Identification of biomarkers and modifiable risk factors for Parkinson's disease dementia

By

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

March 2019

Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide and afflicts approximately 1% of individuals over age 65. The dementia that develops in association with PD is devastating to both the patient and their family and common, with more than 80% of individuals with PD developing dementia by 15 years after symptom onset. Current treatments for both PD and PD-dementia (PDD) have unacceptable side effects and there are no disease modifying therapies. An improved understanding of the pathophysiology of PD and PDD, however, is leading to testing of therapeutics that are targeted to specific pathways that have been implicated in PD pathophysiology. This pathophysiology is reviewed in Chapter 1, with an emphasis in the c-Abl molecule pathways role in PD pathophysiology. A biomarker for both PD and PDD would greatly improve our diagnosis, prognosis, and potentially the efficacy of these new treatment trials. Chapter 2 therefore considers whether poly (ADP-ribose), a molecule downstream in the c-Abl pathway, is a potential biomarker for PDD. Finally, we need to improve the outcomes for our current patients and Chapter 3 explores the role of a modifiable risk factor, namely vascular disease and subsequent vascular pathology, in the development of PDD. Together, these investigations broaden our understanding of PDD and are a step toward improved treatment for individuals with this devastating disease.

Thesis Defense Committee

David Newman Toker, MD, PhD; Ted M. Dawson, MD, PhD

Rebecca Gottesman, MD, PhD; Marie Diener-West, PhD

John McGready, PhD

Acknowledgements

The process of writing this dissertation and completing this research began many years ago when I was neurology resident. At that time, two of my early mentors. Dr. Rebecca Gottesman and Dr. Lori Jordan, told me about a program through which I could receive the training I needed in clinical research while I continued on with my larger career. I was instantly intrigued. The true launch of this research though began at a meeting with Dr. Ray Dorsey, Dr. Ted Dawson and me approximately 8 and a half years ago in late 2010. Dr. Dawson was interested in beginning a translational research program focusing on biomarkers for Parkinson's disease (PD) and I was a first-year movement disorder fellow tapped by Dr. Dorsey to help start the program. At the urging of Dr. Joe Savitt I asked to include my interest in PD-related cognitive impairment into the biomarker investigation. And so a research project was born.

In addition to receiving salary support from the JHU KL2 program, my inclusion in the Graduate Training Program in Clinical Investigation ensured that I would not finish this degree with even more debt. I am also indebted to my many mentors. Dr. Dawson's support of my research and career, including frequent meetings, has been the linchpin of my academic success. He give me the freedom to run things my way and was never overbearing. At the same time, his gentle prodding has frequently been exactly what was needed to move a project along. I remain indebted to his support of my career. The support of my other committee members has also been instrumental to my success. Dr. Newman-Toker never seemed to waver in his belief that I would eventually finish the

degree. Dr. Rebecca Gottesman was always available for the right guidance exactly when I needed it. As a resident she showed me that I could indeed have a successful career and family and her support of my pregnancy while I was chief resident has been a model of how to support trainees and colleagues in all components of their career. Dr. John McGready sat with me for many hours to help me apply what we learned in biostatistics to real-world data. Dr. Diener West was kind enough to jump into my committee at the last minute to ensure that I could indeed complete the PhD.

I have been fortunate to have a number of other mentors. Dr. Zoltan Mari was my "go to" person for many clinical questions while he was here at Johns Hopkins and was a key part of my clinical training. He and Dr. Greg Pontone were instrumental in helping me to develop my early questions regarding patient enrollment and continue to assist me in manuscript preparation and publication. Dr. Arnold Bakker assisted at a key moment regarding research regulatory questions. My current colleagues of Drs. Alex Pantelyat, Emile Moukheiber, Ankur Butala, and Jee Bang have all been amazing in managing my idiosyncrasies and worked with me despite my frequent delays in emailing them back. While he just started as my division director, Dr. Kelly Mills has already shown a gift at encouraging me exactly when needed. I am looking forward to continuing to work with all of these colleagues in the forthcoming years. Throughout my time at Johns Hopkins Neurology the support of Dr. Justin McArthur has also provided the ongoing backdrop to my success.

Critical to the success of this research and my career have been the many people who have worked with me and taught me how to perform the day-to-day activities necessary for a high quality research project. Katerina Salnikova was in the trenches with

me from the beginning of the JHU PDBP cohort, and it is only because of her dedication that everything was ready for that first patient. Nadine Yoritomo was critical to my knowledge of research regulations and the actual implementation of Good Clinical Practice. Her appropriate insistence on performing research according to the explicit regulations was such an incredible gift. In addition, her leadership of the office, including training of new research assistants and serving as a resource for all research and personal questions, helped to create the joyful working atmosphere at Greenspring Station. Catherine Bakker has remained the person to whom I turn for all research-related questions and continues to amaze me with her hidden gifts of editing and writing and organization. I also continue to be amazed at the work of Vanessa Johnson, Katherine Iannuzelli, and AJ Hall. Their knowledge of research practices is unparalleled and the research would not continue without all of their efforts. It is a privilege to work with all of you. While not directly related to this research, Donna DeLeno Neuworth has also been a continued inspiration and reminder of our larger goals of improving the lives and outcomes of our patients. I further had the privilege of working with Carrie Berlett for the past 6 years. She was a critical part of keeping up with all of my patients and a joy to work with, thus allowing me to pursue this research program. With Carrie leaving JHU, I am sure that Jana Arreola will not only continue to fill Carrie's shoes but also carve her own path within the Ataxia Center and our JHU community. I do also need to thank the online Academic Research Mom's Facebook community. You are all "my people" and provided key assistance with Endnote at just the moment needed.

My family provided the foundation under which I have been able to complete this research. My in-laws both raised a wonderful son and showered my children with love,

while at the same time supporting my need to pursue this research. My parents have always been my biggest fans, from when I was in the 5th grade spelling bee through undergraduate school, medical school, and now finally the completion of this graduate training. Your love and support have been the shoulders on which I have stood for my entire life. Finally, this dissertation and my life would be incomplete without my amazing husband, partner and best friend. Josh, thank you for your steadfast support even when it has meant you are responsible for more things at home. Your unwavering support of my career has allowed me to become closer to "having it all" than any woman I know. And while my children are too young to truly understand what it means to complete a project of this magnitude, I nevertheless dedicate all that I do to them and their future. Thank you for sharing their mommy with her patients and her career. My goal is to serve as an example of a life and career well-lived and I am even more excited to be part of their future successes than my own. I hope this research and that of many others leads to a better world for them as adults.

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List of Abbreviations

AD: Alzheimer's Disease

ADP: Adenosine diphosphate

BBB: Blood Brain Barrier

CAA: Cerebral Amyloid Angiopathy

CBS: Corticobasal Syndrome

CDR: Clinical Dementia Rating

CERAD: Consortium to Establish a Registry for Alzheimer's Disease

CI: Confidence Interval

CMA: Chaperone-mediated autophagy

CNS: Central Nervous System

COMT: Catechol-O-methyltransferase

CSF: Cerebrospinal Fluid

CVD: Cardiovascular Disease

DBS: Deep Brain Stimulation

DLB: Dementia with Lewy Bodies

DNA: Deoxyribonucleic acid

ELISA: Enzyme-linked immunosorbent assay

FDA: Food and Drug Administration

HBS: Harvard Biomarker Study

JHU: Johns Hopkins University

LBs: Lewy Bodies

LED: Levodopa Equivalent Dosing

List of abbreviations

LNs: Lewy Neurites

LSVT: Lee Silverman Voice Treatment

MCI: Mild Cognitive Impairment

MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale

MJFF: Michael J. Fox Foundation

MoCA: Montreal Cognitive Assessment

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MSA: Multiple System Atrophy

NINDS: National Institute of Neurological Diseases and Stroke

NMDA: N-methyl-D-aspartate

OH: Hydroxy

PAR: Poly-ADP-ribose

PARIS: Parkin Interacting Substrate

PARP1: Poly-adp-ribose polymerase 1

PD-MCI: Parkinson's disease-Mild Cognitive Impairment

PD: Parkinson's disease

PDBP: Parkinson's Disease Biomarker Program

PDD: Parkinson's disease dementia

PHF-1: Phosphorylated anti-Tau

PMCA: Protein misfolding cyclic amplification

PPMI: Parkinson's Progression Markers Initiative

PSP: Progressive Supranuclear Palsy

REM: Rapid eye movement

RNA: Ribonucleic acid

SD: Standard Deviation

TCDD: Tetrachlorodibenzo-p-dioxin

UK: United Kingdom

UPDRS: Unified Parkinson's Disease Rating Scale

UPS: Ubiquitin proteasome system

UPSIT: University of Pennsylvania Smell Identification Test

VCAM-1: Vascular Cell Adhesion Molecule-1

I: Pathophysiology and current treatment of Parkinson's disease (PD) and PD-dementia (PDD)

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting approximately 1% of the population over the age of 65. With the aging population and improving medical treatment, the number of individuals with PD is expected to grow from its current level of more than 4.5 million people worldwide to more than 8 million people by 2030 (Dorsey et al., 2007). Approximately 1/3 of individuals with PD have PD- dementia (PDD), with another subset of individuals experiencing PD-related mild cognitive impairment (Aarsland & Kurz, 2010). Individuals with PD are less likely to work, have higher nursing home placement and greater mortality than the general population and those with PDD have worse outcomes than those with PD alone (Aarsland, Larsen, Tandberg, & Laake, 2000).

PD is a chronic progressive disorder manifested by at least two of the four motor signs of tremor at rest, bradykinesia, rigidity and postural instability and results from the selective degeneration of the nigrostriatal pathway that provides dopaminergic innervation to the striatum. PDD is applied to individuals who develop dementia in the setting of a PD diagnosis and case series that followed patients through 15 years after symptom onset found a cumulative incidence of PDD of about 80% of the PD population (Aarsland, Andersen, Larsen, Lolk, & Kragh-Sorensen, 2003). PD is currently diagnosed based on patient history and a clinical examination showing the presence of Parkinson's motor signs, coupled with no or minimal "red flags" that indicate atypical parkinsonism (i.e. slow or absent vertical saccades) or other exclusion criteria (i.e. history of repeated strokes or long term exposure to specific medications) (Hughes, Daniel, Kilford, & Lees, 1992; Postuma et al., 2018). Using these diagnostic methods, autopsy series have shown

that even movement disorder specialists are incorrect about the diagnosis of PD approximately 10% of the time (Hughes et al., 1992). Furthermore, it is unfortunately not unusual for patients to have an up to 2-year delay between symptom onset and diagnosis. Diagnosis is complicated by two primary factors. 1) The phenotypic heterogeneity of the disease and 2) the phenotypic overlap between PD and many of the atypical parkinsonisms. In other words, parkinsonism and the associated degeneration of the nigrostriatral circuitry is a final common pathway of neurodegeneration caused by different abnormal proteins including synucleinopathies such as multiple system atrophy (MSA), Dementia with Lewy Bodies (DLB), and PD and tauopathies such as Progressive Supranuclear Palsy (PSP), Corticobasal Syndrome (CBS) and even Alzheimer's disease (AD). Definitive diagnosis of PD can only occur based on autopsy tissue that shows the presence of α-synuclein, the presence of eosinophilic, intracytoplasmic proteinaceous inclusions termed Lewy bodies (LBs) with alpha-synuclein inside them, and dystrophic Lewy neurites (LNs) in surviving nigral neurons (McKeith et al., 2017)

Although the cardinal symptoms of PD can be improved using currently available dopamine replacement strategies, many of these treatments have unacceptable side effects and many of the non-motor features of the disease have minimal treatments. Most importantly, treatments that provide neuroprotection and/or disease modifying effects are an urgent unmet clinical need. Development of these new therapeutics requires looking at the challenge from multiple perspectives, including improving our understanding regarding the pathophysiology of PD and the identification of progression biomarkers for PD. We also need to take a similarly multi-pronged approach to the development of disease modifying therapeutics for PDD, recognizing that development of PDD is

multifactorial such that many but not all of the therapeutics for PD will likely improve PDD.

Two critical steps toward development of these disease modifying therapeutics for PDD are 1) better understanding of the pathologic heterogeneity of and pathologic contributors to PDD and 2) identification of progression markers that are predictive of speed of progression as well as the makeup of the underlying pathophysiology. Development of PDD is certainly dependent on the alpha-synuclein pathology that is the hallmark of PD but PDD development is also influenced by the tau and amyloid beta pathology ((Irwin, Lee, & Trojanowski, 2013; Irwin et al., 2012)). While vascular disease contributions to dementia have been well-documented, the importance of vascular disease and associated vascular risk factors for the development of PDD is less clear. Since vascular risk factors are modifiable, determination of whether vascular disease is associated with development of PDD has direct and immediate impact on patient care. In addition, should vascular disease be shown to contribute to development of PDD, treatment of vascular disease among our patients becomes even more critical and it opens the door to investigation of vascular biomarkers. We can also seek out biomarkers amongst currently known PD pathways, including the parthanatos cell death pathway. These biomarkers will point to the potential efficacy of therapeutics that are under development or approved for a number of different cancers, including c-Abl inhibitors and poly (ADP-ribose) polymerase 1 (PARP1) inhibitors.

In this review, we discuss the current state of PD diagnosis and management, with a focused discussion of abnormal alpha-synuclein and parthanatos as part of PD pathophysiology. We also discuss the spread of this abnormal alpha-synuclein within the

brain and finally there is a brief discussion regarding the environmental contributors to PD pathophysiology. Together, this improved understanding of the many facets of PD pathophysiology seeks to pave the way to more effective and efficient diagnosis of PD and to meet our goal of halting disease progression.

Review of current therapeutics

The current treatment of PD is solely based on symptomatic management. Effective treatment is therefore individualized, within the guidelines of the evidence-based practices discussed below. Patients experience varying degrees of symptoms, with some having more rapid motor impairments while some experience severe psychiatric symptomatology. Put together, this phenotypic heterogeneity is one of the many challenges to diagnosis, treatment, and to the identification of disease modifying therapies.

Treatment of motor symptoms of PD

The mainstay treatment for the motor complications of PD remains dopamine replacement, usually in the form of levodopa. Levodopa was first given to patients in 1961, with growing recognition of its efficacy through the 1960s and the addition of a dopa decarboxylase inhibitor in the early 1970s (Tolosa, Marti, Valldeoriola, & Molinuevo, 1998). Carbidopa/levodopa remains the most effective medication for treating the motoric symptoms of PD. Unfortunately, about 50% of patients develop dyskinesias within about 5 years of treatment (Rascol et al., 2000). Additionally, over time the therapeutic window of the medication narrows (Olanow, Obeso, & Stocchi, 2006), necessitating increased frequency and amount of dosing. Much of the

development of new medications in PD has focused on improving the motor fluctuations, dyskinesias, due to this narrowing of the therapeutic window. A new oral formulation of carbidopa/levodopa was FDA approved in January 2015. Marketed under the brand name Rytary, it contains both control release and immediate release carbidopa/levodopa. Patients therefore take the medication about 3-4 times a day, as opposed to the current carbidopa/levodopa with which patients may eventually require as many as 7-10 doses a day. Carbidopa/levodopa intestinal gel, FDA approved in 2015, follows the same principles of dopamine replacement but has a novel delivery system with a percutaneous jejunostomy tube that enables continuous dosing of the medication. There are additional novel delivery systems currently being tested including an inhaled levodopa (LeWitt et al., 2016) as well as a subcutaneous levodopa (ClinicalTrials.gov). These have great potential to improve the dyskinesias and motor fluctuations that occur with the narrowing of the therapeutic window, but it is the same medication that was identified more than 55 years ago and is not disease modifying. There are several other symptomatic therapies in regular use. Dopamine agonists improve PD symptoms, though they are generally not as effective as carbidopa/levodopa. Currently available agonists include pramipexole, ropinirole and the more recently available transdermal formulation rotigotine. These medications have a place in management, often as monotherapy or even first-line therapy among younger patients and/or to help with the motor fluctuations that occur with carbidopa/levodopa dosing. However, these medications also have unacceptable side effects—about 17% of patients taking dopamine agonists develop compulsive behaviors including compulsive gambling, sex, or shopping (Weintraub et al., 2010). Monoamine oxidase inhibitors, rasagiline and selegeline and the recently FDA approved safinamide,

may also be useful to augment levodopa. COMT inhibitors, namely entacapone and tolcopone, also seek to extend the life of levodopa. Finally, amantadine is useful for treatment of dyskinesias and is sometimes used for symptomatic treatment (Dietrichs & Odin, 2017).

There are also surgical options currently available for PD symptomatic treatment. These surgical treatments are most effective at improving the motor fluctuations due to the narrowing therapeutic window of levodopa and the pharmacokinetics of medication administration. The most frequently performed surgical procedure for PD is deep brain stimulation (DBS) that involves placing electrodes in either the globus pallidus internus or the subthalamic nuclei. The resulting constant stimulation attenuates the motor fluctuations that occur later in the PD course. Prior to the proven effectiveness of DBS, ablative procedures were common. The pallidotomies and thalamatomies were helpful for symptomatic treatment but concerns over side effects and the irreversibility led to DBS becoming more common (Fasano, Daniele, & Albanese, 2012). New approaches to ablative procedures are being explored and such an approach continues to have potential benefit for PD patients. Carbidopa/levodopa intestinal gel (marketed as Duopa in the US) is a newer surgical approach mentioned previously that involves placement of a percutaneous jejunostomy tube and the subsequent infusion of carbidopa/levodopa intestinal gel.

