

Aging is the primary risk factor for the majority of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). There are almost 40 million people aged 65+ in the United States. Statistics predict that if you reach age 65 you can expect to live almost 20 more years. The average age of onset of PD is 60 years. If you are 85 years of age, you have an almost 50% risk of developing AD. The population of persons 85+ is projected to increase from 4.2 million in the year 2000 to 6.6 million in the year 2020. This suggests that these neurodegenerative diseases will reach a prevalence of epidemic proportions.

The current dogma holds that cellular mechanisms that are associated with aging and those that are related to neuron degeneration in PD and AD *are unrelated*. However, more recent evidence suggests that normal aging and the degeneration of specific neuron populations in AD or PD may be linked by the same cellular mechanisms. This remains a topic of debate. For your comprehensive exam, choose either AD or PD and use this disease to address the following questions:

- 1) Discuss what is known about one primary molecular mechanism that creates the pathology that occurs in one of these age-related neurodegenerative conditions. Discuss age-related changes in brain physiology that may promote, enhance, and/or allow the appearance of the disease specific pathology to be exaggerated in middle age and beyond.
- 2) For this section, assume that neurodegenerative disease exists along a continuum with natural aging. The implication of this is that if individuals survive long enough, it is inevitable that they will eventually develop AD or PD. Keep in mind that AD and PD are each viewed as genetically heterogeneous and complex disorders caused or influenced by multiple factors (e.g., specific genes, susceptibility alleles, environmental exposures, gene-environment interactions). Thus the development of "cause-directed therapies" is a goal of future research in these fields. Indeed, one model suggests that elements of lifestyle and genetics that promote healthy aging will decrease the incidence of these diseases in the general population. In this regard, discuss the following issues:
  - A.) how elements of lifestyle, genetics, or an intervention might impact the incidence of AD or PD.
  - B.) whether current data suggests a *single* target to cure AD or PD or if a multi-pronged approach is necessary.
  - C.) In the realm of "personalized medicine," how one could tailor a specific therapy or lifestyle for two patients with different "forms"\* of AD or PD .

James Parkinson first described the clinical symptoms of Parkinson's disease (PD) in his 1817 monograph, "An Essay on the Shaking Palsy." Since this time, PD has come to affect 2% of the 65 year-old population (1) making PD the 2<sup>nd</sup> most common neurodegenerative disease next to Alzheimer's disease. Diagnosis of PD is clinical and is based strongly on motor disability, effects of L-DOPA treatment and imaging techniques to visualize dopaminergic neuronal loss in the basal ganglia.(2) Parkinsonism syndrome is defined by motor disabilities such as tremor, rigidity, slowness or lack of movements (bradykinesia/akinesia), postural instability (impaired balance and frequent number of falls) and episodes of freezing. In addition, patients are presented with non-motor symptoms such as dementia and depression. PD accounts for roughly 80% of parkinsonism syndrome cases.(3) There are two main forms of PD; familial (genetic) and sporadic (no apparent genetic linkage). Sporadic PD accounts for almost 95% of PD cases.(3) Both forms exhibit two main pathological features; loss of dopamine (DA) neurons within the substantia nigra (SN) and the presence of intraneuronal cytoplasmic inclusions called 'Lewy Bodies' (LB). Since 1817 much research has been done to elucidate a cause(s), but the pathogenesis of PD remains unknown.

One of the greatest risk factors for PD is age.(4, 5) However, the current dogma holds that molecular mechanisms underlying aging and PD are not the same. For example, different patterns of DA neuron loss in PD and aging were described.(6) However, more recent evidence supports the idea that aging and PD may both involve similar molecular mechanisms.(7) The complex nature of both aging and PD development and additionally, the lack of fully identifying causes of PD, forces the link between aging and PD to remain controversial. This paper has been split into two sections. The first section is focused on a potential mechanism underlying PD and similar age-related mechanisms that have potential to exaggerate PD pathology. Specifically, I will discuss one primary molecular mechanism underlying PD pathology (I) and age-related changes in brain physiology that may influence PD pathology to be exaggerated in middle age and beyond (II). The second section is focused on the complexity of PD in regards to factors that mediate the incidence of PD, targets for potential cures and tailored therapies. Specifically, I will discuss how elements of lifestyle, genetics or intervention may impact PD incidence (III), current data suggesting a multi-targeted

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approach is necessary for a cure for PD (IV) and how therapies can be tailored for two patients with different forms of PD (V).

