between neurodegeneration, α-synuclein abnormalities, and Lewy body PD.

Although the missense mutations in α-synuclein are rare, they may provide insights into pathogenic mechanisms leading to Lewy body formation. Previous studies have shown that α-synuclein filaments assemble in vitro from recombinant wild-type proteins, and that these filaments closely resemble those in pathologic inclusions, while both the A53T and E46K mutations accelerate fibril formation in test tube assays, suggesting that accelerated polymerization of α-synuclein may be responsible for disease in patients harboring this mutation (Forman et al., 2005; Giasson et al., 2004b; Rochet et al., 2004; Savitt et al., 2006). On the other hand, A30P α-synuclein has been reported to bind poorly to vesicles compared with the wild-type protein, although it is not clear how this is related to disease because the autosomal dominant mode of inheritance suggests a gain of toxic function for α-synuclein. One possibility is that impaired binding of A30P α-synuclein to vesicles hinders axonal transport of the protein, leading to accumulation of, and aggregation of, α-synuclein in cell bodies and processes. Finally, among the multiple other genes that have been implicated in familial parkinsonism, the underlying neuropathology is incompletely characterized for most of these familial forms of PD, although individuals with mutations in the *LRRK2* gene do develop α-synuclein pathologies, but the mechanisms to account for this are not entirely clear (Zimprich et al., 2004). This notwithstanding, the data on patients with LRRK2 mutations suggest that LRRK2, a protein that contains a number of well-defined domains, including Ras/GTPase, tyrosine kinase, WD40, and leucine-rich-repeat domains, could define a potential pathway that can lead to α-synuclein Lewy body formation.

α-Synuclein Fibril Formation

Other than missense mutations in α-synuclein, many other factors and events have been reported to influence the fibrillization of α-synuclein in vitro, and these

could be involved in the formation of α-synuclein inclusions in sporadic PD. Posttranslational modification of α-synuclein (such as C-terminal truncation and phosphorylation), incubation with metals (such as aluminum, copper, and iron), and oxidative/nitrative challenge (such as hydrogen peroxide and peroxynitrite treatment) also may accelerate fibril formation (Giasson et al., 2004b, Forman et al., 2005, Savitt et al., 2006).

However, oxidation does not always lead to an increase in α-synuclein fibril formation, and dopamine itself was shown to inhibit fibrillization, resulting in the formation of α-synuclein spherical oligomers (Conway et al., 2001; Rochet, et al., 2004; Norris et al., 2005). Interestingly, this inhibition is dependent on dopamine autoxidation, but not on α-synuclein oxidation, and the dopamine oxidation product dopaminochrome was identified as a specific inhibitor of α-synuclein fibrillization by inducing conformational changes (Norris et al., 2005). Taken together, these data suggest that dopamine autoxidation can prevent α-synuclein fibrillization and that decreased dopamine levels in substantia nigra neurons might promote α-synuclein aggregation in PD.

Models of α-Synuclein Aggregation and Neurodegeneration

Several α-synuclein animal models have been generated in flies, worms, and mice that add further support to the view that pathological α-synuclein is linked to mechanisms of neurodegeneration in PD (Bilen and Bonini, 2005; Feany, 2004; Giasson et al., 2004a). Expression of wild-type and mutant α-synuclein using promoters in mice has yielded differing results (Giasson et al., 2004a). For example, using the platelet-derived growth factor promoter to drive overexpression of wild-type α-synuclein results in a mild motor phenotype associated with dopaminergic nerve terminal loss in the striatum and inclusion formation in the substantia nigra, cortex, and hippocampus. However, although these inclusions contain α-synuclein and ubiquitin, they are unlike the Lewy bodies of PD because they are granular rather than fibrillar, and some inclusions are nuclear. Expression of wild-type or mutant forms of α-synuclein by the Thy1 promoter also results in a mild motor phenotype with nonfilamentous accumulation of α-synuclein in various brain regions. Surprisingly, no differences between mice expressing wild-type and mutant forms occur. Mice expressing A53T α-synuclein under control of the mouse PrP promoter, however, develop filamentous α-synuclein inclusions, primarily in brainstem and spinal cord, which correlate with the expression of a severe, complex motor phenotype leading to paralysis and death. Although these mice have provided a model that exhibits α-synuclein inclusion formation and associated neuronal dysfunction, the substantia nigra of these mice is unaffected. However, recent studies of transgenic mice expressing a truncated form of α-synuclein that fibrilizes more readily than the full-length protein in vitro did show evidence of substantia nigra Lewy bodies and neurodegeneration (Tofaris et al., 2006). Thus, α-synuclein transgenic mice provide a compelling model of PD-like neurodegeneration.