Non-pharmacological treatments for PD have also been shown to benefit symptoms. Exercise has been proven to be critical to the treatment of the motor symptoms of PD (Goodwin, Richards, Taylor, Taylor, & Campbell, 2008). Specific exercises that have shown benefit include tai chi (F. Li et al., 2012) and a special type of

physical therapy called the LSVT BIG program (Ebersbach et al., 2015). Many patients also benefit from other forms of physical therapy, speech therapy, and occupational therapy (Sturkenboom et al., 2013) that provide tailored exercises and adaptive mechanisms to improve their health and function.

Treatment of non-motor symptoms of PD

The treatment of the non-motor symptoms of PD, similar to the treatment of the motor symptoms, aims to ameliorate the functional impact of changes. One of the most common and debilitating non-motor symptoms of PD are the cognitive changes. Again, there are no disease modifying therapies for the cognitive changes though there is some evidence that exercise may improve cognition (David, FB et al., Mov Dis 2015). Most frequently, clinicians use the medications initially developed for treating Alzheimer's disease (AD). Given the overlap in pathophysiology between AD and PD this is a reasonable approach and subsequent trials support use of acetylcholinesterase inhibitors, specifically rivastigmine, as good pharmacological choices to treat the cognitive changes observe in PD. With these treatments, some patients demonstrate clear improvements while still others demonstrate worsening cognition in the setting of these medications (Svenningsson, Westman, Ballard, & Aarsland, 2012). Memantine, a partial NMDA-receptor antagonist, has shown less benefit in cognition in at least one study, with other studies demonstrating a mild but not always significant improvement in quality of life (Svenningsson et al., 2012). There is also a fluid interaction between cognition and many of the PD motor treatments. Frequently, stopping or decreasing amantadine, any of the dopamine agonists, and sometimes even levodopa can lead to cognitive improvement.

Conversely, some patients require larger and more optimal doses of levodopa to improve their cognition.

Neuropsychiatric manifestations of PD are also very common and debilitating for many of our patients, with depressive symptoms and anxiety affecting at least 40% of individuals with PD (Aarsland, Marsh, & Schrag, 2009) and can lead to significant disability, with patients reporting that depression is a greater determinant of health-related quality of life than motor symptoms (Soh, Morris, & McGinley, 2011). Furthermore, individuals with PD and depression have greater disability compared to those with PD alone (Pontone et al., 2016). Treatment for the depression and anxiety related to PD relies primarily on the selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and norepinephrine reuptake inhibitors (SNRIs) (Pachana et al., 2013). Cognitive behavior therapy has also been shown to have modest benefits (Pachana et al., 2013). For many patients, the anxiety symptoms also correlate with the "off" time in their motor fluctuations. For these patients' adjustment of their dopamine schedule can be very beneficial. PD-related psychosis is also common (Aarsland & Kramberger, 2015), with treatment relying primarily on quetiapine or clozaril due to the prominent extrapyramidal side effects of the other antipsychotics. In addition, the FDA recently approved the novel antipsychotic pimavanserin to treat PD-related psychosis. Pimavanserin is a selective serotonin 5-HT2A inverse agonist without dopaminergic, adrenergic, histaminergic, or muscarinic affinity, therefore potentially leading to fewer side effects (Cummings et al., 2014). Despite these treatment options, the depression, anxiety, and psychosis associated with PD remain debilitating for many patients and new treatments are needed to ease the burden of these symptoms.

Autonomic nervous system changes are also common in later-stage PD (Cersosimo & Benarroch, 2012). For individuals with orthostatic hypotension the usual treatments are midodrine, fludrocortisone, and droxidopa, the latter of which was FDA approved more recently and is specific for treatment of orthostasis in PD. These medications act on the adrenergic receptors, inflammatory cytokines, and peripheral arterial and venous vasoconstriction (Seppi et al., 2011). They are also associated with supine hypertension, though droxidopa is supposed to have less of an instance of that complication. For urinary urgency, frequency, and nocturia, often with accompanying bladder spasms, anticholinergics have been found to be reasonably effective though may have cognitive side effects and some patients may benefit from botulinum toxin injections into the bladder muscle (Giannantoni et al., 2009).

Pain and fatigue are also common in PD. The pain is often due to the motor fluctuations, dyskinesias, off medication times, and central limb pain (Chaudhuri & Schapira, 2009), as well as a neuropathy, which is common in PD (Rajabally & Martey, 2011). The treatment for many of the pain symptoms is therefore adjustment of their levodopa to reduce the fluctuations and total off times, as well as some nighttime carbidopa/levodopa to assist with the pain at night (Chaudhuri & Schapira, 2009). Gabapentin and other neuropathy treatments are also useful for the neuropathic pain. Treatment of the fatigue involves first a comprehensive assessment of the etiology of the fatigue including a good sleep history, as well as an assessment of additional contributors to fatigue including depression and other medical comorbidities such as pulmonary and cardiac function changes. Treatment can then be targeted at the etiology as well as general work toward improvements in endurance.

Challenges to the identification of biomarkers

The lack of disease-modifying therapies is due to both the significant work required to elucidate the pathophysiology of PD and the significant hurdles translating the basic science, pre-clinical and clinical knowledge into effective therapeutics. First and foremost, the phenotypic and likely pathophysiologic heterogeneity of the disease means that a therapy that is potentially efficacious for one subset of the disease is drowned out by its lack of effect on other disease subsets (Lang & Espay, 2018). Importantly, if the potential disease modifying therapy had a large effect on some or all PD pathophysiology pathways, the heterogeneity of the disease would not be as important for determining the efficacy of the therapeutic. In other words, this heterogeneity is an issue because the effect size of the therapeutics tested thus far is either small or nonexistent.

There are also significant challenges related to study design. Since PD is a slowly progressive disease, study designs must follow patients for enough time for them to demonstrate clinical changes. In the absence of a large disease modification or even disease reversal of the therapy, we would anticipate patients in both the therapeutic and placebo arms to be clinically worse at the end than at the beginning of a trial. We therefore need to compare relative differences in disease progression. Different trial designs including a delayed start design have been used to try to overcome this issue, but it remains a challenge in all trials. Again, the known heterogeneity of PD and its rate of progression will complicate this calculation.

Finally, clinical trials rise and fall based on the quality of their outcome measurements. At this time, the primary motor outcome in every PD study is the Unified

Parkinson's Disease Rating Scale (UPDRS) or the more recent Movement Disorder Society-UPDRS (MDS-UPDRS). Both scales rely on patient responses and physician assessments to quantify a patient's non-motor and motor deficits. While there is significant literature validating the tool and an online course that administrators of the tool need to pass, it remains subjective. This subjectivity is made even more challenging in PD by the motor fluctuations of the disease—patients' MDS-UPDRS scores may vary widely within even an hour of testing. Significant research is ongoing into identification of more objective clinical outcomes with the use of wearables and other technologies. Biomarkers would therefore make a fundamental difference in identification of a disease modifying therapy by allowing for the stratification of PD patients into different pathophysiologic categories and would provide objective outcome measurements and measures of medication target engagement.

Biomarkers

A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention." ("Biomarkers and surrogate endpoints: preferred definitions and conceptual framework," 2001). A classic example of a disease biomarker is fasting blood glucose levels for diabetes mellitus—blood glucose levels diagnose diabetes, track disease progression, and monitor response to therapeutics. An ideal biomarker for PD would be similar—it would be an objective measurement that allows us to diagnose PD, follow disease progression, and monitor response the therapeutics. In practice, it is likely that we will need a collection of different compounds for each of

these types of biomarkers. In addition, given the phenotypic heterogeneity of PD, different phenotypes of PD may require different markers. A PD biomarker marker may be biofluid or biochemical, clinical, genetic, or imaging based.

One of the many challenges to biomarker identification is the variability of the methodology used to collect and measure the marker. There are many large research programs within PD that are seeking to address this challenge through the standardization of biofluid acquisition protocols. The Harvard Biomarker Study (HBS), established in 2008 (Mohammadi, 2013), developed a longitudinal biobank of clinical data with associated blood, CSF, RNA, DNA, and ultimately autopsy tissue. Its primary goal is to identify biomarkers for PD and other neurodegenerative diseases. The Michael J. Fox's Foundation's (MJFF) Parkinson's Progression Markers Initiative (PPMI) began in 2010 and has enrolled more than 1400 participants as of January 2019 in the many different subgroups of the study (PPMI). PPMI seeks to identify progression markers of PD and serves as a validation cohort for biomarkers discovered through other research studies, including the HBS, and the newer studies BioFIND and the Parkinson's Disease Biomarker Program (PDBP) (Rosenthal et al., 2016). The BioFIND study, funded by the MJFF and the National Institute of Neurological Diseases and Stroke (NINDS), enrolled individuals with moderate stage PD in a cross sectional, case control study and included blood and CSF collection. The PDBP, also NINDS funded, is a longitudinal biofluid collection from individuals with all stages of PD and controls as well as some atypical parkinsonism patients. Biofluids from each of these investigations are submitted to a central repository and are being used by researchers at numerous institutions to discover and validate new biomarkers.

A detailed review of all assessed biomarkers is beyond the scope of this manuscript and readers are referred to other articles discussing biomarkers including Gwinn et al (Gwinn et al., 2017) and Chen-Plotkin et al (Chen-Plotkin et al., 2018). We will discuss a few of the more promising protein and biofluid-based biomarkers, as these are the potential targets for new therapeutics. The most frequently tested biofluid biomarker in PD is alpha-synuclein, the pathological protein associated with neuronal cell loss in PD. Total alpha-synuclein levels have been found to be lower in the CSF of individuals with PD (Mollenhauer, El-Agnaf, Marcus, Trenkwalder, & Schlossmacher, 2010) though levels do not seem to change with increasing disease severity. Pathological tissue concentration of alpha-synuclein also seems to separate individuals with PD compared to controls. Gastric (Sanchez-Ferro et al., 2015), rectal and colonic (Pouclet et al., 2012) biopsies demonstrated significant concentration of alpha-synuclein in the tissue of individuals with PD compared to controls. Alpha-synuclein has also been found in the skin (Zange, Noack, Hahn, Stenzel, & Lipp, 2015) and peripheral autonomic nerve fibers that innervate the heart (Iwanaga et al., 1999), abdominopelvic organs (Minguez-Castellanos et al., 2007) and the paraspinal sympathetic ganglia and endocrine organs (Beach et al., 2010). In addition, presence of posttranslationally modified alpha-synuclein (p-serine-129) in the CSF (Y. Wang et al., 2012) and skin nerve fibers differentiates between PD and MSA (Zange et al., 2015). Another promising marker for PD is the DJ-1 protein. DJ-1 likely has many roles including inhibiting alpha-synuclein aggregation. Total DJ-1 levels are lower in the CSF of individuals with PD (Hong et al., 2010) and higher total DJ-1 levels were found to correlate with greater disease severity in the saliva of PD patients (Masters, Noyce, Warner, Giovannoni, & Proctor, 2015). However, total

DJ-1 levels in the plasma are not significantly different between those with PD and those with Alzheimer's disease or controls (Shi et al., 2010), limiting DJ-1's utility as a biomarker. Seven DJ-1 post-translationally modified isoforms in the whole blood may be biomarker candidates and further research is forthcoming. Both alpha-synuclein and DJ-1 are also part of protein panels that, when looked at together, had reasonable sensitivity (> 92%) and specificity (up to 60%) for both PD vs controls and PD vs some of the atypical parkinsonisms (sensitivity about 99% and specificity about 90%) (Shi et al., 2011). Other potential markers of PD diagnosis or motor progression include ApoA1 ((Qiang et al., 2013); (Swanson et al., 2015)), Vitamin D (Ding et al., 2013) and urate levels (Ascherio et al., 2009), though none of these markers have held up under significant further scrutiny.

Biochemical markers for PD-dementia include the most common markers for both PD and Alzheimer's disease, specifically amyloid beta, tau and phospho-tau. Lower total alpha-synuclein levels predicted preservation of cognitive function in PD (Stewart et al., 2014). Furthermore, up to one half of individuals with PD-dementia had the biomarker signature of Alzheimer's disease with amyloid-beta42 levels reduced (Montine et al., 2010). Still others have noted that a combination of CSF amyloid beta42 as well as age, REM behavior symptomatology, smell testing, and striatal uptake on DaTScan imaging predicted dementia within two years (Schrag, Siddiqui, Anastasiou, Weintraub, & Schott, 2017). While there are CSF-based markers for vascular injury including most prominently e-selectin and vascular cell adhesion molecule-1 (VCAM-1) (G. Li et al., 2015), neither of these have been tested in the context of PD-related cognitive impairment.

There are many other markers that are undergoing testing and validation in new cohorts. Importantly, none of the markers that we have discussed nor any of these new markers are ready to be used in standard clinical care.

Aberrant proteostasis

The presence of Lewy bodies, intraneuronal and cellular proteinaceous inclusions that are sometimes enriched with ubiquitin, in afflicted regions of the PD brain provides glaring evidence of abnormal protein processing and leads to accumulation of mutant, misfolded or damaged intracellular proteins. Notably, α-synuclein that is prone to form amyloid-like aggregates is a major constituent of Lewy bodies and Lewy neurites in sporadic and inherited PD. Consequently, the aggregation potential of α-synuclein has gained much attention as a possible molecular cause underlying most forms of PD. Supporting this notion are observations that familial PD associated SNCA point mutations, namely A53T, A30P, E46K, and H50Q all increase the propensity of α-synuclein to aggregate *in vitro* (Narhi et al., 1999); (J. Li, Uversky, & Fink, 2001); (Khalaf et al., 2014). SNCA triplications also facilitate the aggregation process, likely as a result of increased protein load and consequent macromolecular crowding (Miller et al., 2004) . Truncated, aggregation prone forms and oligomer forming variants of α-synuclein promote dopaminergic neurotoxicity *in vivo* implicating the aggregated species in pathogenesis (Periquet, Fulga, Myllykangas, Schlossmacher, & Feany, 2007); (Winner et al., 2011). Furthermore, a number of factors presumed to play a contributing role in sporadic PD including oxidative and nitrosative stress accelerate α-synuclein misfolding. Nevertheless, the presence of aggregated α-synuclein by and of itself does not prove if protein misfolding and aggregation exhibit a causal relationship with neuronal death or

represent a secondary step in the course of the disease. In fact, while overexpression of wild-type or mutant α-synuclein produces neurological defects in mice and rats, none of these models recapitulate the entire spectrum of PD phenotypes. Furthermore, Lewy bodies are not common to all genetic forms of PD. While mutations in GBA are associated with Lewy bodies and most cases of LRRK2 have α-synuclein inclusions, some LRRK2 mutations are devoid of Lewy pathology. For as many cases of parkin with α-synuclein inclusions, an equal number without Lewy pathology have been reported. Accumulating evidence indicates that α-synuclein oligomers can themselves have detrimental effects on various physiological functions that culminate in neurotoxicity. While the underlying mechanisms of aggregate toxicity are poorly understood, one possibility is that α-synuclein aggregates composed of more heterogeneous oligomers may expose flexible hydrophobic surfaces that promote aberrant interactions with other cellular proteins resulting in their sequestration and functional impairment (Campioni et al., 2010); (Bolognesi et al., 2010). Such inhibition of central protein quality control and clearance mechanisms can lead to further propagation of folding defects and set forth feed-forward mechanisms of further neuronal injury. Oligomeric intermediates of α-synuclein can also compromise the integrity of various membrane structures in the cell and cause neurotoxicity through pore-like membrane permeabilization (Kayed et al., 2004) or by destabilization of the membrane allowing nonspecific ion-transport (Danzer et al., 2007). Thus, disruptions in basic cellular functions that interface with the unique biology of dopaminergic neurons could result in multifactorial toxicity with prolonged periods of such alterations eventually causing neuronal death.

How α-synuclein aggregation associated proteotoxicity dominates clearance mechanisms and impinges on neuronal survival is an important question to explore, particularly when not one but three protein quality control pathways, namely ubiquitin proteasome system (UPS), chaperone-mediated autophagy (CMA) and macroautophagy are involved in regulating α-synuclein levels. Monomeric α-synuclein is actively degraded by all of these pathways that also compensate each other to maintain steady-state levels of α-synuclein (Kaushik, Massey, Mizushima, & Cuervo, 2008); (Koga, Martinez-Vicente, Macian, Verkhusha, & Cuervo, 2011); (Massey, Kaushik, Sovak, Kiffin, & Cuervo, 2006)). However, oligomers and aggregates are primarily degraded via macroautophagy. Overexpression of wildtype α-synuclein also impairs macroautophagy by inhibiting autophagosome biogenesis in a Rab1a dependent manner (Winslow et al., 2010). Such reduction in autophagy, a major route for clearance of aggregation-prone intracytoplasmic proteins could subsequently increase the cellular concentration of such proteins thereby augmenting their probability of aggregation. In fact, α-synuclein induces fibrillation of tau, which then promotes synergistic fibrillation of both proteins (Giasson et al., 2003) indicating that increased α-synuclein load could serve as a template and initiate feed-forward cycles of indiscriminate aggregate formation. (Tau, of course, is one of the pathologic proteins found in AD, thus demonstrating a pathologic link between the two diseases and explaining some of the reason for the prominence of AD pathology among individuals with PD (Irwin et al., 2013)). Given the prominent role macroautophagy plays in the clearance of dysfunctional mitochondria (a process referred to as mitophagy) (reviewed in (Ryan, Hoek, Fon, & Wade-Martins, 2015)), α-synuclein mediated inhibition of macroautophagy could have far reaching effects on mitochondrial

quality control and increase neuronal susceptibility to proapoptotic insults, all of which are implicated as pathogenic processes in PD.