**A potential molecular mechanism underlying PD and age-related molecular mechanisms  
with potential to exaggerate PD pathology**

$\alpha$ -synuclein is a natively unfolded soluble protein (140 amino acids). It is localized both in the cytoplasm and with synaptic vesicles. For instance, Davidson et al. demonstrated the ability of  $\alpha$ -synuclein to associate with phospholipid membranes, bind to acidic phospholipids and present a greater preference in small unilamellar vesicle (20-25 nm) binding in preparations of small and large unilamellar vesicles.(8) All of these attributes suggest a role of  $\alpha$ -synuclein in synaptic vesicle function. Other studies suggest a role of  $\alpha$ -synuclein in synaptic vesicle recycling, neurotransmitter synthesis and release as well as synaptic plasticity.(9) Despite the previous studies, an exact physiological function of  $\alpha$ -synuclein remains unknown. In PD, the protein  $\alpha$ -synuclein is thought to play a main role in DA neuronal loss. Familial PD has been linked to specific point mutations in the gene encoding for  $\alpha$ -synuclein (SCNA) including an alanine substitution for threonine at position 53 (Ala53Thr), Ala30Pro and Glu46Lys.(10-12) Moreover,  $\alpha$ -synuclein aggregates are a major component of LB's in familial and sporadic PD.(13, 14) Taken together,  $\alpha$ -synuclein is a critical feature associated with PD and remains essential in current PD research. Therefore, I will discuss what is currently known about  $\alpha$ -synuclein misfolding and aggregation relating to degradation dysfunctions that may underlie the pathology of PD.

I. A molecular mechanism of PD:  $\alpha$ -synuclein misfolding, aggregation and degradation

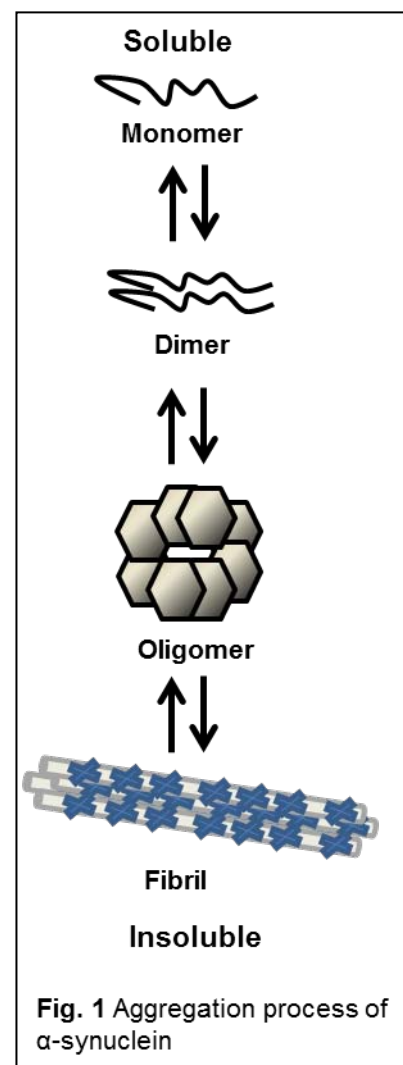
*$\alpha$ -synuclein misfolding and aggregation*

Protein misfolding and aggregation is one possible molecular mechanism underlying the pathology of PD. Protein misfolding may occur naturally (i.e. not in the disease state) as a result of trial and error during the time at which the newly synthesized polypeptide chain attempts to fold into the lowest-energy three-dimensional native state. Quality control mechanisms such as ubiquitin-proteasome system (UPS) and lysosomal pathways differentiate misfolded and correctly folded native proteins guiding misfolded proteins to

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degradation. However, protein aggregation results if misfolded proteins are not properly degraded. Accumulation of protein aggregates is thought to be cytotoxic due to possible protein aggregate-dependent cell deformation, protein-aggregate seizure of necessary cell survival proteins, as well as protein aggregate interference in neuronal intracellular trafficking.

The pathway in which proteins become misfolded is complex and unclear. Under physiological conditions  $\alpha$ -synuclein exists in an unfolded state, but upon changes in the environment  $\alpha$ -synuclein self-assembles into many different conformations.(15) The aggregation process of  $\alpha$ -synuclein (Fig. 1) is not fully understood, but it was demonstrated that the formation of  $\alpha$ -synuclein dimers (one of the first transition states in aggregation process) displays a longer lifetime than  $\alpha$ -synuclein in a monomer state suggesting that  $\alpha$ -synuclein dimers may be more stable.(16) These authors suggest that oligomers form from the stabilized  $\alpha$ -synuclein dimers subsequently forming  $\beta$ -pleated sheets (protofibrils) and upon reaching a certain size they undergo elongation forming insoluble fibrils that are found to be the main component in LB's.  $\alpha$ -synuclein fibril formation can be induced by several protein modifications. Protein modifications can include phosphorylation at Ser129 (17, 18), nitration (18) and truncations at the C-terminus.(19) It is important to note that some studies provide evidence that phosphorylation at Ser129 is *not* necessary for  $\alpha$ -synuclein aggregation (20, 21) suggesting that



phosphorylation at Ser129 alone does not produce  $\alpha$ -synuclein aggregates. Protein modification of  $\alpha$ -synuclein can also result by neurotoxic insults. Neurotoxic insults from oxidation and nitration processes have the capability to modify  $\alpha$ -synuclein.(18) Likewise, introduction of nitric oxide (NO) and superoxide produced nitrated  $\alpha$ -synuclein in accordance with increased  $\alpha$ -synuclein aggregate formation.(22) These studies suggest that various factors can influence  $\alpha$ -synuclein aggregation, but whether the aggregation products, protofibrils or