Results from transgenic *Drosophila* also support a mechanistic role for pathological α-synuclein in mechanisms of PD (Bilen and Bonini, 2005; Feany, 2004). For example, panneuronal overexpression of wild-type or mutant α-synuclein resulted in age-dependent degeneration of a subset of dopaminergic neurons, formation of filamentous α-synuclein inclusions, and locomotor dysfunction. However, bigenic flies expressing human α-synuclein and heat shock protein 70 (HSP70) or Rab1 GTPase were protected from neurodegeneration despite the formation of Lewy body-like α-synuclein inclusions (Bilen and Bonini, 2005; Cooper et al., 2006). Accordingly, these *Drosophila* models exhibit key features of PD and may serve as powerful tools in the discovery of genetic factors or pharmacologic agents that influence the pathogenic process.

Thus, there is overwhelming evidence that α-synuclein pathologies play integral roles in the onset and progression of PD and related disorders. Also, while the nigrostriatal system is the region that commonly undergoes neurodegeneration in PD, multiple additional brain regions are always affected in these disorders, and their clinical manifestations commonly extend beyond those attributable to the nigrostriatal system alone and often include cortical Lewy bodies and Lewy neurites associated with dementia (Forman et al., 2005; Giasson et al., 2004b; McKeith, et al., 2005).

α-Synuclein Pathogenic Pathways and Neurodegeneration

How α-synuclein abnormalities lead to neurodegeneration remains to be fully clarified, but several possibilities have been proposed (Bilen and Bonini, 2005; Cooper et al., 2006; Cookson, 2005; Forman et al., 2005; Giasson et al., 2004b; Rochet et al., 2004; Savitt et al., 2006) as summarized schematically in Figure 1. For example, α-synuclein localizes to the presynaptic terminal and binds to lipid membranes, which makes it plausible that this protein plays a role in the function of synapses or synaptic turnover. Hence, the sequestration of α-synuclein into aggregates or amyloid fibrils in the disease state disable it from performing its normal functions. Moreover, these effects could be amplified if other synaptic proteins that interact with α-synuclein are also sequestered or trapped in Lewy bodies and Lewy neurites, thereby resulting in the loss of their critical synaptic functions. Additionally, the demonstration that Lewy body pathology is composed of fibrillar α-synuclein, and that several autosomal dominant mutations in *SNCA* lead to enhanced rates of protein fibrillization, makes it plausible that conformational alterations in the structure of α-synuclein lead to a gain of neurotoxic properties by these pathological species of α-synuclein. However, other investigators have proposed that small, prefibrillar oligomers of α-synuclein are the toxic species leading to neuron dysfunction and degeneration by forming structures with pore-like morphologies. It is further hypothesized that these pore-like structures contribute to cytotoxicity in neurodegenerative diseases by disrupting organelle membranes and altering permeability that could alter mitochondrial or other organelle functions. Additionally, misfolding of α-synuclein in the endoplasmic reticulum/Golgi network has been hypothesized to launch a cascade of events culminating in PD. However, further studies of purified α-synuclein amyloid pores and the effects of pathological

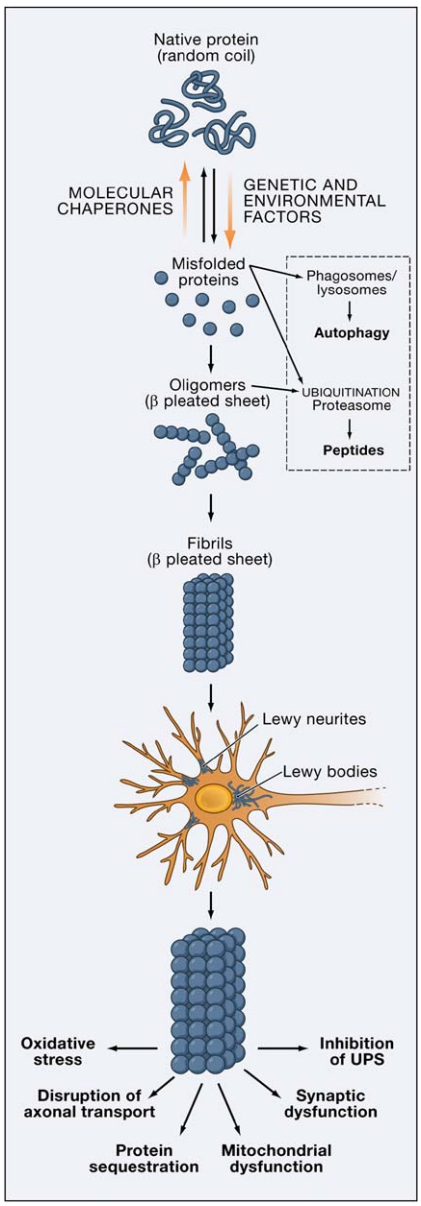

Figure 1. Model of α-Synuclein Misfolding and Aggregation and the Downstream Consequences