Post-translational modifications of PD linked proteins also appear to impact its autophagic clearance. In the case of α-synuclein, PLK2 kinase mediated phosphorylation of α-synuclein at (S129) facilitates its autophagic clearance (Inglis et al., 2009); (Oueslati, Schneider, Aebischer, & Lashuel, 2013)) while phosphorylation of α-synuclein at tyrosine 39 (Y39) by the tyrosine kinase, c-Abl increases its propensity to aggregate (Mahul-Mellier et al., 2014); (Brahmachari et al., 2016). Of note, phospho-Y39 α-synuclein accumulation is observed in substrantia nigra and striatum in postmortem PD brains as well as in Lewy bodies of PD patients. Moreover, c-Abl overexpression in wild type mice leads to dopamine neuron degeneration with concomitant elevation of phospho-Y39 α-synuclein and pathogenic α-synuclein accumulation. While c-Abl overexpression accelerates behavioral abnormalities and pathology of human A53T transgenic mice, these defects are ameliorated by c-Abl knockout, indicative of the pathogenic contribution of c-Abl to α-synuclein neurodegeneration (Brahmachari et al., 2016). Various oxidative, nitrosative and dopaminergic stressors implicated in PD are known to activate c-Abl (Sun et al., 2000); (Ko et al., 2010); (Imam et al., 2011)) and could in part explain the nigrostriatal neuronal injury and pathogenic process underlying sporadic forms of PD. These studies indicate that selective inhibition of c-Abl could be neuroprotective. Indeed, the brain penetrant c-Abl inhibitor nilotinib ameliorates striatal motor deficits in a MPTP mouse model of PD (Tanabe et al., 2014) and dopamine neuron loss in a viral mouse model of α-synuclein toxicity (Hebron, Lonskaya, & Moussa, 2013) and human studies of nilotinib are ongoing as described below. Early neuroinflammatory

responses to α-synuclein also appear to be modulated by c-Abl inhibitors (Hebron et al., 2014). A major caveat to be considered with these studies is that the inhibitors used are non-specific kinase inhibitors with broad activity profiles towards a wide range of kinases and are thus likely to have unforeseen side effects with substantial toxicity. The presence of markers of c-Abl activation like phospho-Y39 α-synuclein in the PD brain nevertheless, provide a rationale for developing specific c-Abl inhibitors with better safety records. By preventing α-synuclein Y39 phosphorylation and its propensity to aggregate, therapeutics based on c-Abl inhibition could promote synuclein clearance and thereby counteract toxicity associated with synucleinopathies. In a parallel pathway, stress-induced activation of c-Abl also has an inhibitory effect on the multifunctional E3 ligase parkin (Ko et al., 2010); (Imam et al., 2011), mutations in which are the most common cause of autosomal recessive PD (Khan et al., 2003). Tyrosine phosphorylation of parkin by c-Abl inactivates a catalytic function of parkin resulting in toxic accumulation of parkin substrates like aminoacyl-tRNA synthetase-interacting multifunctional protein type 2 (AIMP2) (Y. Lee et al., 2013), fuse-binding protein 1 (FBP1) (Ko, Kim, Sriram, Dawson, & Dawson, 2006); (Ko et al., 2010)) and PARIS (Parkin Interacting Substrate) (Shin et al., 2011)) that cause dopaminergic neurodegeneration. Of note, PARIS and AIMP2 accumulate in familial PD with parkin mutations, sporadic PD, conditional parkin knockout mice and MPTP intoxicated mice and are thus pathologically relevant parkin substrates (Y. Lee et al., 2013); (Shin et al., 2011)). Thus, modifying the phosphorylation status of parkin by interfering with c-Abl activation provides unique opportunities to maintain parkin in a catalytically active state. In this regard, c-Abl inhibitors Imatinib, INNO-406 have an effect towards reducing

tyrosine phosphorylation of parkin and suppressing upregulation of parkin substrates like FBP1 and AIMP2 thereby slowing the progression of PD (Imam et al., 2011); (Ko et al., 2010); (Imam et al., 2013). Other c-Abl inhibitors like Nilotinib reduce c-Abl activation and levels of PARIS in a MPTP-induced model of PD (Karuppagounder et al., 2014). In other words, blocking or stopping c-Abl activation has significant impact on a number of pathways that together may slow the progression of PD. Nilotinib, imatinib, and other c-Abl inhibitors are already FDA approved for the treatment of acute lymphocytic leukemia and other disorders with Philadelphia chromosome translocations. Given their potentially promising mechanism of action, studies are currently underway to test the c-Abl inhibitors in PD patients. A small, open labeled study of nilotinib in PD showed both a reasonable clinical response and a reduction in α-synuclein levels in the CSF (Pagan et al., 2016). The pilot data also indicated that nilotinib had the potential to be safe in this population. There are now two randomized controlled trials of nilotinib vs placebo in PD patients to determine the safety and potential efficacy of this medication. One study seeks to enroll 75 individuals at a single site and is currently enrolling. The other study is also underway and is a collaboration of the Michael J. Fox Foundation, the Van Andel Research Institute, and the Cure Parkinson's Trust (ClinicalTrials.gov). In addition, as discussed above, development of more targeted c-Abl inhibitors would facilitate further explorations as to the utility of this pathway as potential disease-modifying therapy in PD.

Other components of the c-Abl pathway also provide potential therapeutic targets. The elevation of AIMP2 activates poly-ADP-ribose polymerase 1 (PARP1), leading to an increase in the poly-ADP-ribose (PAR) polymer. This activation of PARP1 and

subsequently PAR, leads to the accumulation of significantly more toxic alpha-synuclein oligomers. Moreover, PARP1 inhibitors stopped the accumulation of this toxic alpha-synuclein in a mouse PD model (Kam et al., 2018). PARP1 inhibitors are also FDA approved treatments for a variety of gynecological cancers and are undergoing testing for other cancers as well. Furthermore, if markers of c-Abl activation for instance, Y245 of c-Abl, Y39 of α-synuclein, Y143 of parkin, and PAR levels are detectable in biological fluids like the CSF of PD patients, they could be utilized as biomarkers of disease progression as well as to monitor the efficacy of c-Abl inhibition and PARP1 inhibition-based treatments (Brahmachari et al., 2016). See Figure 1 for schematic of c-Abl pathway.

Intercellular transmissibility of α-Synuclein

Accumulating evidence indicates that many neurodegenerative diseases involve cell-to-cell spreading of disease related proteins that form the hallmark lesions in their respective neurodegenerative disorders (Guo & Lee, 2014). While such lesions were initially thought to arise in a cell-autonomous manner and confined to selectively vulnerable brain regions, the notion that a prion-like mechanism could underlie the intercellular transmissibility of such proteins is gaining momentum. This hypothesis also fits nicely with the Braak model of PD progression (Braak et al., 2003). In the Braak model, abnormal α-synuclein forms Lewy bodies in specific, susceptible neuronal types, beginning in the peripheral and enteric nervous system and moving caudally through the brainstem, midbrain, and then eventually the cortex. Indeed, neuropathological

examination of α-synuclein aggregates in postmortem PD brain tissues indicate the presence of Lewy bodies in several other regions of the brain apart from the nigra (Surmeier & Sulzer, 2013), implying that the pathogenic changes spread within the brain as the disease progresses, affecting multiple functional networks. Several recent studies have implicated the cell-to-cell spread of α-synuclein likely via the extracellular milieu as a potential mechanism underlying such a pathological progression. This spread of the pathologic protein may go between vulnerable cell populations or between loci that are connected as part of the neuronal circuitry. Consistent with the impairment of multiple neural circuitry during the disease process, PD patients describe constipation and REM behavioral disorder that predate their motor symptoms and localize to the brainstem. Following onset of motor symptoms due to substantia nigra involvement, patients may subsequently develop cognitive impairment in association with cortical involvement (Jankovic, 2008).

Even though α-synuclein is abundant in neuronal cytoplasm, a small amount of α-synuclein is, for unknown reasons, constitutively released from neuronal cells. In humans, nanomolar concentrations of α-synuclein is detected in the blood plasma, brain interstitial fluid and CSF (El-Agnaf et al., 2003); (El-Agnaf et al., 2006). A fraction of cellular α-synuclein is partitioned into cytosolic vesicles and secreted through an unconventional exocytic pathway even from healthy mammalian cells and primary neurons in culture (H. J. Lee, Patel, & Lee, 2005). Intriguingly, α-synuclein secretion is increased under various stress conditions including proteasomal, mitochondrial, lysosomal dysfunctions and oxidative stress (Jang et al., 2010); (H. J. Lee et al., 2011); (H. J. Lee et al., 2013). Under such conditions, vesicular translocation of α-synuclein is

also increased and vesicular α-synuclein appears to be more prone to aggregation than cytosolic α-synuclein (Jang et al., 2010); (H. J. Lee et al., 2005). Consequently, much of the α-synuclein secreted from cells under stress conditions is in oligomeric forms.

Recent work also demonstrates that aspects of α-synuclein transmission and aggregation can be recapitulated in murine model systems by exogenous introduction of pathological α-synuclein derived from diseased tissues or *in vitro* generated preformed fibrils (PFFs) of α-synuclein (Luk et al., 2012); (Volpicelli-Daley et al., 2011); (Osterberg et al., 2015); (Jones et al., 2015). The presence of miniscule quantities of aggregated or fibrillar α-synuclein in these scenarios has been demonstrated to serve as nucleation sites that seed the aggregation of endogenous α-synuclein, and this aggregation spreads along synaptically connected pathways likely through sequential events of exocytosis and endocytosis, reminiscent of a prion-like mechanism. Moreover, embryonic mesencephalic neurons grafted into the neostriatum of PD patients also develop α-synuclein positive Lewy bodies and Lewy neurites that are associated with functional decline of the grafted dopaminergic neurons (Kordower, Chu, Hauser, Freeman, & Olanow, 2008); (Kordower, Chu, Hauser, Olanow, & Freeman, 2008); (Hely, Reid, Adena, Halliday, & Morris, 2008), indicating that cell-cell propagation and misfolding of α-synuclein could underlie the CNS spread of Lewy bodies and Lewy neurites.

The emergence of α-synuclein transmission hypothesis has provided a viable explanation for the stereotypical spreading of neuropathology and progressive deterioration of multiple functional networks in PD. However, there are still a number of open-ended questions that should be carefully considered to be able to evaluate the

applicability of a prion-like mechanism to the underlying disease process. One of the more elusive challenges for this transmission hypothesis is to define the seed. In PD research, the presence of α-synuclein containing seed has been empirically defined by showing that extracts from pathogenic tissues or pre-aggregated forms of *in vitro* generated α-synuclein catalyze aggregate formation (Golde, Borchelt, Giasson, & Lewis, 2013). However, *in vitro* generated variants of synthetic α-synuclein fibrils may not necessarily be identical to fibrils formed in the human brain which limits the establishment of a convincing link between aggregate forms with 'seeding' potential and disease pathogenesis. A recent study reported the existence of two different morphologically distinct oligomeric α-synuclein aggregates in post-mortem PD brain tissues (Xin et al., 2015). Distinct α-synuclein strains that differ in their conformation and activity have also been observed to differ in their propensity to cross-seed tau aggregation (Guo et al., 2013), implying that conformational variants of α-synuclein can possess varied biological activities and thus, tremendous heterogeneity likely underlie synucleinopathies. Future studies should therefore be aimed at isolating the pathogenic protein species originating from the diseased brain to identify the pathogenic species of α-synuclein. In this regard, protein misfolding cyclic amplification (PMCA), a biochemical diagnostic procedure was recently demonstrated to be highly sensitive in its detection of α-synuclein oligomers in biological fluids of PD patients (Shahnawaz et al., 2017). Intriguingly, PMCA has been employed for detection of prions in biological fluids (Saborio, Permanne, & Soto, 2001); (Saa, Castilla, & Soto, 2006) and has also been used to gain mechanistic understanding of factors involved in prion transmission (Morales, Duran-Aniotz, Diaz-Espinoza, Camacho, & Soto, 2012). Seeding competent β-amyloid

oligomers have also been detected with high precision in the CSF of AD patients using PMCA (Salvadores, Shahnawaz, Scarpini, Tagliavini, & Soto, 2014). The PMCA platform adapted for detection of α-synuclein appears to be rather specific in its detection (Shahnawaz et al., 2017) which raises the possibility that techniques like these could facilitate identification of the pathogenic α-synuclein species that could then be subjected to detailed biochemical and biophysical analyses to characterize their transmission properties. Notably, the feasibility of detecting α-synuclein in PD biological fluids highlights the utility of diagnostic procedures like PMCA in monitoring disease progression as well as facilitate preclinical identification of patients likely to develop PD.

Studies of the transmissible property of α-synuclein have important therapeutic implications. Cell-based replacement therapies to replace degenerating dopamine neurons are emerging strategies to restore dopamine delivery and abate the debilitating symptoms in PD. However, findings from the transmission studies indicate that these approaches when used alone may not be successful as the implanted tissues eventually develop Lewy pathology owing to synuclein transmission from host tissues. The prevalence of extracellular α-synuclein raises the possibility that strategies that promote enhanced clearance of extracellular α-synuclein could be beneficial. In fact, the neuroprotective effects observed with α-synuclein immunotherapy (J. S. Lee & Lee, 2016) suggest that this would be a viable option to curb the intercellular transmission of α-synuclein and numerous alpha-synuclein vaccine studies are underway, though none have reported their safety and efficacy at this time. However, careful consideration is required to determine if diverse pathological strains of α-synuclein exist which would necessitate extensive screening for antibodies with higher binding affinity and specificity for distinct

pathogenic strains. In this regard, broader applicability can be achieved with antibodies that recognize shared conformations of multiple strains, especially those with cross-seeding potential (Guo & Lee, 2014). Studies on Aβ passive immunotherapy show that only 0.1% of circulating antibodies manage to cross the blood-brain barrier (BBB) and reach the brain (Yu & Watts, 2013). Therefore, considerable efforts should be directed at devising strategies that promote uptake of antibodies across the BBB to maximize therapeutic effects.

Additional environmental contributors to disease progression

Dietary factors and toxin exposures are also associated with modulating disease progression. There has been significant evidence showing that higher caffeine intake is associated with decreased risk of PD development (Hamza et al., 2011; Popat et al., 2011) and that caffeine intake helps individuals with their PD symptoms, likely due to the caffeine targeting the adenosine receptor. Elevated Vitamin D levels have also been found to be associated with PD development and a number of lines of research support these findings. Researchers have found that 1) there is an increased expression of the Vitamin D Receptor gene among individuals with PD (Scherzer et al., 2007), 2) 25-hydroxy Vitamin D3 reduces 6-hydroxydopamine induced neurotoxicity (J. Y. Wang et al., 2001), and 3) there are lower levels of total Vitamin D and Vitamin D metabolites among individuals with PD (Ding et al., 2013). Total Vitamin D is a combination of 25-hydroxy-vitamin D2 (25[OH]D2) and 25-hydroxy-vitamin D3 (25[OH]D3). While Vitamin D2 is obtained from exogenous sources, most frequently in the U.S. from fortified milk and cereal, Vitamin D3 is endogenous and obtained from conversion of

cholesterol in the skin following sun exposure. Vitamin D3 levels are thought to be the primary driver of the negative association between Vitamin D levels and PD development and severity, further underscoring the importance of sun exposure among individuals with PD and/or Vitamin D supplementation (Ding et al., 2013).

Nicotine may also change PD risk with some research suggesting that nicotine use is protective (Ma, Liu, Neumann, & Gao, 2017). To that end, there is an ongoing study of transdermal nicotine patch as a disease modifying therapy in PD (ClinicalTrials.gov) to determine if individuals who are randomized to receive the nicotine patch will have slower disease progression. Alternatively, others postulate that individuals who are in the prodromal phase of PD and are going to go on to develop PD are more risk averse or maybe even less sensitive to tobacco's effects, therefore making them less likely to smoke (or be able to quit easier) (Ritz, Lee, Lassen, & Arah, 2014). Overall, given the well-proven risks of smoking tobacco, cigarette smoking is not a good method of PD disease prevention.

There are other exposures that have been shown to be toxic and accelerate disease development. One of the more clinically important toxins is Agent Orange (2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) along with contaminant 2,3,7,8-TCDD). Agent Orange was used extensively by US forces during the Vietnam War as part of a wider crop destruction efforts believed to benefit US war goals. Agent Orange exposure has subsequently been linked to the development of numerous diseases, including likely increased risk of PD. One large multicenter case-control study found a more than 2 fold increased odds of PD among those exposed to Agent Orange (Tanner et al., 2009) but other studies found no association (Kamel et al.,

2007). The exact mechanism of neurotoxicity of the chemicals in Agent Orange has not been worked out in great detail but it is believed that 2,4-D inhibits microtubule assembly and TCDD may increase oxidative stress. Importantly for patient the Veterans Administration lists PD on the list of diseases due to Agent Orange exposure and therefore patients are eligible for increased VA benefits.

There are numerous other toxins that have been shown to be associated with PD including MPTP, paraquat, and rotenone. Polychlorinated biphenyls used as lubricants and coolants have also been associated with PD as has specific solvents. Please refer to the review by Goldman (2014) for further discussion of environmental exposures contributing to PD (Goldman, 2014).