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fibrils, cause cytotoxicity of DA neurons remains unclear. For example, Ala53Thr mutant  $\alpha$ -synuclein increased the rate of  $\alpha$ -synuclein aggregation such that fibrils were observed within a short time-frame (2 wk) *in vitro*.(23) However, in the same study, the rate of  $\alpha$ -synuclein aggregation caused by another mutation, Ala30Pro mutant  $\alpha$ -synuclein, was impaired as only spherical assemblies and not fibrils were seen. Since the Ala30Pro mutant  $\alpha$ -synuclein is implicated in PD and the relative rate of fibril formation was diminished, it suggests that protofibrils may be cytotoxic and mediate PD progression. This result can also be viewed in a way that fibrils are cytotoxic since the rate of Ala53Thr mutant  $\alpha$ -synuclein fibrils formation was much greater than the Ala30Pro mutant. Furthermore, another study concluded that amyloid proteins induced apoptosis in the dopaminergic neuroblastoma cell line SH-SY5Y.(24) However, these conclusions are solely based on *in vitro* work. In summary, toxic end-products of  $\alpha$ -synuclein misfolding and aggregation, either protofibrils or fibrils, are mediated by various processes including protein modifications and oxidative/nitrative reactions and may contribute to a molecular mechanism underlying the pathology of PD.

*$\alpha$ -synuclein aggregation: increased production and dysfunctional degradation systems*

Accumulation of protein aggregates may result from an increase in protein aggregate production. For example,  $\alpha$ -synuclein aggregation may be enhanced by molecular crowding, metals and polycations.(25) Likewise, mutations in the gene encoding for  $\alpha$ -synuclein increased the tendency of  $\alpha$ -synuclein aggregation.(23) Protein aggregate accumulation may also result from dysfunctional degradation mechanisms. The UPS degrades short-lived and misfolded proteins through a succession of events. Firstly, an ubiquitin molecule is activated by the ubiquitin-activating E1 enzyme that drives the thiol ester bond formation between the carboxy-terminal glycine on ubiquitin and the cysteine residues on E1 in an ATP-dependent manner. Secondly, the activated ubiquitin molecule is transferred to one of many ubiquitin-conjugating E2 enzymes then subsequently to the target protein that is bound to an ubiquitin-protein ligase E3 enzyme. E3 enzymes present selectivity in the degradation process by binding specifically to targeted proteins. Thirdly, the activated ubiquitin molecule is ligated to a lysine residue on the target protein by E3. At this stage, polyubiquitin chain formation from addition of other activated ubiquitin molecules to lysine residues

present within the ubiquitin molecule is necessary for proteasome recognition. The 26S proteasome complex is required for polyubiquitinated protein degradation. The 26S proteasome complex is formed from a catalytic core (20S proteasome) made of both  $\alpha$  and  $\beta$  subunits that is capped by two 19S complexes.

The UPS is the primary degradation process and thus it is likely that  $\alpha$ -synuclein may be degraded by this pathway. As such, Bennet et al. have shown that the degradation pathway for  $\alpha$ -synuclein is the UPS by significantly inhibiting the degradation of wildtype and Ala53Thr mutant  $\alpha$ -synuclein transfected in SH-SY5Y cells with application of the proteasome inhibitor,  $\beta$ -lactone.(26) The authors also showed that  $\beta$ -lactone significantly reduced degradation of endogenous  $\alpha$ -synuclein in non-transfected SH-SY5Y cells suggesting that  $\alpha$ -synuclein is degraded via the UPS pathway *in vivo*. A dysfunction in the UPS process has potential to induce protein aggregate formation and therefore increase PD incidence. Dysfunctions occurring in the UPS events have been implicated in PD. For example, McNaught et al. determined by Western Blot analysis that patients with sporadic PD have a greater loss of the  $\alpha$ -subunit in DA neurons of the SN than controls.(27) The  $\alpha$ -subunit is important in regulating the stability and assembly of the 26S proteasome complex and such loss of the  $\alpha$ -subunit would result in proteasome destabilization and the lack of assembly; both of which would cause impairments in protein degradation leading to protein accumulation. This study suggests that misfolded proteins in PD would not be degraded in a sufficient manner due to the nature of the 26S proteasome complex. Likewise, observation of stable ubiquitin-conjugates localized within PD brains(28) suggests that proteasome activity is impaired where targeting and ubiquination of misfolded proteins is not. In addition, blocking proteasome activity in PC12 cells with the proteasome inhibitor PSI led to accumulation of ubiquitinated synuclein-1 (rat homologue of human  $\alpha$ -synuclein) inclusions *in vitro*.(29) Together, these studies provide evidence that dysfunctional proteasome activity is capable of impairing  $\alpha$ -synuclein degradation and hence can lead to the formation of  $\alpha$ -synuclein aggregates. On the other hand, Ala53Thr mutant  $\alpha$ -synuclein transfected in PC12 cells was capable of diminishing proteasome activity.(30) In addition,  $\alpha$ -synuclein inclusions diminished proteasome activity(31) thus, creating a feed-forward cycle in which dysfunctional proteasome activity leads to  $\alpha$ -synuclein aggregates that can in turn act to impair proteasome activity. It is important to