Schematic illustration of the stepwise process whereby normal, highly soluble α-synuclein misfolds and is converted into pathological oligomers and higher-order aggregates that fibrillize and deposit into Lewy bodies and Lewy neurites in affected neurons of the PD brain. The genetic abnormalities mentioned in the text, as well as poorly understood environmental factors (e.g., pesticides, head trauma), may accelerate this process and overwhelm the ability of normal quality-control systems (molecular chaperones, ubiquitin proteosome system (UPS), phagosome/lysosome system) to prevent or reverse protein misfolding or eliminate proteins that have misfolded or assembled into pathological aggregates and amyloid fibrils. Several of the proposed toxic consequences of the accumulation of fibrillar deposits of α-synuclein are illustrated in the lower part of the figure. The therapeutic interventions discussed in the text are designed to abrogate the pathways depicted here that lead to the misfolding, fibrillization, aggregation, and toxicity of α-synuclein that can culminate in PD and related α-synucleinopathies.

α-synuclein on intracellular transport are needed to test the validity of these hypotheses further.

Thus, although the precise mechanism of pathogenesis remains hotly debated, there is strong evidence implicating increased expression of α-synuclein and pathologically altered forms of this protein in the pathogenesis of both familial and sporadic PD. Thus, duplication as well as triplication of *SNCA* results in a dose-dependent increase in the severity and/or age of onset of PD/PDD (Singleton et al., 2003), while genetic polymorphisms in the *SNCA* gene appear to confer risk for sporadic PD by increasing the expression of α-synuclein (Savitt et al., 2006). Thus, genetic mechanisms are implicated in mechanisms of PD by increasing expression, aggregation, and accumulation of α-synuclein in the CNS (Giasson et al., 2004b; Forman et al., 2005; Savitt, et al., 2006; Skovronsky et al., 2006).

However, environmental factors may also play a crucial role in the pathogenesis of α-synucleinopathies, and epidemiological studies suggest an association of PD with environmental toxins such as pesticides. Indeed, chronic systemic treatment of rats with rotenone, a pesticide known to inhibit mitochondria, causes selective nigrostriatal dopaminergic degeneration with associated inclusions containing fibrillar α-synuclein (Greenamyre and Hastings, 2004; Savitt et al., 2006). Thus, rotenone treatment may induce an increase in oxidative stress in the dopaminergic neurons, which in turn may facilitate fibrillization of α-synuclein, providing a link between oxidative stress and pathogenesis of α-synucleinopathies.

Accordingly, based on the data summarized above and in other recent reviews (Bilen and Bonini, 2005; Rochet, et al., 2004; Savitt et al., 2006; Skovronsky et al., 2006), there are multiple reasons to target pathological species of α-synuclein for the discovery of novel disease-modifying therapies for PD and related α-synucleinopathies. A list of the most compelling reasons to embark on PD drug discovery that focuses on pathways leading to α-synuclein pathologies follows here, and some of these are discussed in further detail below:

1) Mutations/multiplications in the *SNCA* gene cause familial PD/PDD/DLB.
2) Lewy bodies and Lewy neurites are hallmark PD/PDD/DLB amyloid inclusions detected by α-synuclein-specific antibodies.
3) Epitope mapping demonstrates that regions spanning the entire α-synuclein molecule are present in Lewy bodies.
4) Insoluble, filamentous α-synuclein aggregates in Lewy bodies and Lewy neurites contain abnormally nitrated, phopshorylated, and ubiquitinated residues.
5) α-Synuclein filaments are recovered from PD/PDD/DLB brains as well as from Lewy bodies purified from these brains.
6) Recombinant α-synuclein forms Lewy body-like amyloid fibrils.
7) α-Synuclein single transgenic mice/worms/flies develop a neurodegenerative disease with filamentous α-synuclein amyloid deposits.

8) Coexpression of HSP70 or Rab1 with α-synuclein in flies and β-synuclein with α-synuclein in mice ameliorates the disease phenotype, while induction of heat shock proteins (HSPs) with geldanamycin in α-synuclein transgenic flies attenuates degeneration of Lewy body-containing neurons.