Conclusion

While described by James Parkinson's as a single disease, PD and the dementia that often develops in conjunction with the disease, is a clinically and pathophysiologically variable disease. Bridging the connection between the underlying pathophysiology and the clinical heterogeneity is a necessary next step toward the development of disease modifying therapies. Biomarkers will help us toward this goal by informing clinical trials but identification of better biomarkers will also be informed by our growing understanding of the different disease mechanisms. Aberrant α-synuclein is emerging as a common endpoint to a number of different diseases pathophysiologies and the inciting factor toward others, including inflammatory pathways and increased stress on the neuron. The c-Abl pathway may be of particular importance due to its role in both sporadic and genetic PD and possible PDD and due to the large number of c-Abl and

PARP1 inhibitors that are both FDA approved and in development. Finally, genetic and environmental influences overlay and contribute to all of the pathophysiologic changes in ways that we are still investigating.

The profound pathophysiologic heterogeneity is reflected in the phenotypic heterogeneity of the disease. Our patients have variable symptoms ranging from bothersome tremors to severe rigidity to significant changes in balance to profound and relatively rapid cognitive impairment versus only a mild executive dysfunction. Determining the degree to which these different phenotypes are reflective of specific underlying pathophysiologies is unknown and would be incredibly instructive toward disease modifying therapies. The next two chapters will work toward this goal by first, determining if PAR levels in the CSF are related to motor or cognitive decline in PD and then by determining whether vascular pathology contributes to cognitive impairment in PD.

II: Poly(ADP) ribose (PAR) is a possible progression marker for PD and PD-dementia

Introduction

A biomarker for Parkinson's disease (PD) cognitive progression is urgently needed to support the development of disease modifying therapeutics. The dementia that develops in patients with PD is a devastating and common non-motor symptom of PD, with more than 80% of individuals developing PD-dementia (PDD) by 15 years after diagnosis (Aarsland et al., 2003). Individuals with PDD have significantly greater morbidity and mortality than those with PD alone. Current therapeutic options for both PD and PDD have limited efficacy and do not change disease course. An ideal biomarker is part of the pathophysiology of PD, thus confirming its relevance, and has known therapeutics that block its pathologic activity.

The parthanatos cell death pathway is a regulated cell death mechanism that has been implicated as a critical pathway in PD pathophysiology (Kam et al., 2018; Y. Lee et al., 2013). The pathway likely begins with abnormal alpha-synuclein strains entering the neuron, which in turn leads to both a direct and indirect activation of PARP1. This double activation of PARP1 leads to an increase in PAR, which subsequently results in DNA fragmentation and dopamine neuron cell death (Kam et al., 2018). PARP1 inhibitors are currently FDA approved for a number of gynecologic cancers and currently in Phase II studies for diseases as diverse as prostate cancer (ClinicalTrials.gov) and stroke (ClinicalTrials.gov) and have been considered for testing in PD (Martire, Mosca, & d'Erme, 2015; Scott, Dawson, & Dawson, 2017). Furthermore, we have previously shown that cerebrospinal fluid (CSF) PAR levels are different between PD and control patients in two separate cohorts (Kam et al., 2018).

We now further develop the role of PAR as a potential biomarker to look at its behavior over time and ability to predict disease change. Specifically, we hypothesize that CSF PAR levels 1) continue to separate PD and control participants over time, 2) PAR levels at baseline and over time predict cognitive decline, and 3) PAR levels predict cognitive progression.

Methods

Study population

This study was approved by the Johns Hopkins Institutional Review Board. We utilized clinical data and CSF from the Johns Hopkins site of the NINDS Parkinson's Disease Biomarker Program (PDBP) (Gwinn et al., 2017; Rosenthal et al., 2016). The PDBP is a consortium of sites investigating a biomarker for PD, with each site completing common assessments but each investigator having control over additional data collected and biomarkers investigated. For the JHU PDBP, individuals diagnosed with PD and controls were recruited from numerous sources including existing longitudinal, observational investigations and the movement disorder clinics at Johns Hopkins and affiliated neurologists. Controls were further recruited through asking the spouses of PD participants. All PD patients met UK Brain Bank criteria for diagnosis of PD and were taking medication to treat the symptoms of PD at the time of enrollment. Control participants must have been cognitively normal at the time of enrollment (Montreal Cognitive Assessment (MoCA) score of equal to or greater than 26) and did not have a first-degree relative with parkinsonism. Efforts were made to ensure that control and PD participants were similar in age and education levels, but the control

participants were not explicitly age-matched. All participants had to agree to an annual lumbar puncture and therefore control and PD participants for whom a lumbar puncture could not be safely obtained were excluded from the investigation. We did continue to follow individuals who opted out of subsequent lumbar punctures, became ineligible to undergo a lumbar puncture due to anticoagulation therapy, and those whose lumbar punctures we were unable to obtain.

Clinical diagnosis was checked at each follow up visit and individuals who no longer fit diagnostic criteria for PD were excluded from this analysis (n=2, 1 individual was subsequently diagnosed with Progressive Supranuclear Palsy-Parkinson's subtype and 1 individual has concomitant hydrocephalus). Enrollment began in 2012 and was completed in 2017 and follow up ended in August of 2018. Between 2012 and August of 2017, visits were every 6 months and then participants underwent a final annual visit during the last year of the investigation. Throughout the study the annual visits incorporated detailed assessments of cognition, psychiatric symptomatology, as well as motor evaluations and review of medications, smell function, and family history. Participants also underwent their annual lumbar puncture in conjunction with this visit as well as a blood draw and urine collection. The 6-month visits included review of medication and primarily motor testing as well as a blood draw and urine collection. Since lumbar punctures and comprehensive cognitive testing were obtained annually, this investigation includes solely data obtained at the annual visits.

Outcome variable: Cognitive diagnosis

At each annual visit participants underwent approximately 80 minutes of cognitive testing, which included two tests in each of five cognitive domains. Each participants' cognitive testing was normed to age and education standards. In addition, each participant identified a study partner that was willing and able to report on the participants cognitive and functional state. This informant underwent a structured interview that allowed us to determine a Clinical Dementia Rating (CDR) Scale score. This CDR was modified from what is typically used in an Alzheimer's Disease population to account for functional difficulties due to motor impairment such that we could focus on any challenges the participant may have due to cognitive impairment. The cognitive diagnosis was subsequently based on consensus conference, during which the movement disorder physicians, clinical psychologists, nurses and research coordinators in attendance at each meeting considered both the testing data and the information obtained from the informant to stratify the participant's cognition into normal, mild cognitive impairment, and dementia (Cholerton et al., 2013). We evaluated PAR levels as a predictor for change between strata, as well as a predictor of development of any form of cognitive impairment and development of dementia. Participants were considered to have converted to MCI or dementia either following consistent stratification to that new level. If the conversion to the new strata occurred at their last follow up visit, they were also considered to have converted.

Explanatory variables: PAR assay

The PAR polymer assay is a sandwich ELISA. Two different clones of monoclonal anti- PAR antibody were used, with the first antibody coated on the 96-well

microtiter plate and the biotinylated anti- PAR antibody added after the PD patient and control patient CSF were added. Following incubation, the color change was detected with the HRP-conjugated streptavidin saturated at 50 nM.

Additional variables

Demographic and disease-specific variables were stratified to improve interpretation of results. Education was divided into 5 categories (completed high school, some college, completed college, completed post-graduate degree) and race was divided into Caucasian and Persons of Color. Levodopa Equivalent Dosing for each participant was computed according to Tomlinson et al. (Tomlinson et al., 2010). Disease duration was computed as age at visit 1 minus age at diagnosis. Depression symptomatology was treated as a continuous variable based on Hamilton Depression Scale score and anxiety symptomatology was also treated as a continuous variable based on Hamilton Anxiety Scale score.

Statistical analysis

We first looked for baseline demographic differences in our PD versus control participants and between the cognitive strata in our PD participants to inform which variables we needed to consider to statistically control for in addition to the variables that are part of the known pathophysiology of our biomarker. We further assessed the behavior of each of our variables with stem plots and subsequent Q-Q plots, allowing us to confirm a normal distribution of our data. Patterns of missingness were evaluated with regards to both individuals who were lost to follow up by comparing historical trends in

clinical data and the behavior of our cohort. Patterns of missingness were also considered for individuals for whom we continued to follow but were unable to obtain CSF PAR levels by using student t-tests to evaluate demographic and clinical differences.

To address question 1: Student t-test were used to compare PAR levels at each visit both between PD and controls and between the cognitive strata of our PD cohort. To address question 2: To determine whether PAR levels at visit 1 predict cognitive decline, we first determined whether PAR levels were different at baseline and follow up visits between individuals with PD and normal cognition and those with PD and dementia. We subsequently sought to determine whether PAR levels at Visit 1 predicted decline by establishing two logistic regressions, each of which controlled for age, gender, race, education, cognition at baseline, PD-disease duration, depression and anxiety symptomatology and follow up time. A multinomial logistic regression used all three cognitive strata as its outcome and a logistic regression used cognitive progression to dementia as its outcome. To determine whether PAR levels vary over time consistent with changes in cognition, the same logistic regressions were set up with the outcome variable being cognitive change and the explanatory variables including both PAR at visit 1 and PAR levels at each visit. To address question 3: We defined individuals whose cognitive status progressed as those whose consensus conference diagnosis was consistently one or two steps higher than their baseline (ie for individuals who started as PD with normal cognition, cognitive status progressed if the individuals was diagnosed with PD-mild cognitive impairment for two visits) or someone who was diagnosed with the next step higher on the last research visit. Individuals who were diagnosed with dementia at the baseline visit were excluded from this analysis. For both question 2 and

3, our analysis took into account intra-subject correlations of repeated measures. Stata 14 was used for all statistical analyses and significance was set at <0.05.

Results

Demographics

Our PD and control participants were well matched with regards to age, race, and education levels (Table 1). Our PD patients were more likely to be men and had slightly higher depression and anxiety symptomatology. As expected, the cohorts also differed with regards to their PD-based motor symptoms and baseline cognitive status. We also compared the characteristics of our PD cohort, stratified by cognitive diagnosis at baseline. Individuals who were demented at baseline were more likely to be older, male and have a longer disease duration than individuals with MCI or normal cognition (Table 2).

Patterns of missingness indicate that those who were lost to follow up were clinically more advanced than those who remained in the investigation. There were no differences between those for whom CSF PAR values were obtained and those for whom we were unable to obtain CSF PAR levels (data in appendix).

Cognitive change

At baseline, we had 25 individuals with PD-normal cognition, 45 individuals with PD-MCI and 15 individuals with PDD. Among the 25 individuals who began with normal cognition, 8 of them converted to PD-MCI during the course of the investigation and 1 individual converted to PD-MCI and then PDD. Among the 45 individuals who

began the investigation with PD-MCI, 17 converted to PDD during the course of the investigation. Those who converted their cognitive status differed from those who did with regards to their age, disease duration, Movement Disorder Society – Unified Parkinson's Disease Rating Scale (MDS-UPDRS) total scores, baseline Montreal Cognitive Assessment (MoCA) scores, and disease duration ($p< 0.05$). (Table 3).

Question 1: PAR levels at each visit

PAR levels at visit 1 were not significantly different between PD and controls (PD 109.49 (53.51), Control 88.51(49.42), $p=0.06$). These levels are different than previously published (Kam et al., 2018) due to the exclusion of the two individuals that no longer meet UKBB criteria. PAR levels at visit 2-4 were different between PD and controls (Visit 2: PD 148.28 (76.93), Control 103.36 (52.98), $p<0.01$; Visit 3: PD 132.27 (76.15), Control 85.33 (52.36), $p=0.01$; Visit 4: 157.25 (56.98) Control 96.91 (40.13), $p<0.01$)(Figure 2). In addition, levels among our control population remain relatively stable between visit 1 and visit 3 and visit 4, whereas PAR levels among our PD population increase over that follow up time.

We also compared PAR levels at each visit between those with PD-normal cognition, PD-MCI and PDD. There was a significant difference between PAR levels at visit 1 between the cognitive strata (PD-normal cognition PAR levels 132.92 (52.96), PD-MCI PAR levels 104.46 (48.17), PDD PAR levels 83.72 (56.78), $p=0.01$) but no difference between PAR levels at the other visits (Visit 2: PD-normal cognition PAR levels 155.46 (64.75), PD-MCI PAR levels 132.28 (79.62), PDD PAR levels 170.34 (79.58), $p=0.23$; Visit 3: PD-normal cognition PAR levels 143.82 (55.08), PD-MCI PAR

levels 125.56 (75.36), PDD PAR levels 146.09 (102.73), p=0.71; Visit 4: PD-normal cognition PAR levels 133.57 (28.36), PD-MCI PAR levels 163.81 (56.86), PDD PAR levels 168.74 (76.80), p=0.45). If we evaluate PAR levels at each visit with the patients stratified according to their baseline cognition, it is again only visit 1 that demonstrates a significant relationship (Visit 1, as above; Visit 2: PD-normal cognition PAR levels 135.86 (52.84), PD-MCI PAR levels 148.45 (85.92), PDD PAR levels 172.53 (84.56), p=0.48; Visit 3: PD-normal cognition PAR levels 119.96 (62.34), PD-MCI PAR levels 144.33 (83.10), PDD PAR levels 77.31 (44.30), p=0.26; Visit 4: PD-normal cognition PAR levels 146.13 (26.18), PD-MCI PAR levels 156.96 (65.10), PDD PAR levels 273.18 (n=1, no SD), p=0.10).

Question 2: PAR at Visit 1 as a predictor of cognitive decline

In a simple multinomial logistic regression, for every 10 units increase in PAR levels at Visit 1 there is a 12% (95% CI -21% to -1%) reduction in the odds of a diagnosis of dementia compared to normal cognition (p=0.03). The diagnosis of normal cognition to PD-MCI was not significant (1% reduction in odds, 95% CI -8% to +7%, p=0.87). Controlling for MoCA levels at visit 1, total levodopa equivalent daily dosing, disease duration, age, gender, education, race and depression symptomatology continued to demonstrate a significant relationship between increased PAR levels at visit 1 and reduction in the odds of the diagnosis of PDD versus normal cognition (for every 10 units increase in PAR levels at Visit 1, there is a 29% reduction in the odds of diagnosis to

dementia from normal cognition, 95% CI -45% to -7%, p=0.01). Controlling for anxiety, eliminated the significance of this relationship (for every 10 units increase in PAR levels at Visit 1, there is a 21% reduction in the odds of diagnosis of dementia versus normal cognition, 95% CI -39% – 3%, p=0.08).

When taking into account PAR levels at each visit (instead of just Visit 1, as above), the relationship between PAR concentration and cognition was similar once we controlled for our independent variables but different at the simple multinomial logistic regression level. That is, in a simple multinomial logistic regression, for every 10 units increase in PAR levels there is a not significant reduction in the odds of a diagnosis of dementia compared to normal cognition (OR < 1%, p=0.94, 95% CI -5% to + 5%). The diagnosis of normal cognition to PD-MCI was also not significant (4% reduction in odds, 95% CI -8% to +1%, p=0.16). Controlling for MoCA levels at visit 1, total levodopa equivalent daily dosing, disease duration, age, gender, education, race and depression symptomatology continued to demonstrate a significant relationship between increased PAR levels and reduction in the odds of the diagnosis of PDD versus normal cognition (for every 10 units increase in PAR levels, there is a 29% reduction in the odds of diagnosis of dementia versus normal cognition, 95% CI -45% to -7%, p=0.01). Additionally controlling for anxiety, eliminated the significance of this relationship (for every 10 units increase in PAR levels, there is a 21% reduction in the odds of diagnosis of dementia versus normal cognition, 95% CI -37% to +2%, p=0.08).

<u>Question 3: PAR as a predictor of cognitive change</u>

We subsequently evaluated whether PAR levels were associated with change in cognition from PD-normal to PD-MCI or PD-MCI to PDD. In both the simple logistic regression and when controlling for the same dependent variables as above, PAR levels at Visit 1 and PAR levels throughout our follow up time did not significantly correlate with change of cognition status. See Table 4 for complete model.

Conclusion

To our knowledge this is the first investigation that tests c-Abl pathway molecules in the CSF to determine if they predict cognitive decline. We have provided evidence that PAR levels separate individuals with PD from controls at most visits after Visit 1, but only separate individuals within different cognitive strata at Visit 1. PAR at Visit 1 may predict a diagnosis of PDD both before and after controlling for a number of variables but PAR levels do not predict change in cognitive status from PD-normal cognition to PD-MCI or PDD.

There is significant preclinical data that highlights the importance of PAR in the pathophysiology of PD as reviewed in Chapter 1. Briefly, poly ADP ribose synthetase activity has been implicated as an important player in DNA damage as early as 1995 (Zhang, Pieper, & Snyder, 1995). Subsequently, significant investigation has demonstrated the importance of PAR and PARP1 in oxidative stress (Virag, 2005), progressive neurodegeneration (Hanai et al., 2004), and in the MPTP-based PD model (Mandir et al., 1999). Most recently, we determined that misfolded alpha-synuclein induces PARP1 activity, thus increasing the levels of PAR and leading to neuronal cell death (Kam et al., 2018). PAR is therefore potentially an ideal marker given its role in PD

pathophysiology and its importance in cell death. Despite this strong data, the CSF PAR levels do not predict cognitive change in this cohort, though this conclusion is mitigated by the overall small number of individuals that exhibited cognitive change during our current follow up period as well as the loss to follow up of more clinically advanced individuals.