note that studies also suggest that  $\alpha$ -synuclein degradation is regulated by the lysosomal system and under conditions of elevated  $\alpha$ -synuclein levels the lysosomal pathway becomes predominant as the UPS pathway becomes dysfunctional as a result of proteasome inhibition.(32) In summary,  $\alpha$ -synuclein aggregates may result from interactions promoting  $\alpha$ -synuclein aggregation and/or by an impaired degradation processes such as the UPS system.

Since  $\alpha$ -synuclein aggregates have the ability to form *in vitro* when proteasome activity was impaired and are associated with diminished 20S proteasome immunoreactivity in dopaminergic SN neurons in PD brains (31), then it is likely that inhibition of proteasome activity and therefore increased  $\alpha$ -synuclein aggregation has the ability to produce motor disabilities in an animal model mimicking those in PD patients. Likewise, McNaught et al. treated rats with both a synthetic (PSI) and naturally occurring (epoxomicin) proteasome inhibitor and observed distinct motor dysfunctions.(33) The authors observed significant motor deficits presented as severe bradykinesia and rigidity within 8 weeks of treatment and L-DOPA treatment improved these deficits. However, other studies present evidence that proteasome inhibition does *not* cause motor dysfunctions. Kordower et al. treated both rats and monkeys with PSI in an attempt to replicate results from the McNaught study.(34) The authors show that there are no significant differences in motor skills,  $\alpha$ -synuclein immunoreactivity and tyrosine hydroxylase (TH) immunoreactivity in SN cells between PSI and vehicle-treated animals. The authors suggest that a lack of significant findings may have resulted from improper preparation and administration of PSI. However, they note that McNaught personally instructed the authors on the correct preparation and administration of PSI. In summary,  $\alpha$ -synuclein aggregates are associated with diminished proteasome activity, but it remains unclear if diminished proteasome activity *in vivo* in association with  $\alpha$ -synuclein aggregates can lead to motor dysfunctions implicated in PD.

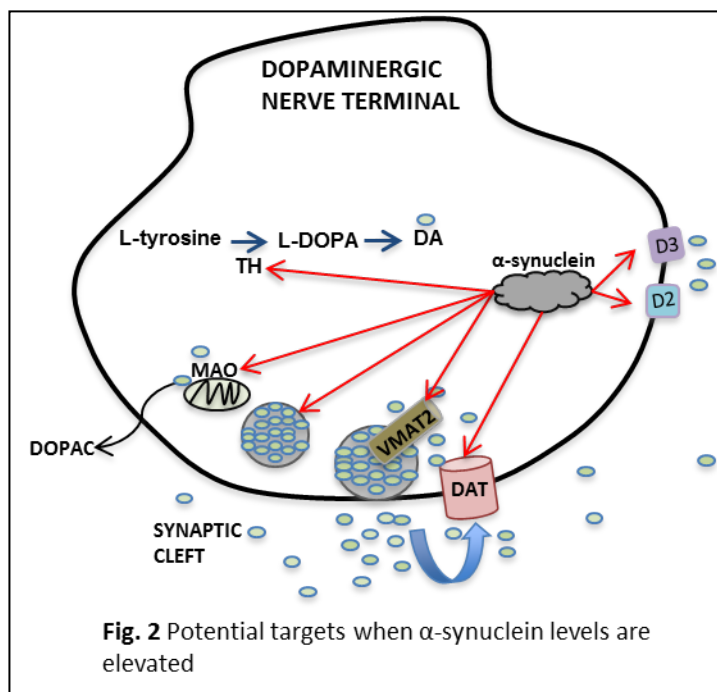
In conclusion, a likely candidate that influences the pathology of PD is  $\alpha$ -synuclein. The process of  $\alpha$ -synuclein aggregation is complex, but studies have shown that several protein modifications and gene mutations promote  $\alpha$ -synuclein aggregation. In addition,  $\alpha$ -synuclein aggregation can result from dysfunctional

degradation systems. Taken together,  $\alpha$ -synuclein aggregation can result by several cellular processes and impairments in these processes may underlie the pathology of PD.