Targeting α-Synuclein-Mediated Neurodegeneration for Parkinson's Disease Drug Discovery

It is becoming increasingly evident that several approaches to drug discovery appear compelling based on the current understanding of α-synuclein-mediated mechanisms of neurodegeneration (Figure 1). For example, inhibition of α-synuclein aggregation is an attractive target for drug development, and several groups have identified small-molecule and peptide-based inhibitors of aggregation (Rochet, et al., 2004; Savitt et al., 2006; Skovronsky et al., 2006). Interestingly, a number of catecholamines, including dopamine, have been found to inhibit α-synuclein fibrillization, and the inhibitory activity of dopamine depends on its oxidation and leads to accumulation of α-synuclein protofibrils (Norris et al., 2005; Rochet et al., 2004). Hence, this link between dopamine and α-synuclein provides insights into the potential mechanisms underlying the selective vulnerability of dopaminergic neurons in PD, although many types of neurons other than those that are dopaminergic, as well as astrocytes and oligodendroglial cells, also are selectively affected by accumulations of α-synuclein inclusions.

Moreover, the ameliorative effects of HSPs on the PD phenotype in model systems discussed above were extended by followup studies showing that treatment of transgenic α-synuclein-expressing flies with the drug geldanamycin protects neurons against α-synuclein toxicity through augmenting chaperone activity (Bilen and Bonini, 2005). An alternative approach would be to harness other normal cellular mechanisms that counteract the accumulation of intracellular misfolded or conformationally altered proteins, including normal proteolytic pathways, the ubiquitin proteasome system, and autophagic system (Cuervo et al., 2004). Finally, while the biochemical signatures of pathological α-synuclein are abnormal protein phosporylation, ubiquitination, insolubility and aggregation, the abnormal phosphorylation and ubiquitination of α-synuclein have not yet been shown to be required for fibrillization (Giasson et al., 2004b). Nonetheless, abnormal phosphorylation may be a target for the discovery of disease-modifying therapies of PD, since this posttranslational modification may contribute to stabilizing α-synuclein aggregates.

However, a major impediment to conducting cost-effective and informative clinical trials of potential disease-modifying therapies for PD and related α-synucleinopathies is the absence of robust biomarkers for both early diagnosis of these disorders when therapy is likely to have the greatest impact and monitoring the responses of patients to new therapeutic interventions in clinical trials. A challenge to overcoming this obstacle is the complexity of neurodegenerative movement disorders and the overlap of α-synucleinopathies with other diseases, including Alzheimer's disease (AD), the most common subtype of which is the Lewy body variant of AD. As in the AD field, which is at the vanguard of neurodegenerative disease biomarker research (Thal et al., 2006), all biomarkers for PD and related α-synucleinoapthies should be developed and validated based on criteria similar to those proposed by the Work Group On Biological Markers of Alzheimer's Disease (Ronald and Nancy Regan Institute, 1998). The consensus report from this group of biomarker experts specified that ideal AD biomarkers should be linked to fundamental features of AD neuropathology, be validated in neuropathologically confirmed AD cases, be able to detect AD early in its course and distinguish it from other dementias, and be reliable, noninvasive, simple to perform, and inexpensive. Furthermore, it was recommended that all AD biomarkers undergo evaluation of their sensitivity, specificity, prior probability, positive predictive value, and negative predictive value, as well as undergo validation by independent groups who publish their findings in peer-reviewed journals. Finally, to be informative for diagnosis, it was recommended that biomarkers have a sensitivity and specificity of >85%. The same recommendations of this AD biomarker work group should also be applied to PD biomarkers, which could be grounded in a better understanding of α-synuclein-mediated neurodegeneration. Indeed, there are emerging data to suggest that the measurement of normal or pathological α-synuclein in cerebrospinal fluid or blood could lead to the development of diagnostic tests (Tokuda et al., 2006). However, while hypothesis-driven candidate biomarkers such as normal and abnormal α-synuclein proteins should be a major focus of PD biomarker research, it is timely to pursue the identification of biomarkers using unbiased strategies based on proteomics or related technologies (Abdi et al., 2006).

These significant challenges to PD drug discovery notwithstanding, the advances in PD research reviewed here suggest that therapeutic agents designed to inhibit or reverse the fibrillization and aggregation of α-synuclein in PD and related α-synucleinopathies could have potential disease-modifying effects. The realization of this PD drug discovery goal would certainly revolutionize the treatment of PD for the second time since the introduction of L-dopa for the symptomatic therapy of PD 50 years ago, thereby offering prospects for not only arresting and/or reversing the progression of PD, but also preventing the onset of this and related neurodegenerative α-synucleinopathies.

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