The inverse relationship between PAR levels and a diagnosis of dementia implies a complex pattern of PAR concentration over disease course. Based on preclinical data, we would hypothesize that PAR levels would rise earlier in the disease course only to then decrease as the neurons "burn out" and the rate of neurodegeneration slows down. As such, the inverse relationship implies that we are catching our patients on the downward slope of their PAR levels, and thus the stage of the disease where their overall rate of clinical change would be less than previously. In this scenario, the heterogeneity of our cohort would prevent us from identifying this relationship across all of our analyses. The implications of this hypothesis are that those with the lowest PAR levels among the PD cohort are actually the most clinically demented or advanced patients. Subsequent investigations will investigate this hypothesis further as we need a larger cohort to determine the pattern of CSF PAR levels overtime and cross sectionally.

The role and importance of anxiety symptomatology is intriguing. Anxiety among individuals with PD is very common, with a lifetime prevalence as high as 49% reported among individuals with PD (Pontone et al., 2009). Even more common, though, are anxiety symptoms that do not meet the threshold for diagnosis of an anxiety disorder but rather symptomatology that impacts function and quality of life (Pontone et al., 2009). This is certainly the situation in our cohort, where the mean Hamilton Anxiety Scale

score is 10.4 (SD 6.06) even amongst our most anxious group (A score of less than 17 on the Hamilton Anxiety Scale is considered mild anxiety (Hamilton, 1959)). Anxiety is thought to heighten concentration and attention to tasks that are considered threat-related, to the detriment of attention and working memory in other areas (Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013). Among individuals with new-onset PD, those with anxiety were three times more likely to have cognitive impairment (Dissanayaka et al., 2017). The relationship between anxiety and levodopa is also complex—patients frequently report a heightened anxiety state as their levodopa wears off and as the disease worsens, so does anxiety symptomatology (Martinez-Fernandez, Schmitt, Martinez-Martin, & Krack, 2016). In our cohort, anxiety symptomatology, but not depression symptomatology, mitigated the relationship between PAR levels and diagnosis of dementia. This is likely due to the overall worsening of anxiety that occurs in conjunction with increased disease duration and worsening of cognition. More research is needed in this area and in a larger cohort we will be able to explore this complex relationship further.

The search for a biomarker for PD-related cognitive decline has previously included primarily the biomarkers used for Alzheimer's disease (Aasly et al., 2012; Backstrom et al., 2015; Biundo, Weis, & Antonini, 2016; Hall et al., 2012; Kang, 2016; Kang et al., 2016) as well as alpha-synuclein (Shi et al., 2012; Shi et al., 2014; Simonsen et al., 2016). The use of AD-based biomarkers is problematic due to the co-pathology of amyloid beta, tau, and alpha-synuclein observed in approximately 40% of individuals with PD at autopsy and the known potentiation of abnormal tau and abnormal alpha-synuclein with each other (Irwin et al., 2013). The positive AD-based markers therefore

may represent the AD pathology among our patients more than the PD pathology. By contrast, PAR has clearly been shown to be part of the abnormal alpha-synuclein pathway. PAR's role in AD is less well understood and warrants further investigation.

One of the many things that makes PAR intriguing as a biomarker are the availability of PARP1 inhibitors. Three PARP1 inhibitors are currently FDA approved for use in gynecologic cancers and are being tested for use in a wide variety of other cancers as well as diseases as diverse as pulmonary arterial hypertension and as adjunct therapy with tPA in stroke. There are also a number of PARP1 inhibitors that are under development for use in cancer and other diseases. These diseases all feature DNA fragmentation as a common endpoint and as such benefit from PARP1 inhibition. These FDA-approved PARP1 inhibitors also inhibit PARP2 and PARP3 and have various amounts of blood brain barrier penetration, thus implying that some of the PARP1 inhibitors would be more effective than others for a neurodegenerative central nervous system disease. The strong preclinical data for PARP1 inhibition continues to make it an interesting therapeutic despite the less promising results of CSF PAR as a biomarker presented here. Since PARP1 activates PAR, levels are likely responsive to PARP1 activity. Therefore, PAR levels may be a biomarker for target engagement and we have demonstrated the ability to test PAR levels on a reasonably large scale.

This is the first investigation to our knowledge of whether PAR levels in the CSF is a potential biomarker of PDD. Subsequent analysis should evaluate the role of PAR as a marker of motor progression and confirm the results presented herein regarding cognitive progression. Efforts should also be made to evaluate the role of serum PAR as a marker of cognitive and motor decline in PD. While PAR in the CSF is not clearly a

marker for cognitive progression, this investigation is nevertheless an exciting step into furthering our understanding of the utility of PAR as a biomarker of PDD.

III. Vascular burden contributes to the development of PD-dementia

Introduction

Individuals with Parkinson's disease dementia (PDD) have increased mortality above those with Parkinson's disease (PD) alone. In addition, individuals with PDD are less likely to continuing working and more likely to require assisted living. Approximately 80% of individuals with PD will have developed PDD by about 15 years after symptom onset (Aarsland et al., 2003), with many individuals having developed dementia even sooner in their disease course. The pathologic etiology of PDD that has been identified thus far includes individuals with only cortical alpha-synuclein and individuals who have both cortical alpha-synuclein and cortical amyloid and beta. Individuals with these two pathologic types have an earlier mortality compared to individuals with alpha-synuclein pathology alone (Irwin et al., 2017; Irwin et al., 2012).

Vascular pathology has been shown to contribute to dementia in older populations outside of those with PD. Autopsy investigations of older adults demonstrate that those with vascular pathology, including lacunar and larger infarcts (Breteler, 2000) as well as vessel disease and cerebral amyloid angiopathy (Pontes-Neto, Auriel, & Greenberg, 2012), demonstrate increased rates of dementia compared to those without that pathology. In addition, the addition of vascular pathology to underlying Alzheimer's disease (AD) pathology significantly increases the risk of developing dementia. The amyloid and tau that make up AD pathology potentiate the development of vascular pathology and vice versa (Love & Miners, 2016), thus leading to an overall worsening of the individual's cognition. Finally, the relationship is likely reciprocal with mid-life vascular risk factors increasing brain amyloid deposition and development of dementia (Gottesman, Albert, et al., 2017; Gottesman, Schneider, et al., 2017)

Despite the importance of vascular pathology in AD dementia and the development of dementia in general, the contribution of vascular pathology to the development of PDD has not been fully elucidated. Irwin et al showed no contribution of vascular pathology to the development in dementia in alpha-synucleinopathies (Irwin et al., 2012) while others have reported that vascular risk factors worsened cognition and freezing of gait among individuals with PD (Stojkovic et al., 2018). This investigation sought to determine the role of vascular pathology in the disease duration of PDD, and secondarily the role of vascular pathology on the development of PDD. We hypothesized that vascular pathology would shorten the disease duration and that individuals with a heavier burden of vascular pathology would have an even shorter disease duration than those with a more modest vascular burden. We also evaluated whether individuals with vascular pathology in general and those with a heavier vascular burden were more likely to develop PDD. Finally, we compared our cohort to previous investigations by determining whether individuals with Alzheimer's disease-based pathology, specifically amyloid and tau, had both increased odds of dementia or shorter disease duration than those without the vascular pathology.

Methods

Participants

Participants were part of the Morris K. Udall Parkinson's Disease Research Center of Excellence research study. This investigation is approved by the Johns Hopkins School of Medicine Institutional Review Board. The Udall Center enrolls individuals with PD, atypical PD, and controls and obtains autopsies at the time of their death.

Participants included in this analysis participated in the Udall Center's Longitudinal study, which also includes clinical, motor, cognitive, and psychiatric assessments from the time of enrollment and every other year until the time of autopsy. The research investigation began in 1999 and has been ongoing since that time. This analysis included only those individuals who had autopsy-proven PD pathology at the time of their death and used the cognitive, motor, and psychiatric testing from their in-person research visit closest to their time of death.

Cognitive impairment

Dementia was defined according to the criteria laid out by the 2007 Movement Disorder task force (Emre et al., 2007). Briefly, that manuscript states that individuals probable PDD must have the core criteria of first be diagnosed with PD according to UK Brain Bank Criteria, and then also have a dementia syndrome with insidious onset and slow progression. They also must have impairment in more than one cognitive domain that is a decline from premorbid level with cognitive deficits severe enough to impair daily life. Diagnosis may also include behavioral symptoms that support the diagnosis. We operationalized these criteria by dividing the diagnosis into specific categories and then insuring that any participant we defined as dementia met the probable PDD dementia diagnostic criteria. All those that did not meet the definition of dementia were defined as simply not demented. Given the small numbers, we did not differentiate between those with mild cognitive impairment and those with normal cognition. During this process, we did not include reports of cognitive change that occurred within 2 months of death.

Disease duration and other variables

Disease duration was defined as the time from disease diagnosis to death. For the majority of the analyses, disease duration was treated as a continuous variable. We also divided disease duration into quartiles and compared the highest and lowest quartile with respect to the role of vascular pathology increasing the odds of being in the higher or lower disease duration group.

Pathology

Autopsies were conducted by the Division of Neuropathology at Johns Hopkins using the most up-to-date staining and diagnostic criteria at the time of autopsy. Autopsy methods have been described elsewhere (Mills et al., 2016). Briefly, after fixing in 10% buffered formaldehyde tissue blocks were processed, cut at 10-micrometers and stained with H&E. Subsequently, selected sections were silver-stained (Hirano method) and immunostained against phosphorylated anti-Tau (PHF-1)(a gift of Dr. Pater Davies) as part of our efforts to identify AD pathology and alpha-synuclein (Transduction laboratories) as part of our efforts to identify PD pathology. The subsequent determination of pathologic type and severity followed the 2012 McKeith Criteria (McKeith et al., 2017for the Lewy body pathology and we used both CERAD criteria ; Mirra et al., 1991) and Braak levels (Braak, Alafuzoff, Arzberger, Kretzschmar, & Del Tredici, 2006)for the AD-based pathology. For the CERAD criteria, participants were stratified into possible, probable and definite AD contributing to their cognitive change, with additional analysis grouping the probable and definite AD groups together. For the

Braak staging-based analysis, we grouped those with low (Braak stage 0-II), middle (Braak stage III-IV) and high (Braak stage V- VI) neurofibrillary pathology together.

For the vascular pathology, the neuropathologists noted the presence or absence of atherosclerosis and arteriosclerosis, significant obstruction of large vessels, gross evidence of other vascular lesions including aneurysms, infarcts less than and larger than 10 mm, parenchymal hemorrhages, and sometimes vascular lesion location. They also looked specifically at cerebral atherosclerosis, cerebral amyloid angiopathy, and the presence of other microvascular disease or microinfarcts not noted elsewhere. They also determined, along with the other pathologic data and cognitive testing data, whether they believed that vascular lesions contributed to the development of antemortem cognitive impairment. We operationalized these criteria in the following manner: First, we grouped together as a common endpoint any brain that showed vessel obstruction, parenchymal vascular lesions, large or lacunar infarcts, hemorrhages cerebral atherosclerosis, and other microvascular disease and other microvascular infarcts. Presence of any of these pathologies was termed as presence of CVD pathology. We did not include general atherosclerosis since we have a variable for the more specific and relevant cerebral vessel atherosclerosis, nor did we include arteriosclerosis or cerebral amyloid angiopathy (CAA) in this initial analysis. In the case of CAA, we do not have any data on the severity of CAA and the presence of CAA was very common in our cohort. We therefore created an additional composite variable that included everything in our initial cerebrovascular disease positive group plus those who were CAA positive, thus allowing us to consider the contribution and importance of CAA in general in our population both separate from and in combination with other forms of cerebrovascular disease. To

determine cerebrovascular disease severity, individuals were given 1 point for the presence of each of the vascular pathologies we have been calculating (vessel obstruction greater than 50%, parenchymal vascular lesions, large infarcts, lacunar infarcts, hemorrhages, cerebral atherosclerosis, other microvascular disease, other microinfarcts, cerebral amyloid angiopathy) and the total vascular burden was a sum of each person's vascular pathology. This CVD burden score therefore ranged from 0 to 9.

Statistical Analysis

Demographic and pathologic characteristics between individuals with PD pathology versus those with PD and AD pathology were compared using Student's t-test or Chi-squared as appropriate. For this analysis, those with AD pathology were considered to be those with probable or definite AD based on the CERAD criteria. We restricted our initial analysis in which disease duration was our outcome to those with PDD at their last clinical visit. After confirming that disease duration was normally distributed, we subsequently set up a simple linear regression of disease duration and our three forms of vascular pathology (CVD presence, amyloid angiopathy, and CVD burden). With each of these, we subsequently set up an additional linear regression controlling for age, gender, education, and UPDRS motor subscore. We set up the same simple and multiple logistic regression comparing the odds of being in the highest quartile of disease duration to being in the lowest quartile of disease duration. Finally, to evaluate whether vascular disease increased the odds of developing dementia, we set up a simple and then multiple logistic regression looking at each of our vascular disease subtypes and then controlling for the variables discussed above as well as time from last research visit to autopsy.

Results

Demographics

Since 1999, we have had 67 individuals from the Longitudinal Study with sufficient information to confirm cognitive diagnosis come to autopsy with pathologically proven PD. Among those individuals, 56 had confirmed dementia at the time of their last research visit while the remaining 11 had MCI at that time. Among those with PDD at the time of the last research visit, there was no difference in age, gender, race, disease duration, UPDRS motor score, anxiety and depression symptomatology, disease duration, and time from last research visit to autopsy ($p>0.05$) between those with PD pathology (n=31) and those with PD and AD pathology (n=25) (Table 5). AD pathology was defined as possible or definite AD based on CERAD criteria.

We further compared those with PD-MCI versus those with PDD. Individuals with PD-MCI were more less likely to be men (PD-MCI 27.28% men, PDD 71,43% men, $p<0.01$), had lower UPDRS motor sub scores (PD-MCI 29.22 (SD 12.09), PDD 46.11 (SD 18.26), $p=0.01$), had lower UPDRS total scores (PD-MCI 46.22 (SD 13.20), PDD 85.52 (SD 26.03), $p<0.01$), and a shorter disease duration (PD-MCI 13.09 years (SD 5.20), PDD 17.36 years (SD 6.24), $p=0.03$). The PD-MCI and PDD groups were evenly matched with regards to education (PD-MCI 15.64 years (SD 2.29), PDD 16.23 years (SD 3.47), $p=0.58$), race (PD-MCI 90% Caucasian, PDD 96% Caucasian, $p=0.13$), age at death (PD-MCI 81.82 years (SD 7.93), PDD 78.30 years (SD 7.41), $p=0.16$), Hamilton Anxiety scale total score (PD-MCI 6.80 (SD 5.07), PDD 9.75 (SD 6.20), $p=0.16$), Beck Depression Inventory score (PD-MCI 6.11 (SD 3.76), PDD 9.63 (SD 6.84), $p=0.14$), and

months from last visit to autopsy (PD-MCI 35.00 months (SD 28.04), PDD 25.02 months (SD 17.99), p=0.13).

Pathology summary

Among individuals with PDD at their last research visit prior to autopsy, 22 had no AD pathology per CERAD criteria, 9 had possible AD, 20 and probable AD, and 5 had definite AD. Using the Braak staging for neurofibrillary tangles, 5 individuals had Braak stage 1, 15 individuals had Braak stage II, 14 individuals had Braak stage III, 19 individuals had Braak stage 4, and 1 individuals had Braak stage VI (no autopsy showed Braak stage V, Braak staging data was not available for 2 individuals). Among individuals with probable or definite AD by CERAD criteria, 4 individuals had Braak stage II, 7 individuals had Braak stage III, 13 individuals had Braak stage IV and 1 individuals had Braak stage IV. The overwhelming majority of autopsies from both the PD path and the PD and AD path group had cortical level Lewy bodies (41 of the 56 autopsies, 73%). About 45% of the cohort had some form of vascular pathology, with that number increasing to 71% when CAA is included. There was a significant difference between the PD path and the PD and AD path with regards to the percent of autopsies with CAA (p=0.05). CVD burden was similar between the two groups, with the largest number of individuals demonstrating 2 different forms of cerebrovascular disease (18 of the 56 individuals with PDD, or 32%). See Table 6 for additional details regarding the pathologic characteristics of our cohort.

Disease duration

In separate simple linear regressions, neither cerebrovascular disease, CAA, or overall CVD burden were significantly associated with disease duration among our PDD cohort (CVD beta 1.16 (95% CI -2.22, 4.59), p=0.49; CAA beta -1.04 (95% CI -4.61, 2.53), p=0.56, CVD burden beta 0.36 (95% CI -1.03, 1.74), p=0.61). When controlling for age, education, race UPDRS motor sub scores, Lewy body level, and different forms of AD pathology (both CERAD and Braak staging), cerebrovascular disease, CAA, and overall CVD burden continued to not contribute to changes in disease duration (Table 7). Our model also did not show a significant relationship between Lewy body level or AD pathology and disease duration.

We further looked at disease duration in strata, comparing the highest and lowest quartile of disease duration. In separate simple logistic regression, presence of cerebrovascular disease, CAA, or overall CVD burden did not increase the odds of being in the lowest or highest quartile of disease duration ((CVD OR 1.52 (95% CI -0.36, 6.60), p=0.57; CAA OR 0.86 (95% CI 0.19, 3.88), p=0.84, CVD burden OR 1.21 (95% CI 0.67, 2.17), p=0.53). When controlling for age, education, race, UPDRS motor sub scores, Lewy body level, and different forms of AD pathology (both CERAD and Braak staging), cerebrovascular disease, CAA, and overall CVD burden did not increase the odds of being in either the lowest or highest quartile of disease duration (data not shown, similar to Table 7). There was a trend toward individuals with a higher AD CERAD score having an increased odds ratio of being in the highest quartile of disease duration (i.e. the longer disease duration) but this was not significant.