Consequences of  $\alpha$ -synuclein overexpression: changes in DA neurotransmission

Experiments using  $\alpha$ -synuclein knock-out (KO) mice describe a role for  $\alpha$ -synuclein in mediating DA neurotransmission.(35) Hence, elevated levels of  $\alpha$ -synuclein may contribute to DA neuronal impairments (Fig. 2) and loss in the SN of PD patients. Lundblad et al. performed an elegant study in which they were able to observe functional changes in DA neurotransmission in rats overexpressing  $\alpha$ -synuclein *in vivo* using high-speed chronoamperometry.(36) High-speed chronoamperometry is an electrochemical detection method that

enables DA neurotransmission changes to be measured in real-time as a function of an oxidation current. The authors observed impairments in DA reuptake rates in rats expressing  $\alpha$ -synuclein levels 4-5 fold greater than endogenous levels even before observable cell loss and overt motor deficits occurred, suggesting DAT was impaired. Immunohistochemistry revealed no significant changes in DAT immunoreactivity; however, this technique fails to identify any occurring functional changes. To determine DAT function, the authors injected DA



exogenously and observed the extracellular DA elimination rate was hindered in rats overexpressing  $\alpha$ -synuclein. To further elucidate DAT dysfunction, the authors could compare the effects of a DAT inhibitor on reuptake rates in control rats to reuptake rates present in rats overexpressing  $\alpha$ -synuclein. In addition to reduced reuptake rates, DA release was significantly reduced in rats overexpressing  $\alpha$ -synuclein within the timeframe of DA neurodegeneration. Overall, results of this *in vivo* study coincide with previous *in vitro* results on  $\alpha$ -synuclein effects on DA storage and release mechanisms and DA neurodegeneration. For example,



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Bisaglia et al. have shown a human neuroblastoma cell line overexpressing Ala30Pro mutant  $\alpha$ -synuclein had greater propensity for DA toxicity presented as diminished cell viability with elevated apoptotic and necrotic cells numbers when exposed to concentrations of DA (and DOPA).(37) The authors suggest that the toxic effects induced by Ala30Pro mutant  $\alpha$ -synuclein could be due to reactions between  $\alpha$ -synuclein and DA oxidation products. In accordance, adduct formation between DA-*ortho*-quinone and  $\alpha$ -synuclein hinders fibril formation while prolonging the protofibril state (38) possibly leading to increased cytotoxicity. Consistent with a role in DA cytotoxicity,  $\alpha$ -synuclein has the capability to permeabilize synaptic vesicles (39), which could ultimately lead to increased cytosolic DA levels. Moreover, impairments in DA synaptic vesicles could result in alterations in DA release. Impaired DA release has been shown in Ala53Thr mutant  $\alpha$ -synuclein transfected PC12 cells, primary neuronal cultures overexpressing  $\alpha$ -synuclein and hippocampal slices of transgenic mice overexpressing the human  $\alpha$ -synuclein, maintained *in vitro*.(30, 40) Overexpression of  $\alpha$ -synuclein is cytotoxic primarily in DA neurons since  $\alpha$ -synuclein functions to protect other cell types against glutamate excitotoxicity.(41) In summary, studies reveal elevated  $\alpha$ -synuclein is cytotoxic to DA neurons resulting in increased susceptibility to DA toxicity and altered DA release and reuptake mechanisms both *in vivo* and *in vitro*.

Elevated  $\alpha$ -synuclein-dependent changes have the potential to affect other areas of DA neurotransmission.  $\alpha$ -synuclein localization is found within the cytosol and with synaptic vesicles and hence  $\alpha$ -synuclein may target other pre-synaptic components such as TH, monoamine oxidases (MAO), DA autoreceptors (D2 and D3) and VMAT2. For example, evidence suggesting that  $\alpha$ -synuclein may bind to and permeabilize synaptic vesicles (described above) implies that elevated  $\alpha$ -synuclein may have an indirect effect on VMAT2. In addition, the association between elevated  $\alpha$ -synuclein and diminished TH immunoreactivity suggests that consequences of elevated  $\alpha$ -synuclein may directly/indirectly target TH. Cell culture experiments have demonstrated that overexpression of  $\alpha$ -synuclein mediates processes of TH regulation.(42) Moreover, it has been demonstrated that  $\alpha$ -synuclein interaction with TH both in cell line and *in vivo* reduced TH activity resulting in reduced DA synthesis.(43) Overexpression of  $\alpha$ -synuclein may also affect intracellular MAO. MAO

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is a mitochondrial bound enzyme necessary for converting DA into 3,4-dihydroxyphenyl-acetic acid (DOPAC) and in doing so creates possible neurotoxic by-products like hydrogen peroxide. If overexpression of  $\alpha$ -synuclein is able to increase MAO activity, this will lead to an elevated level of oxidative stress that could promote DA neurotoxicity and increase  $\alpha$ -synuclein aggregation. In summary, elevated levels of  $\alpha$ -synuclein may work directly or indirectly to affect components of DA biosynthesis and metabolism.