We next sought to evaluate these same demographic and pathologic variables regarding their association with development of PDD over MCI. In simple logistic

regression, presence of cerebrovascular disease and presence of CAA were not associated with development of PDD over MCI (CVD OR 1.61 (95% CI 0.43, 5.98), p=0.48; CAA OR 6.46 (95% CI 0.74, 56.45)) but presence of 2 forms of vascular pathology (i.e. a CVD burden score of 2) resulted in a 9 times greater odds ratio of development of PDD over PD-MCI (OR 9.00, (95% CI 1.03 – 78.57), p=0.04) and for every one point increase in CVD burden, participants had a 2.10 increased odds of having PDD over PD-MCI (OR 2.10, (95% CI 1.07 – 4.12), p=0.03). When controlling for age at time of death, gender, education, race Lewy body level, AD CERAD score, disease duration, Beck Depression Inventory score, Hamilton Depression Score, and time from last research visit to autopsy, increased CVD burden continued to increase the odds of developing PDD over PD-MCI (OR 4.50 (95% CI 1.14 – 17.68), p=0.03) (Table 8).

Discussion

While we failed to find a relationship between vascular pathology and disease duration, we did find that cerebrovascular burden increased the odds of developing dementia over mild cognitive impairment in our PD cohort. The presence of vascular pathology and overall vascular burden did not shorten the time from symptom onset to death nor did increased Lewy body level and CERAD level. The increased Lewy body level and CERAD level also did not predict conversion to PDD from PD-MCI.

The importance of CVD burden is intriguing as it adds on to others more recent findings regarding the role of cerebrovascular risk. Schwartz et al found that pallor in the Globus Pallidus internus was significantly associated with higher Hoehn and Yahr scores and that the number of vascular risk factors (i.e. hypertension, etc.) predicted dementia

(Schwartz, Halliday, Soh, Cordato, & Kril, 2018). They did not find an association between small vessel disease, defined as perivascular pallor, gliosis, hyaline thickening, and enlargement of perivascular spaces, and the presence of dementia. Our findings of the role of cerebrovascular burden and development of dementia differ from this manuscript for a number of reasons. 1) The clinical analysis of our cohort is more uniform as the same set of raters (YS, CB, LR) reviewed each autopsy case regarding their final cognitive strata, 2) the participants in the research study were evaluated by movement disorder experts and 3) most importantly our investigation evaluated all forms of cerebrovascular disease, not just small vessel disease. It is therefore more comprehensive with regards to the impact of cerebrovascular risk factors on pathology and, ultimately, on cognition. We suspect that their finding that pathology did not correlate with dementia is because they were not looking at the many varied forms of cerebrovascular pathology.

The important clinical question then is how to go about mitigating CVD risk factors and there is growing evidence that what truly matters in the importance of vascular risk factors and cognition, is middle age health. Growing evidence indicates that the pathophysiology of middle age hypertension, hyperlipidemia, and diabetes alters the vascular architecture in the brain, thus causing cognitive impairment 10, 20, and even 30 years later. While we have a few individuals in our autopsy cohort that we have been following for more than 15 years, the numbers are not large enough to form any meaningful conclusions and there are no PD-based cohorts with ongoing follow up that can address these questions. The field would benefit from such an investigation both to better understand vascular modification of cognitive impairment and to address other

long-term questions. Even in the absence of a larger, long term cohort study, future research should consider vascular risk factors as they contribute to the development of dementia.

Our results should be interpreted with caution given the overall low numbers of individuals with PD-MCI and the differences between our cohort and the cohorts of other earlier investigations of pathology and PDD. Specifically, our cohort was not demonstrating the importance of Lewy body level and AD disease burden on the development of PDD and on shortening disease duration. We suspect this is because the majority of our patients had cortical Lewy bodies and probable AD, thus decreasing our ability to detect differences. The CVD burden score was more evenly distributed across the cohort.

The analysis of vascular pathology as a contributor to PDD continues to be a valuable exploration. Vascular disease and vascular risk factors are modifiable and therefore are currently the most treatable part of the PD pathology puzzle. This investigation indicates a role of vascular burden with regards to development of dementia therefore emphasizing the potential role of risk factor modification in the treatment and prevention of cognitive impairment in our patients. It is worth further pursuit of this topic as addressing vascular risk has an immediate impact on patient care and could potentially improve outcomes. Further exploration of both our and other cohorts are a critical future need.

IV. Concluding Remarks

The fear that patients experience when they are diagnosed with PD is frequently palpable in the examination room when having that discussion. The concerns focus primarily on what is going to happen to them in the future, how quickly will their disease progress and what modifications to their life will need to be made. In addition to understandable concerns over worsened quality of life and increased mortality, the biggest fear of our patients is worsening of their cognition and development of dementia. This cognitive change robs patients and their families of their quality of life and who they are as individuals arguably more so than the physical manifestations of the disease. The investigations presented here sought to improve the diagnosis, prognostication, and understanding of the development of PDD.

We have learned that PAR may not be an ideal biomarker for PDD but that CVD burden may influence development of PDD. As such, these studies both move the field forward. Specifically, the investigation of PAR continues. It is clear that the relationship between the c-Abl pathway and PD pathophysiology is complex and likely not linear and larger cohorts are needed to determine with certainty that PAR is or is not a biomarker of cognitive decline. This further investigation should be done concurrently with investigation into PARP1 inhibitors—our current patients demand no less than our full commitment to moving toward better treatments as soon as possible. It is also clear that the role of vascular pathology and vascular risk factors is more complex in our PD patients than has been shown in Alzheimer's disease patients and the general aging population. Larger cohorts and cohorts that specifically target groups that have been

poorly represented in our and other autopsy studies would be incredibly beneficial to the PD research community, allowing us to address these and other critical questions.

V. Tables

Table 1. Baseline demographic and clinical characteristics of Parkinson's disease and control participants in the JHU PDBP cohort

	Parkinson's disease (n=86)	Healthy Controls (n=35)	p-value
Age, years (SD)	66.08 (8.17)	66.84 (8.86)	0.65
Education, years (SD)	16.82 (2.39)	16.84 (2.33)	0.95
Gender, % Male	69.8	31.42	<0.01
Race, % Caucasian	95.34	88.57	0.17
MDS-UPDRS motor score, mean (SD)	32.13 (11.86)	1.51 (2.02)	<0.01
MDS-UPDRS total score, mean(SD)	60.30 (24.19)	6.8 (6.18)	<0.01
Total LED, mean (SD)	731.00 (497.88)	--	--
Hamilton Anxiety Score total, mean (SD)	7.77 (4.31)	4.23 (4.01)	<0.01
Hamilton Depression Score total, mean (SD)	6.32 (4.62)	3.29 (3.75)	<0.01
MoCA total score, mean (SD)	25.46 (4.54)	27.77 (1.24)	0.04
Cognition, number of individuals	25 Normal Cognition 45 Mild Cognitive Impairment 15 Dementia	27 Normal Cognition 8 Mild Cognitive Impairment	<0.01
UPSIT total score, mean (SD)	18.74 (7.10)	34.34 (3.89)	<0.01

Mean follow-up time, years (SD)	2.96 (1.56)	3.02 (1.54)	0.71
Mean disease duration, years (SD)	6.79 (4.83)	--	--

MDS-UPDRS=Movement Disorder Society-Unified Parkinson's Disease Rating Scale; LED=Levodopa Equivalent Dose; MoCA=Montreal Cognitive Impairment; UPSIT=University of Pennsylvania Smell Identification Test. See appendix for score range of each assessment.

Table 2. Baseline demographic and clinical characteristics of Parkinson's disease-normal cognition, Parkinson's disease-mild cognitive impairment and Parkinson's disease dementia participants in the JHU PDBP cohort

	PD-normal cognition (n=25)	PD-MCI (n=45)	PDD (n=15)	p-value
Age, years (SD)	60.67 (7.84)	66.29 (6.17)	73.84 (7.56)	<0.01
Education, years (SD)	16.76 (2.18)	16.78 (2.57)	16.93 (2.89)	0.97
Gender, % Male	52	73	93	0.02
Race, % Caucasian	100	93	93	0.42
MDS-UPDRS motor score, mean (SD)	27.00 (8.76)	30.22 (9.71)	47.47 (9.83)	<0.01
MDS-UPDRS total score, mean(SD)	51.72 (11.67)	54.91 (17.78)	91.33 (32.62)	<0.01
Total LED, mean (SD)	788.83 (529.57)	687.70 (435.22)	764.50 (630.90)	0.69
Hamilton Anxiety Score total, mean (SD)	6.72 (5.00)	7.33 (3.60)	10.8 (4.77)	0.50
Hamilton Depression Score total, mean (SD)	5.76 (4.39)	5.23 (3.32)	10.40 (6.06)	<0.01
MoCA total score, mean (SD)	28.2 (1.65)	26.09 (2.10)	19.00 (6.76)	0.01

UPSIT total score, mean (SD)	20.76 (6.05)	19.12 (7.07)	14.00 (7.13)	0.01
Mean follow-up time, years (SD)	3.05 (1.67)	3.06 (1.43)	2.30 (1.62)	0.43
Mean disease duration, years (SD)	6.91 (4.20)	5.65 (4.04)	9.04 (5.99)	0.04

MDS-UPDRS=Movement Disorder Society-Unified Parkinson's Disease Rating Scale; LED=Levodopa Equivalent Dose; MoCA=Montreal Cognitive Impairment; UPSIT=University of Pennsylvania Smell Identification Test

Table 3. Baseline demographic and clinical characteristics comparing individuals whose cognition remained stable and those whose cognition progressed to either PD-MCI or PDD during follow-up time among individuals in the JHU PDBP cohort

	Stable cognition (n=44)	Cognitive decline (n=26)	p-value
Age, years (SD)	63.57 (6.97)	65.50 (7.78)	0.29
Education, years (SD)	16.97 (2.31)	16.42 (2.32)	0.34
Gender, % Male	70	58	0.28
Race, % Caucasian	85	96	0.89
MDS-UPDRS motor score, mean (SD)	27.63 (9.64)	31.50 (8.75)	0.10
MDS-UPDRS total score, mean(SD)	50.50 (15.38)	59.31 (15.36)	0.02
Total LED, mean (SD)	655.18 (482.62)	839.97 (431.54)	0.11
Hamilton Anxiety Score total, mean (SD)	6.86 (3.40)	7.52 (4.76)	0.51
Hamilton Depression Score total, mean (SD)	5.05 (2.97)	6.08 (4.77)	0.27
MoCA total score, mean (SD)	27.36 (2.10)	25.96 (2.09)	<0.01
UPSIT total score, mean (SD)	20/26 (7.34)	18.80 (5.66)	0.40
Mean disease duration, years (SD)	5.05 (3.59)	7.89 (4.40)	<0.01

MDS-UPDRS=Movement Disorder Society-Unified Parkinson's Disease Rating Scale; LED=Levodopa Equivalent Dose; MoCA=Montreal Cognitive Impairment; UPSIT=Univ of Pennsylvania Smell Identification Test

Table 4. Results of the final multiple regression analysis evaluating which independent variable predicted change in cognitive status. PAR concentration did not predict change in cognition.

Independent variables	Odds Ratio	95% CI	p-value
PAR at Visit 1	1.01	0.99, 1.02	0.30
PAR concentration throughout follow up time	1.01	0.99, 1.02	0.30
MoCA at Visit 1	0.57	0.40, 0.82	<0.01
Total LED	1.00	0.99, 1.00	0.61
Disease duration	1.25	0.99, 1.58	0.06
Age at baseline visit	1.01	0.91, 1.11	0.86
Gender	0.39	0.09, 1.60	0.19
Education	0.87	0.49, 1.55	0.64
Race	0.19	0.02, 2.24	0.19
Hamilton Depression Scale	1.00	0.78, 1.29	0.98
Hamilton Anxiety Scale	1.03	0.78, 1.37	0.83

PAR=Poly (ADP-ribose); MoCA=Montreal Cognitive Assessment; LED=Levodopa Equivalent Dosing

Table 5. Demographic and clinical characteristics of individuals with pathologically proven Parkinson's disease, comparing those with and without additional AD pathology.

	PD pathology (n=31)	PD and AD pathology (n=25)	p-value
Age at death, years (SD)	77.77 (8.09)	78.96 (6.57)	0.56
Time between last research visit and autopsy, months (SD)	24.94 (17.59)	25.12 (18.84)	0.97
Education, years (SD)	16.65 (3.80)	15.72 (3.01)	0.33
Gender, % Male	71.07	72.00	0.93
Race, % Caucasian	96.80	96.00	0.88
UPDRS motor score at last visit, mean (SD)	43.67 (15.56)	48.90 (20.98)	0.34
UPDRS total score, mean(SD)	85.52 (20.47)	85.52 (31.55)	0.99
Hamilton Anxiety Score total, mean (SD)	10.26 (6.11)	9.10 (6.41)	0.52
Beck Depression Inventory total, mean (SD)	8.9 (7.92)	10.22 (5.96)	0.60
Mean disease duration, years (SD)	18.13 (6.41)	19.52 (6.97)	0.44

MDS-UPDRS=Movement Disorder Society-Unified Parkinson's Disease Rating Scale; LED=Levodopa Equivalent Dose; MoCA=Montreal Cognitive Impairment; UPSIT=Univ of Pennsylvania Smell Identification Test

Table 6. Pathological characteristics of individuals with pathologically proven Parkinson's disease, comparing those with and without additional AD pathology

	PD only (n=31)	PD and AD pathology (n=25)	p-value
Lewy Body level, number of individuals	1 Brainstem 9 Limbic 21 Cortical	0 Brainstem 5 Limbic 20 Cortical	0.25
Presence of CVD, %*	45	44	0.93
Presence of CAA, %	36	64	0.05
CVD presence and CAA, %*	81	60	0.09
*CVD burden**, *number of individuals with each burden level*	0, 1 individual 1, 12 individuals 2, 9 individuals 3, 4 individuals 4, 3 individuals 5, 2 individuals	0, 2 individuals 1, 4 individuals 2, 9 individuals 3, 8 individuals 4, 1 individuals 5, 1 individual	0.27

**CVD (Cerebrovascular disease) defined as presence of any of the following: vessel obstruction greater than 50%, parenchymal vascular lesions, large infarcts, lacunar infarcts, hemorrhages, cerebral atherosclerosis, other microvascular disease, other microinfarcts. **CVD burden is the sum of each of these pathology types, including CAA*

Table 7. Results of three final multiple regressions analyzing which independent variable predicted disease duration. Model 1 includes CVD presence, Model 2 includes amyloid angiopathy, Model 3 includes CVD burden.

Independent variables	Beta coefficient	95% CI	p-value	Beta coefficient	95% CI	p-value	Beta coefficient	95% CI	p-value
	Model 1			Model 2			Model 3		
Age	-0.06	-0.34, 0.23	0.68	0.06	-0.28, 0.39	0.74	-0.05	-0.34, 0.24	0.72
Education	0.31	-0.44, 1.07	0.41	0.19	-0.59, 0.97	0.62	0.18	-0.55, 0.93	0.61
Gender	-0.68	-5.49, 4.14	0.77	-1.40	-6.88, 4.09	0.61	-0.88	-5.79, 4.03	0.71
Race	6.25	-7.06. 19.57	0.35	Omitted because collinearity with CAA			6.49	-7.24, 20.22	0.34
UPDRS motor subscore	0.13	0.02, 0.25	0.02	0.11	-0.02, 0.25	0.10	0.14	0.02, 0.26	0.02
Lewy body level	-4.18 -5.31	-17.71, 9.36 -18.50, 7.88	0.54 0.41	-3.61 -2.69	-17.35, 10.14 -16.00, 10.63	0.60 0.68	-3.43 -4.35	-17.19, 10.31 -17.71, 9.00	0.62 0.51
Probable or Possible AD[1]	2.95	-1.10, 7.00	0.14	3.94	-0.68, 8.56	0.09	2.63	-1.40, 6.76	0.20
*CVD presence**	2.99	-1.09, 7.07	0.15						
CAA				-1.83	-6.64, 2.97	0.44			
*CVD burden***							0.74	-0.98, 2.47	0.39
Adjusted R^2			0.05			0.02			0.01

[1]Possible or probable AD based on CERAD criteria. UPDRS=Unified Parkinson's Disease Rating Scale; AD=Alzheimer's disease; CAA=Cerebral amyloid angiopathy

*CVD (Cerebrovascular disease) defined as presence of any of the following: vessel obstruction greater than 50%, parenchymal vascular lesions, large infarcts, lacunar infarcts, hemorrhages, cerebral atherosclerosis, other microvascular disease, other microinfarcts.

**CVD burden is the sum of each of these pathology types, including CAA

Table 8. Results of the final multiple regression analyses evaluating which independent variable predicted conversion from PD-MCI to PDD.

Independent variables	Odds Ratio	95% CI	p-value
Age	0.91	0.76, 1.09	0.31
Education	0.82	0.50, 1.37	0.45
Gender	0.04	0.00, 1.02	0.05
Race	2.79	0.11, 72.48	0.54
Disease Duration	1.11	0.90, 1.36	0.33
Time from last research visit to autopsy	1.00	0.93, 1.08	0.91
Beck Depression Inventory	1.00	0.99 , 1.01	0.10
Hamilton Anxiety Scale	1.00	0.99, 1.01	0.11
Lewy body level	3.97 5.17	0.04, 374.35 0.15, 189.66	0.55 0.37
Probable or Possible AD[1]	1.50	0.41, 5.38	0.54
CVD burden	4.50	1.15, 17.69	0.03

[1]*Possible or probable AD based on CERAD criteria. CVD=cerebrovascular disease.*

VI. Figures

Figure 1. Proposed c-Abl pathway**.** c-Abl activation leads to increase in poly-ADP-ribose (PAR) levels and ultimately dopamine cell death.

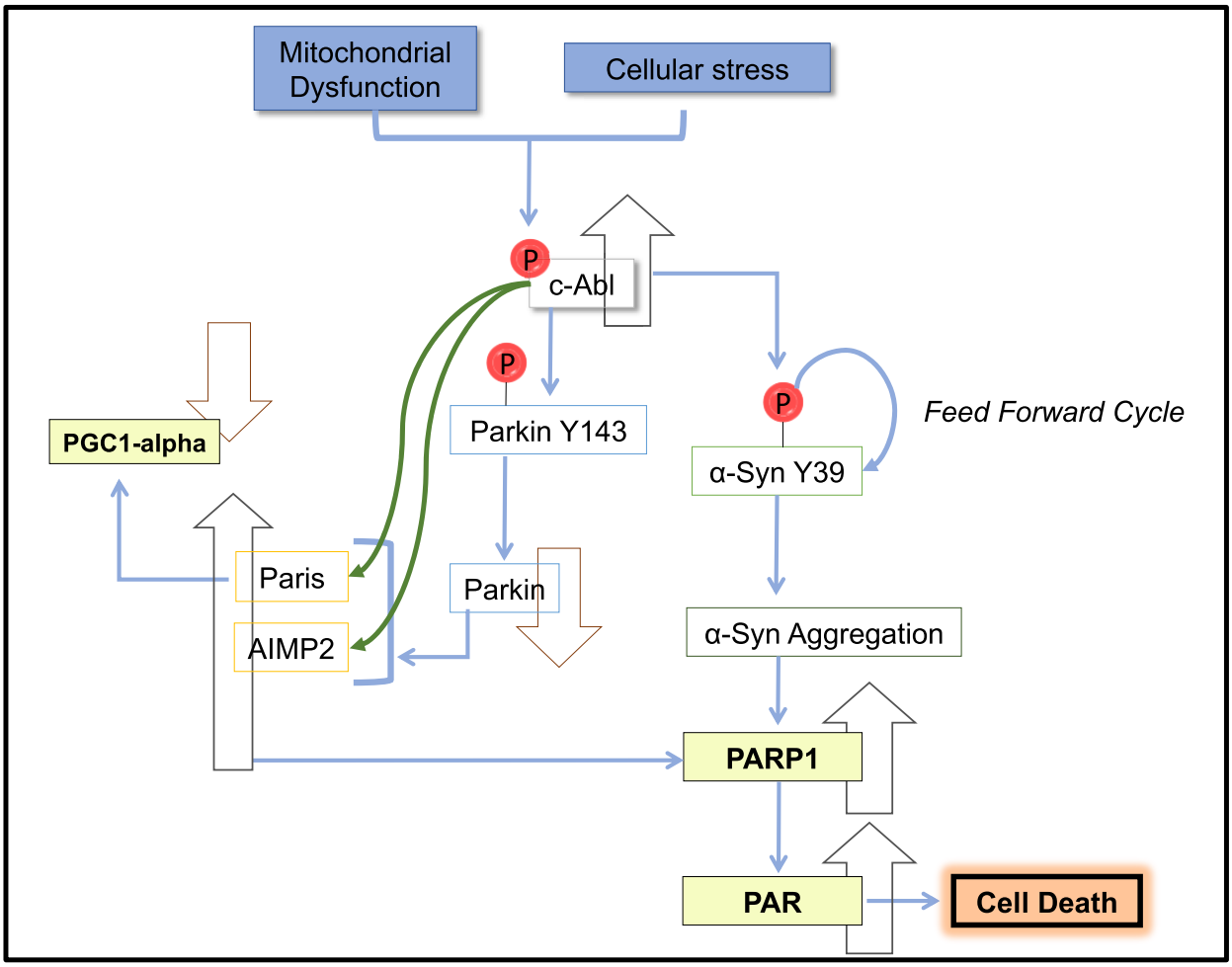
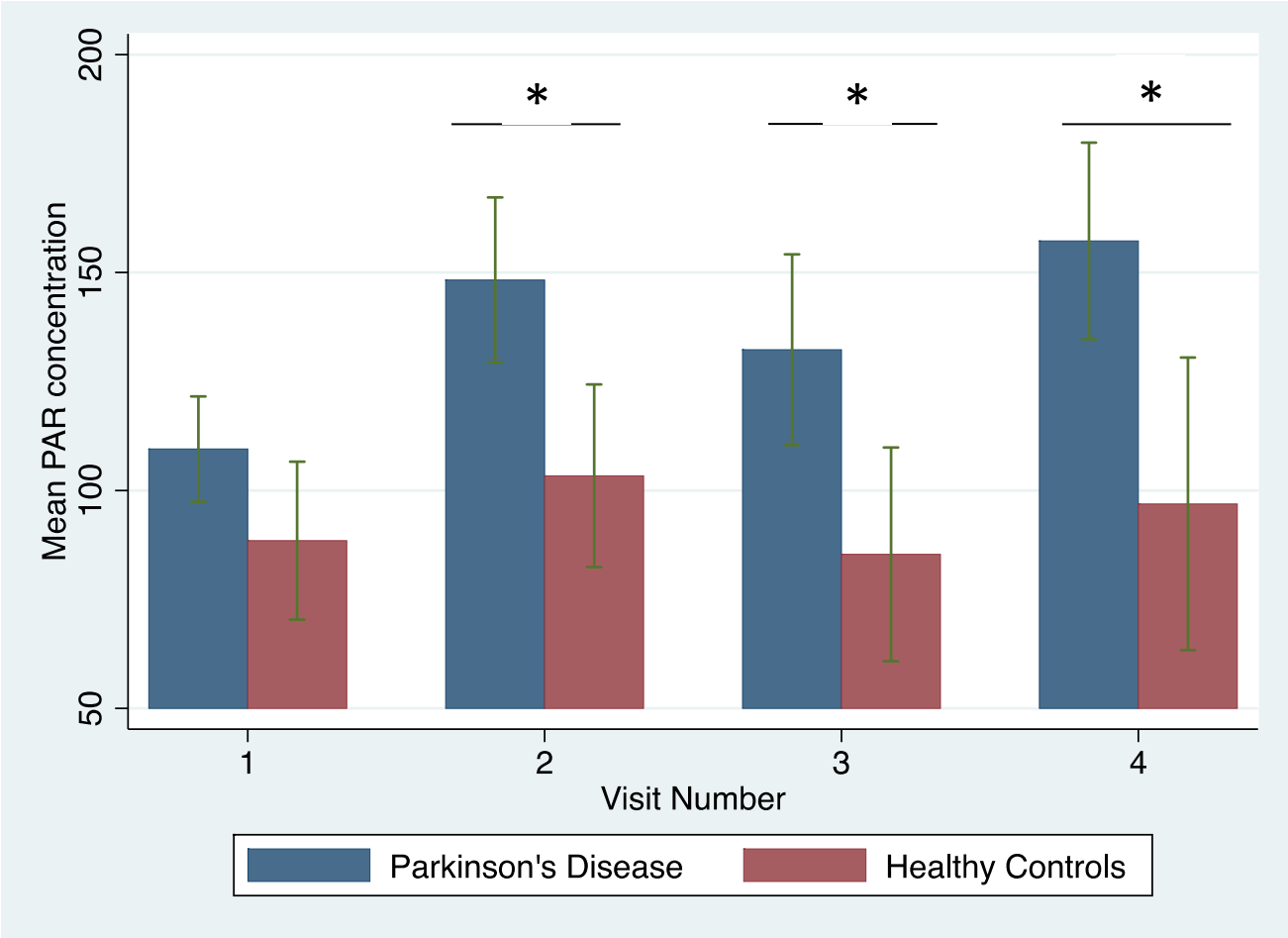


Figure 2. Comparison of mean poly (ADP-ribose) concentration between individuals with Parkinson's disease and controls, by visit number. * indicates $p<0.05$



VIII. Appendix

Assessment of PAR missingness

```
*    Missing-value patterns for PAR
 *       (1 means complete)

             * |   Pattern
   *  Percent   |  1  2  3  4    5  6
   *------------+--------------------
   *     <1%    |  1  1  1  1    1  1
   *            |
   *     31     |  1  1  1  0    0  0
   *     23     |  1  1  1  1    0  0
   *     19     |  1  1  0  0    0  0
   *     11     |  1  0  0  0    0  0
   *      7     |  0  0  0  0    0  0
   *      3     |  1  1  0  1    0  0
   *      2     |  1  0  1  0    0  0
   *      2     |  1  0  1  1    0  0
   *     <1     |  0  1  0  1    0  0
   *     <1     |  0  1  1  0    0  0
   *------------+--------------------
   *    100%    |
```

Assessment of differences between those with no PAR levels (n=10) and those with PAR levels (n=111):

No difference in
-age (mean no PAR level, 64.13 years SD 9.38 years; mean with PAR levels 66.49 years SD 8.26 years; p=0.39)
-gender (10% of women have no PAR levels, 9.8% of men have no PAR levels, p<0.01)
-case/control status (7% of cases do not have PAR levels, 11.4% of controls do not have PAR levels, p=0.42)

Assessment of follow up and subsequent bias:
Mean age of PD patients:
Visit 1: 66.08 years
Visit 2: 67.21 years
Visit 3: 67.68 years
Visit 4: 68.34 years
Visit 5: 69.41 years
Visit 6: 70.19 years
In other words, over 6 years of follow up our mean age only increased by about 4 years. Our older participants were more likely to drop out.

% male of PD patients:
Visit 1: 69.77%
Visit 2: 71.60%
Visit 3: 69.86%
Visit 4: 65.08%
Visit 5: 63.26%
Visit 6: 53.84%
In other words, over 6 years our % males decreased from about 70% to about 53%. Our male participants were more likely to drop out.

MoCA score of PD patients:
Visit 1: 25.46
Visit 2: 25.64
Visit 3: 26.52
Visit 4: 26.38
Visit 5: 26.18
Visit 6: 25.42
In other words, no change in MoCA score over the course of the investigation. This seems unlikely among PD patients. More likely, the individuals with lower MoCA scores dropped out.

MDS-UPDRS part III score of PD patients:
Visit 1: 32.13
Visit 2: 31.03
Visit 3: 31.12
Visit 4: 32.58
Visit 5: 30.14
Visit 6: 29.92
In other words, over 6 years of follow up our cohort improved by more than 2 points in the MDS-UPDRS part III score. Our more advanced patients clearly dropped out of the investigation.

Supplemental Table 1. Normal score ranges for the assessments in these investigations

Test	Range
MDS-UPDRS part III (motor)	0-132
MDS-UPDRS total score (part I, II, III, IV)	0-260
Hamilton Anxiety Rating Scale	0-56
Hamilton Depression Rating Scale	0-50
MoCA	0-30
UPDRS part III (motor)	0-108
UPDRS total (pats I, II, III, IV)	0-199
UPSIT	0-40
Beck Depression Scale	0-63
Abbreviations: UPDRS: Unified Parkinsons Disease Rating Scale; MDS-UPDRS: Movement Disorder Society-Unified Parkinsons Disease Rating Scale; MoCA: Montreal Cognitive Impairment; UPSIT: University of Pennsylvania Smell Identification Test Note: For both the MoCA and the UPSIT, higher scores reflect better performance. For all other assessments, higher scores reflect greater impairment.	

MDS-UPDRS Permissions

Permission is required to use the MDS-developed Rating Scales (with the exception of personal/individual use). Reproduction, translation, modification, sale, or distribution of any portion of the MDS Rating Scales is strictly prohibited. MDS Rating Scales may not be incorporated into clinical trials, training or certification programs or materials, software programs, or otherwise except through use of the Permissions Request Form and payment of applicable fees.

Continue to p. 2 to view the MDS-UPDRS

MDS-UPDRS

The *Movement* Disorder Society (MDS)-sponsored new version of the UPDRS is founded on the critique that was formulated by the Task Force for Rating Scales in Parkinson's disease (*Mov Disord* 2003;18:738-750). Thereafter, the MDS recruited a Chairperson to organize a program to provide the Movement Disorder community with a new version of the UPDRS that would maintain the overall format of the original UPDRS, but address issues identified in the critique as weaknesses and ambiguities. The Chairperson identified subcommittees with chairs and members. Each part was written by the appropriate subcommittee members and then reviewed and ratified by the entire group. These members are listed below.

The MDS-UPDRS has four parts: Part I (non-motor experiences of daily living), Part II (motor experiences of daily living, Part III (motor examination) and Part IV (motor complications). Part I has two components: IA concerns a number of behaviors that are assessed by the investigator with all pertinent information from patients and caregivers, and IB is completed by the patient with or without the aid of the caregiver, but independently of the investigator. These sections can, however, be reviewed by the rater to ensure that all questions are answered clearly and the rater can help explain any perceived ambiguities. Part II is designed to be a self-administered questionnaire like Part IB, but can be reviewed by the investigator to ensure completeness and clarity. Of note, the official versions of Part IA, Part IB and Part II of the MDS-UPDRS do not have separate on or off ratings. However, for individual programs or protocols the same questions can be used separately for on and off. Part III has instructions for the rater to give or demonstrate to the patient; it is completed by the rater. Part IV has instructions for the rater and also instructions to be read to the patient. This part integrates patient-derived information with the rater's clinical observations and judgments and is completed by the rater.

The authors of this new version are:

Chairperson: Christopher G. Goetz
Part I: Werner Poewe (chair), Bruno Dubois, Anette Schrag
Part II: Matthew B. Stern (chair), Anthony E. Lang, Peter A. LeWitt
Part III: Stanley Fahn (chair), Joseph Jankovic, C. Warren Olanow
Part IV: Pablo Martinez-Martin (chair), Andrew Lees, Olivier Rascol, Bob van Hilten
Development Standards: Glenn T. Stebbins (chair), Robert Holloway, David Nyenhuis
Appendices: Cristina Sampaio (chair), Richard Dodel, Jaime Kulisevsky
Statistical Testing: Barbara Tilley (chair), Sue Leurgans, Jean Teresi,
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July 1, 2008

Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

Overview: This portion of the scale assesses the non-motor impact of Parkinson's disease (PD) on patients' experiences of daily living. There are 13 questions. Part 1A is administered by the rater (six questions) and focuses on complex behaviors. Part 1B is a component of the self-administered Patient Questionnaire that covers seven questions on non-motor experiences of daily living.

Part 1A:
In administering Part IA, the examiner should use the following guidelines:

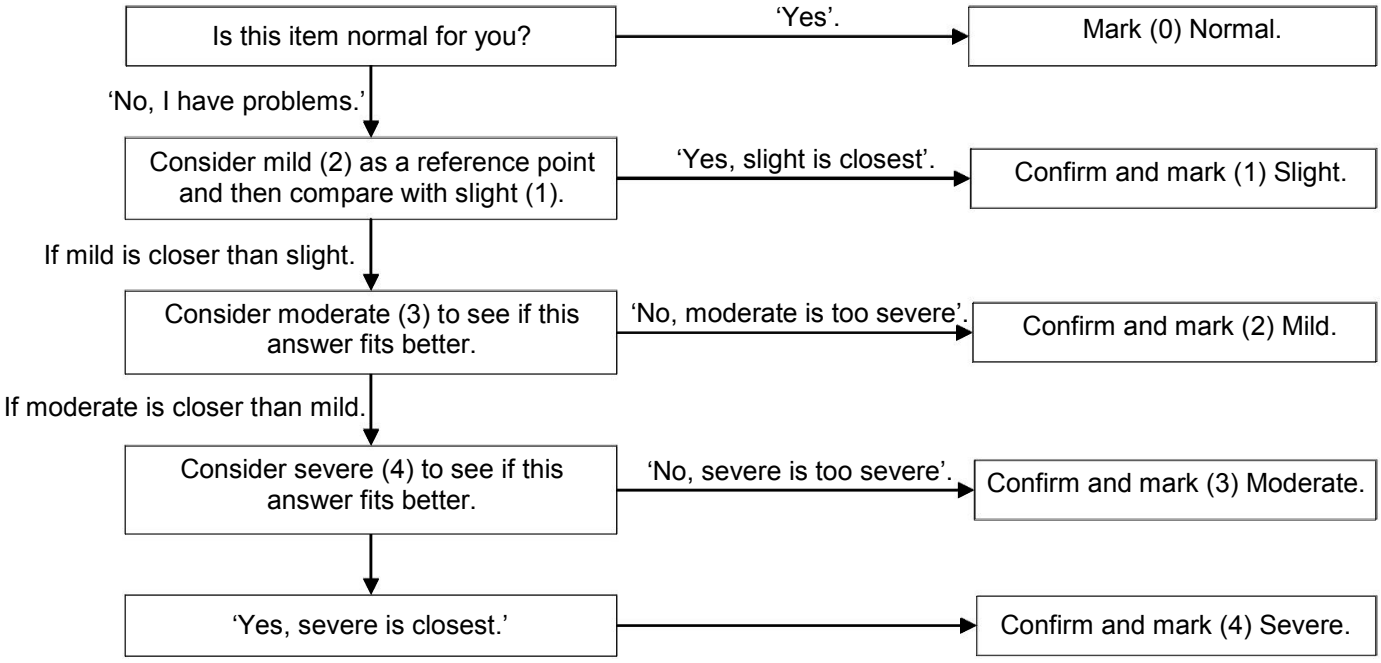
1. Mark at the top of the form the primary data source as patient, caregiver, or patient and caregiver in equal proportion.
2. The response to each item should refer to a period encompassing the prior week including the day on which the information is collected.
3. All items must have an integer rating (no half points, no missing scores). In the event that an item does not apply or cannot be rated (e.g., amputee who cannot walk), the item is marked UR for Unable to Rate.
4. The answers should reflect the usual level of function and words such as "usually", "generally", "most of the time" can be used with patients.
5. Each question has a text for you to read (Instructions to patients/caregiver). After that statement, you can elaborate and probe based on the target symptoms outlined in the Instructions to examiner. You should NOT READ the RATING OPTIONS to the patient/caregiver, because these are written in medical terminology. From the interview and probing, you will use your medical judgment to arrive at the best response.
6. Patients may have co-morbidities and other medical conditions that can affect their function. You and the patient must rate the problem as it exists and do not attempt to separate elements due to Parkinson's disease from other conditions.