In conclusion, an elevated level of  $\alpha$ -synuclein has an increased tendency to form aggregates. Consequences of overexpression of  $\alpha$ -synuclein include disruptions in DA neurotransmission, either by direct or indirect interactions, which can include alterations in DA biosynthesis, storage, release, reuptake and metabolism mechanisms. Most work thus far has been concentrated on *in vitro* systems and it is of necessity to determine if these changes exist *in vivo*.

## II. Age-related changes in brain physiology promoting PD pathology: oxidative stress, compromised cellular processes and degradation pathways

One of the greatest risk factors for development and rapid progression of PD is age (4, 5) and thus changes in brain physiology associated with aging may promote, enhance and/or allow PD pathology to be exaggerated in middle age and beyond. As discussed above, molecular changes underlying PD may involve  $\alpha$ -synuclein-related changes in DA toxicity and DA neurotransmission. Age-related changes in brain physiology can also affect  $\alpha$ -synuclein production and aggregation as well as DA neurotransmission, thus exaggerating PD pathology in middle age and beyond. As stated above,  $\alpha$ -synuclein aggregation can be enhanced by oxidation. Elevations in oxidant production, impaired capability of repair and defense mechanisms and increased levels of reactive oxygen species (ROS) are all positively correlated with age as described by the free radical theory of aging.(44) Increased oxidant or ROS production during aging(45) may act to promote  $\alpha$ -synuclein aggregation. As described above, through various events, ROS exposure nitrates  $\alpha$ -synuclein promoting the formation of  $\alpha$ -synuclein aggregates. In aged monkeys, the number of 3-nitrotyrosine (3-NT) immunoreactive cells was increased in the ventral tier of the SN (vtSN) correlating with DA neurodegeneration.(46) 3-NT is formed as a result of oxidative and nitrative reactions and is used as a marker

of cellular damage. The authors also demonstrated vtSN DA neurons in aged monkeys that showed the highest 3-NT level also had the highest ratio of DAT/VMAT. Thus, it can be implemented that with aging, oxidative and nitrative associated damage increases in accordance with an increase in the likelihood of cytosolic DA-dependent toxicity given a high DAT/VMAT ratio. Both processes can work in concert to increase vtSN DA neuronal susceptibility to degeneration. In addition, oxidation of cytosolic DA to DA-*ortho*-quinone leads to formation of adducts with  $\alpha$ -synuclein and can increase DA neurotoxicity. In summary, age-dependent elevations of oxidants and ROS have the potential to contribute to  $\alpha$ -synuclein aggregation and thus increase DA neuron vulnerability to PD development and progression.

Especially in familial PD, age-related onset suggests that cells are able to combat adverse effects caused by mutated genes for a long period until, during aging, the cells ability to overcome protein accumulation associated with PD is compromised. One way in which cells lose ability to deal with accumulated proteins is by impaired induction of chaperone proteins. Chaperone proteins, in addition to reducing the likelihood of protein aggregation, also work to rescue misfolded proteins. In aging, the induction of chaperone proteins is likely jeopardized. Indeed, induction of chaperone proteins such as heat shock proteins (Hsp) deteriorates with age.(47) Specifically, the Hsp70 protein, important in the UPS pathway, is directly related to DA neuronal survival in aged  $\alpha$ -synuclein-treated *Drosophila* and direct Hsp70 expression halted  $\alpha$ -synuclein-dependent DA neuronal loss.(48) Taken together, induction of chaperone proteins (e.g. Hsp70) is compromised with age and necessary for arresting DA neuronal loss associated with elevated  $\alpha$ -synuclein levels. Such compromise in Hsp70 induction has the potential to lead to DA neuronal loss in PD.

Aging affects the degradation pathways, impairing both the lysosomal and UPS pathways.(49, 50) As described above, both pathways are involved in the degradation of  $\alpha$ -synuclein and age-dependent changes in the UPS pathway may exacerbate  $\alpha$ -synuclein aggregation. Ebrahimi-Fakhari et al. demonstrated that aged mice (> 9 mo) exhibited greater accumulation of  $\alpha$ -synuclein that was dependent upon the UPS pathway.(32) This suggests that as mice age, the UPS pathway efficiency in protein degradation becomes hindered allowing for  $\alpha$ -synuclein accumulation. Furthermore, there is evidence of an age-related hindrance in these degradation

pathways.(50) Overall, degradation pathways that are important in degrading  $\alpha$ -synuclein seem to be impaired with aging and may contribute to enhanced DA vulnerability to PD pathology.

In summary, age-dependent changes in ROS evolution, chaperone protein induction and degradation pathways likely contribute to promote and/or enhance DA susceptibility to insult associated with  $\alpha$ -synuclein aggregation. Changes in brain physiology associated with aging and PD appear to involve similar mechanisms and may work synergistically allowing the appearance of motor deficits of PD to be exaggerated in middle age and beyond.

**PD complexity governed by multiple factors influences  
incidence, targets for potential cures and therapies**

The greatest risk factor for both the development and progression of PD is age (4, 5) and such a causal relationship between the ability to live long enough and PD development can be implemented. The 'stochastic acceleration hypothesis' described by Collier et al. suggests such an outcome.(7) The basis of this hypothesis is that age-dependent modifications made at the SN DA cellular level sets the stage for PD development. These cellular modifications develop by a process of variable and random exposure/pre-deposition to genetic and environmental factors that contribute to enhancing DA neuronal susceptibility to PD. This hypothesis integrates evidence that molecular mechanisms mediating DA neuron loss are in principle the same in aging and PD and changes in molecular mechanisms are variable. In addition to suggesting that PD development is inevitable with increasing age, the 'stochastic acceleration hypothesis' also suggests that PD development is evitable with increasing age by lifestyle and genetic factors that aim towards healthy aging. With this, there are various lifestyle, genetics, and interfering elements that have the ability to mediate the incidence of PD.

III. Lifestyle, genetics or an intervention may impact the incidence of PD

There have been a range of environmental and lifestyle factors contributing to both the increased risk and decreased risk in PD development. Factors that have been associated with an increased risk include but are not limited to the following: age, male gender, Caucasian, well water, pulp mills, MPTP and MPTP-like compounds, insecticides, herbicides, as well as heavy metals and carbon monoxide.(3, 51, 52) MPTP is the

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neurotoxic compound of the common street drug of the 1980's, MPPP. MPTP has been shown to induce Parkinsonism symptoms as well as SN DA neuron loss. MPTP is able to cross the blood brain barrier and when it does, it is oxidized to its active toxic form, MPP<sup>+</sup> (MPP<sup>+</sup> has high affinity for DAT). Environmental exposure to insecticides like rotenone and paraquat may increase the risk of PD incidence. Structure function of both rotenone and paraquat greatly resembles that of MPP<sup>+</sup>. Administration of both rotenone and paraquat to animals induces SN DA neuronal loss. SN DA neuronal loss has been shown to be mitigated by the use of caffeine, tobacco smoke, anti-oxidants (Vitamins C and D), estrogens, and anti-inflammatory agents.(53, 54) Likewise, compounds intended for neuroprotection such as coenzyme Q10 (mitochondrial action), inosine (anti-oxidant production) and isradipine (Ca<sup>2+</sup> channel blocker) are being used in multicenter trails for PD therapy.(55) In summary, several environmental and lifestyle factors have potential to mediate PD incidence.

The finding that a mutation in the SCNA gene encoding for  $\alpha$ -synuclein (10) prompted researchers to look for other gene mutations that may predispose individuals to PD. To date, there are several identified monogenic forms of Parkinsonism. *LLRK2* (leucine-rich repeat kinase 2) gene mutations are now recognized as the most prevalent gene mutation associated with both familial and sporadic Parkinsonism accounting for roughly 7% of familial cases.(56) Mutations of this gene are associated with late-onset Parkinsonism. Mutations in the *Parkin* gene have been associated with early-onset Parkinsonism.(57) *Parkin* is an ubiquitin-protein ligase E3 enzyme important in the UPS degradation pathway and a mutation in the *Parkin* gene impairs the activity of the E3 enzyme. Duplication and triplication of the SCNA gene governs the severity of the disease. Lastly, *DJ-1* gene mutations have been associated with early-onset Parkinsonism.(58) These gene mutations and possible others underlie the pathology of familial PD and predispose individuals to PD.

Given the importance of  $\alpha$ -synuclein in PD pathology, it is a likely target for intervention. A reduction in  $\alpha$ -synuclein expression will likely reduce the rate and probability of  $\alpha$ -synuclein aggregation possibly leading to a reduced PD incidence and diminished symptoms. Studies using a RNA interference approach have demonstrated reduction in the expression of  $\alpha$ -synuclein in rodents and primates.(59, 60) These studies used a silencing RNA directed towards  $\alpha$ -synuclein and were able to reduce protein levels and expression of

endogenous  $\alpha$ -synuclein in the SN with little toxicity shown by normal numbers of DA neurons and levels of DA and DA metabolites. However, it is unknown if effects of reducing  $\alpha$ -synuclein will reduce or reverse motor disabilities or other changes associated with PD.

#### IV. Current data suggests a multiple targeted approach is necessary for PD cure

The main pathology underlying motor disabilities in both familial and sporadic PD is a selective degeneration of DA neurons in the SN. The degeneration of these neurons is multi-faceted and indefinite. Moreover, a well-defined biomarker for PD diagnosis has yet to be determined. Current research in cells, animal models, primates and humans show several factors are likely to contribute to the pathology of PD including, protein misfolding and aggregation, dysfunctional degradation pathways, mitochondrial dysfunction, gene mutations, gene-gene interaction, environmental insults, oxidative stress, inflammation, as well as the aging process.(61) It is also clear that these factors may work in concert to produce the pathology. The general consensus is that many aforementioned factors may contribute to PD development in the vast majority of PD cases, yet it is important to note that some Parkinsonism cases are caused by one factor (e.g. single gene mutation, MPTP). However, these cases are extremely rare. In summary, current data suggests that causes of PD are multi-factorial and hence a multi-based approach to cure PD is necessary.

#### V. Personalized medicine: specific therapy/lifestyle for patients with different forms of PD

Two major clinical subtypes of PD are classified in terms of age of onset and rate of motor dysfunction progression and thus are termed early-onset and late-onset. Symptoms of early-onset are typically tremor predominant where motor deficits are much more evident in late-onset consisting of akinesia, rigidity, gait, and imbalance impairment. The classification of these sub-types focuses only on motor features; however, PD patients are presented with non-motor dysfunctions such as but not limited to urinary dysfunction, constipation, sleep disturbances, fatigue, dementia, anxiety and depression.(2) Therapies that tailor these different subtypes should be built upon common symptoms and age of onset. Current pharmacological therapies for the treatment of symptoms are those that enhance DA neurotransmission. The most effective drug to date to treat motor disabilities is L-DOPA. However, with chronic L-DOPA use dyskinesias begin to develop and most

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predominantly in younger adults. Therefore, L-DOPA may be used to treat symptoms in the late-onset subtype whereas a DA agonist such as pramipexole (Mirapex), ropinirole (Requip) or bromocriptine (Parlodel) may be used to treat symptoms in the early-onset patients. In early-onset patients, tremors may be treated with use of anti-cholinergic drugs like trihexyphenidyl (Artane, Trihexane) or beztropine (Cogentin). However, anti-cholinergic drugs do not treat motor symptoms common in late-set patients and therefore would not be used. Since PD patients also display non-motor features such as depression and dementia, pharmacological therapy may be used to treat these. Depression occurs in roughly half of PD patients(62) and has been suggested to be more predominant in early-onset patients.(63) Therefore, if both clinical sub-types present symptoms of depression, anti-depressants may be used. Anti-depressant use has been known to be efficient in treating symptoms in PD patients with minor side-effects.(2) It is important to note that treating motor symptoms and dementia in PD patients is much more complex and drugs to treat dementia have the ability to cause deleterious side-effects. Non-pharmacologic therapy may also be used to address symptoms of PD. Studies show that it is important to keep patients educated.(2) In addition, exercise has been proved to be beneficial to mitigate motor deficits in PD patients.(64) In conclusion, therapies for two sub-types of PD patients may be formed based on age of onset and degree of symptoms. Pharmacological therapies may differ for the two sub-types based on age and symptoms whereas non-pharmacological therapies may be used to address symptoms in both sub-types.

In conclusion, current research suggests that PD is a complex disease that has the potential to result from many lifestyle, environmental and genetic factors. From this, it is clear that a multi-targeted approach to cure PD is necessary but complicated in that current pharmacologic therapies have implications for different sub-types of PD. Therefore, potential successful therapies for PD sub-types should be built upon the age of onset as well as both motor and non-motor symptoms.

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## **Student 1**

### **Overall Analysis: Pass**

The document was overall very well written, easy to read, good use of explanations; logical flow of thoughts; good organization. Good insight into many complex topics; good attention to details, even when relatively minor (e.g.; page 6 McNaught personally instructing authors on rebuttal manuscript...). The use of summary statement at the end of topics within sections was very helpful. Section III, asked the student to discuss how altering a single factor (lifestyle, genetic mediation or an intervention) might lead to a change in the incidence (rate of new cases/year) of PD. The student did not answer this question. Instead the student listed a series of factors that are known to affect it already. Figures used in the answer don't give credit to a source publication. In addition, the last part of question 2 where it asks for discussion of how, in the realm of personalized medicine, one might tailor a specific therapy or lifestyle for two patients with 'different forms' of AD or PD was not quite what the question asked. However, the interpretation of the question this way still provided a scholarly answer. We were looking for something along the line of if patient 'A' had a form of PD caused by alpha-synuclein and patient 'B' had a form of PD caused by exposure to pesticides, how you might design a therapy to treat each. For patient 'A' you might design an approach to silence or knockdown the excessive a-syn; and for patient 'B', you might give an MAO inhibitor. But again, the student did a very fine job based on their interpretation of the question.

Overall Evaluation: Generally a good effort. It would be advised for the student to more closely analyze the question and ask for clarifications prior to writing.