EXAMPLE OF NAVIGATING THROUGH THE RESPONSE OPTIONS FOR PART 1A

Suggested strategies for obtaining the most accurate answer:
After reading the instructions to the patient, you will need to probe the entire domain under discussion to determine Normal vs. problematic: If your questions do not identify any problem in this domain, record 0 and move on to the next question.

If your questions identify a problem in this domain, you should work next with a reference anchor at the mid-range (option 2 or Mild) to find out if the patient functions at this level, better or worse. <u>You will not be reading the choices of responses to the patient as the responses use clinical terminology. You will be asking enough probing questions to determine the response that should be coded.</u>

Work up and down the options with the patient to identify the most accurate response, giving a final check by excluding the options above and below the selected response.



Patient Name or Subject ID	Site ID	Assessment Date (mm-dd-yyyy)	Investigator's Initials
_____	_____	_____ - _____ - _____	_____

MDS UPDRS
Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

Part 1A: Complex behaviors: [completed by rater]

Primary source of information:

☐ Patient ☐ Caregiver ☐ Patient and Caregiver in Equal Proportion

To be read to the patient: I am going to ask you six questions about behaviors that you may or may not experience. Some questions concern common problems and some concern uncommon ones. If you have a problem in one of the areas, please choose the best response that describes how you have felt MOST OF THE TIME during the PAST WEEK. If you are not bothered by a problem, you can simply respond NO. I am trying to be thorough, so I may ask questions that have nothing to do with you.

1.1 COGNITIVE IMPAIRMENT — **SCORE**

Instructions to examiner: Consider all types of altered level of cognitive function including cognitive slowing, impaired reasoning, memory loss, deficits in attention and orientation. Rate their impact on activities of daily living as perceived by the patient and/or caregiver.

Instructions to patients [and caregiver]: Over the past week have you had problems remembering things, following conversations, paying attention, thinking clearly, or finding your way around the house or in town? [If yes, examiner asks patient or caregiver to elaborate and probes for information.]

0: Normal:	No cognitive impairment.	
1: Slight:	Impairment appreciated by patient or caregiver with no concrete interference with the patient's ability to carry out normal activities and social interactions.	☐
2: Mild:	Clinically evident cognitive dysfunction, but only minimal interference with the patient's ability to carry out normal activities and social interactions.	
3: Moderate:	Cognitive deficits interfere with but do not preclude the patient's ability to carry out normal activities and social interactions.	
4: Severe:	Cognitive dysfunction precludes the patient's ability to carry out normal activities and social interactions.	

	SCORE
1.2 HALLUCINATIONS AND PSYCHOSIS Instructions to examiner: Consider both illusions (misinterpretations of real stimuli) and hallucinations (spontaneous false sensations). Consider all major sensory domains (visual, auditory, tactile, olfactory and gustatory). Determine presence of unformed (for example sense of presence or fleeting false impressions) as well as formed (fully developed and detailed) sensations. Rate the patient's insight into hallucinations and identify delusions and psychotic thinking. *Instructions to patients [and caregiver]: Over the past week have you seen, heard, smelled or felt things that were not really there?* [If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No hallucinations or psychotic behavior. 1: Slight: Illusions or non-formed hallucinations, but patient recognizes them without loss of insight. 2: Mild: Formed hallucinations independent of environmental stimuli. No loss of insight. 3: Moderate: Formed hallucinations with loss of insight. 4: Severe: Patient has delusions or paranoia.	☐
1.3 DEPRESSED MOOD Instructions to examiner: Consider low mood, sadness, hopelessness, feelings of emptiness or loss of enjoyment. Determine their presence and duration over the past week and rate their interference with the patient's ability to carry out daily routines and engage in social interactions. *Instruction to the patient (and caregiver): Over the past week have you felt low, sad, hopeless or unable to enjoy things? If yes, was this feeling for longer than one day at a time? Did it make it difficult for you carry out your usual activities or to be with people?* [If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No depressed mood. 1: Slight: Episodes of depressed mood that are not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions. 2: Mild: Depressed mood that is sustained over days, but without interference with normal activities and social interactions. 3: Moderate: Depressed mood that interferes with, but does not preclude, the patient's ability to carry out normal activities and social interactions. 4: Severe: Depressed mood precludes patient's ability to carry out normal activities and social interactions.	☐

	SCORE
1.4 ANXIOUS MOOD Instructions to examiner: Determine nervous, tense, worried or anxious feelings (including panic attacks) over the past week and rate their duration and interference with the patient's ability to carry out daily routines and engage in social interactions. *Instructions to patients [and caregiver]: Over the past week have you felt nervous, worried or tense? If yes, was this feeling for longer than one day at a time? Did it make it difficult for you to follow your usual activities or to be with other people*? [If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No anxious feelings. 1: Slight: Anxious feelings present but not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions. 2: Mild: Anxious feelings are sustained over more than one day at a time, but without interference with patient's ability to carry out normal activities and social interactions. 3: Moderate: Anxious feelings interfere with, but do not preclude, the patient's ability to carry out normal activities and social interactions. 4: Severe: Anxious feelings preclude patient's ability to carry out normal activities and social interactions.	☐
1.5 APATHY Instructions to examiner: Consider level of spontaneous activity, assertiveness, motivation and initiative and rate the impact of reduced levels on performance of daily routines and social interactions. Here the examiner should attempt to distinguish between apathy and similar symptoms that are best explained by depression. *Instructions to patients (and caregiver): Over the past week, have you felt indifferent to doing activities or being with people?* [If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No apathy. 1: Slight: Apathy appreciated by patient and/or caregiver, but no interference with daily activities and social interactions. 2: Mild: Apathy interferes with isolated activities and social interactions. 3: Moderate: Apathy interferes with most activities and social interactions. 4: Severe: Passive and withdrawn, complete loss of initiative.	☐

1.6 FEATURES OF DOPAMINE DYSREGULATION SYNDROME

SCORE

Instructions to examiner: Consider involvement in a variety of activities including atypical or excessive gambling (e.g. casinos or lottery tickets), atypical or excessive sexual drive or interests (e.g., unusual interest in pornography, masturbation, sexual demands on partner), other repetitive activities (e.g. hobbies, dismantling objects, sorting or organizing), or taking extra non-prescribed medication for non-physical reasons (i.e., addictive behavior). Rate the impact of such abnormal activities/behaviors on the patient's personal life and on his family and social relations (including need to borrow money or other financial difficulties like withdrawal of credit cards, major family conflicts, lost time from work, or missed meals or sleep because of the activity).

Instructions to patients [and caregiver]: Over the past week, have you had unusually strong urges that are hard to control? Do you feel driven to do or think about something and find it hard to stop? [Give patient examples such as gambling, cleaning, using the computer, taking extra medicine, obsessing about food or sex, all depending on the patients.]

0: Normal: No problems present.

1: Slight: Problems are present but usually do not cause any difficulties for the patient or family/caregiver.

2: Mild: Problems are present and usually cause a few difficulties in the patient's personal and family life.

3: Moderate: Problems are present and usually cause a lot of difficulties in the patient's personal and family life.

4: Severe: Problems are present and preclude the patient's ability to carry out normal activities or social interactions or to maintain previous standards in personal and family life.

The remaining questions in Part I (Non-motor Experiences of Daily Living) [Sleep, Daytime Sleepiness, Pain and Other Sensation, Urinary Problems, Constipation Problems, Lightheadedness on Standing, and Fatigue] are in the **Patient Questionnaire** along with all questions in Part II [Motor Experiences of Daily Living].

Patient Questionnaire:

Instructions:

This questionnaire will ask you about your experiences of daily living.

There are 20 questions. We are trying to be thorough, and some of these questions may therefore not apply to you now or ever. If you do not have the problem, simply mark 0 for NO.

Please read each one carefully and read all answers before selecting the one that best applies to you.

We are interested in your average or usual function over the past week including today. Some patients can do things better at one time of the day than at others. However, only one answer is allowed for each question, so please mark the answer that best describes what you can do most of the time.

You may have other medical conditions besides Parkinson's disease. Do not worry about separating Parkinson's disease from other conditions. Just answer the question with your best response.

Use only 0, 1, 2, 3, 4 for answers, nothing else. Do not leave any blanks.

Your doctor or nurse can review the questions with you, but this questionnaire is for patients to complete, either alone or with their caregivers.

Who is filling out this questionnaire (check the best answer):

☐ Patient ☐ Caregiver ☐ Patient and Caregiver in Equal Proportion

Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

SCORE

1.7 SLEEP PROBLEMS

Over the past week, have you had trouble going to sleep at night or staying asleep through the night? Consider how rested you felt after waking up in the morning.

0: Normal: No problems.

1: Slight: Sleep problems are present but usually do not cause trouble getting a full night of sleep.

2: Mild: Sleep problems usually cause some difficulties getting a full night of sleep.

3: Moderate: Sleep problems cause a lot of difficulties getting a full night of sleep, but I still usually sleep for more than half the night.

4: Severe: I usually do not sleep for most of the night.

☐

1.8 DAYTIME SLEEPINESS

Over the past week, have you had trouble staying awake during the daytime?

0: Normal: No daytime sleepiness.

1: Slight: Daytime sleepiness occurs but I can resist and I stay awake.

2: Mild: Sometimes I fall asleep when alone and relaxing. For example, while reading or watching TV.

3: Moderate: I sometimes fall asleep when I should not. For example, while eating or talking with other people.

4: Severe: I often fall asleep when I should not. For example, while eating or talking with other people.

☐

	SCORE
1.9 PAIN AND OTHER SENSATIONS Over the past week, have you had uncomfortable feelings in your body like pain, aches tingling or cramps? 0: Normal: No uncomfortable feelings. 1: Slight: I have these feelings. However, I can do things and be with other people without difficulty. 2: Mild: These feelings cause some problems when I do things or am with other people. 3: Moderate: These feelings cause a lot of problems, but they do not stop me from doing things or being with other people. 4: Severe: These feelings stop me from doing things or being with other people.	☐
1.10 URINARY PROBLEMS Over the past week, have you had trouble with urine control? For example, an urgent need to urinate, a need to urinate too often, or urine accidents? 0: Normal: No urine control problems. 1: Slight: I need to urinate often or urgently. However, these problems do not cause difficulties with my daily activities. 2: Mild: Urine problems cause some difficulties with my daily activities. However, I do not have urine accidents. 3: Moderate: Urine problems cause a lot of difficulties with my daily activities, including urine accidents. 4: Severe: I cannot control my urine and use a protective garment or have a bladder tube.	☐

	SCORE
1.11 CONSTIPATION PROBLEMS Over the past week have you had constipation troubles that cause you difficulty moving your bowels? 0: Normal: No constipation. 1: Slight: I have been constipated. I use extra effort to move my bowels. However, this problem does not disturb my activities or my being comfortable. 2: Mild: Constipation causes me to have some troubles doing things or being comfortable. 3: Moderate: Constipation causes me to have a lot of trouble doing things or being comfortable. However, it does not stop me from doing anything. 4: Severe: I usually need physical help from someone else to empty my bowels.	☐
1.12 LIGHT HEADEDNESS ON STANDING Over the past week, have you felt faint, dizzy or foggy when you stand up after sitting or lying down? 0: Normal: No dizzy or foggy feelings. 1: Slight: Dizzy or foggy feelings occur. However, they do not cause me troubles doing things. 2: Mild: Dizzy or foggy feelings cause me to hold on to something, but I do not need to sit or lie back down. 3: Moderate: Dizzy or foggy feelings cause me to sit or lie down to avoid fainting or falling. 4: Severe: Dizzy or foggy feelings cause me to fall or faint.	☐

	SCORE
1.13 FATIGUE Over the past week, have you usually felt fatigued? This feeling is <u>not</u> part of being sleepy or sad. 0: Normal: No fatigue. 1: Slight: Fatigue occurs. However it does not cause me troubles doing things or being with people. 2: Mild: Fatigue causes me some troubles doing things or being with people. 3: Moderate: Fatigue causes me a lot of troubles doing things or being with people. However, it does not stop me from doing anything. 4: Severe: Fatigue stops me from doing things or being with people.	☐

Part II: Motor Aspects of Experiences of Daily Living (M-EDL)

	SCORE
2.1 SPEECH Over the past week, have you had problems with your speech? 0: Normal: Not at all (no problems). 1: Slight: My speech is soft, slurred or uneven, but it does not cause others to ask me to repeat myself. 2: Mild: My speech causes people to ask me to occasionally repeat myself, but not everyday. 3: Moderate: My speech is unclear enough that others ask me to repeat myself every day even though most of my speech is understood. 4: Severe: Most or all of my speech cannot be understood.	☐

	SCORE
2.2 SALIVA AND DROOLING Over the past week, have you usually had too much saliva during when you are awake or when you sleep? 0: Normal: Not at all (no problems). 1: Slight: I have too much saliva, but do not drool. 2: Mild: I have some drooling during sleep, but none when I am awake. 3: Moderate: I have some drooling when I am awake, but I usually do not need tissues or a handkerchief. 4: Severe: I have so much drooling that I regularly need to use tissues or a handkerchief to protect my clothes.	☐
2.3 CHEWING AND SWALLOWING Over the past week, have you usually had problems swallowing pills or eating meals? Do you need your pills cut or crushed or your meals to be made soft, chopped or blended to avoid choking? 0: Normal: No problems. 1: Slight: I am aware of slowness in my chewing or increased effort at swallowing, but I do not choke or need to have my food specially prepared. 2: Mild: I need to have my pills cut or my food specially prepared because of chewing or swallowing problems, but I have not choked over the past week. 3: Moderate. I choked at least once in the past week. 4: Severe: Because of chewing and swallowing problems, I need a feeding tube.	☐

	SCORE
2.4 EATING TASKS Over the past week, have you usually had troubles handling your food and using eating utensils? For example, do you have trouble handling finger foods or using forks, knives, spoons, chopsticks? 0: Normal: Not at all (no problems). 1: Slight: I am slow, but I do not need any help handling my food and have not had food spills while eating. 2: Mild: I am slow with my eating and have occasional food spills. I may need help with a few tasks such as cutting meat. 3: Moderate: I need help with many eating tasks but can manage some alone. 4: Severe: I need help for most or all eating tasks.	☐
2.5 DRESSING Over the past week, have you usually had problems dressing? For example, are you slow or do you need help with buttoning, using zippers, putting on or taking off your clothes or jewelry? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need help. 2: Mild: I am slow and need help for a few dressing tasks (buttons, bracelets). 3: Moderate: I need help for many dressing tasks. 4: Severe: I need help for most or all dressing tasks.	☐

	SCORE
2.6 HYGIENE Over the past week, have you usually been slow or do you need help with washing, bathing, shaving, brushing teeth, combing your hair or with other personal hygiene? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need any help. 2: Mild: I need someone else to help me with some hygiene tasks. 3: Moderate: I need help for many hygiene tasks. 4: Severe: I need help for most or all of my hygiene tasks.	☐
2.7 HANDWRITING Over the past week, have people usually had trouble reading your handwriting? 0: Normal: Not at all (no problems). 1: Slight: My writing is slow, clumsy or uneven, but all words are clear. 2: Mild: Some words are unclear and difficult to read. 3: Moderate: Many words are unclear and difficult to read. 4: Severe: Most or all words cannot be read.	☐
2.8 DOING HOBBIES AND OTHER ACTIVITIES Over the past week, have you usually had trouble doing your hobbies or other things that you like to do? 0: Normal: Not at all (no problems). 1: Slight: I am a bit slow but do these activities easily. 2: Mild: I have some difficulty doing these activities. 3: Moderate: I have major problems doing these activities, but still do most. 4: Severe: I am unable to do most or all of these activities.	☐

	SCORE
2.9 TURNING IN BED Over the past week, do you usually have trouble turning over in bed? 0: Normal: Not at all (no problems). 1: Slight: I have a bit of trouble turning, but I do not need any help. 2: Mild I have a lot of trouble turning and need occasional help from someone else. 3: Moderate: To turn over I often need help from someone else. 4: Severe: I am unable to turn over without help from someone else.	☐
2.10 TREMOR Over the past week, have you usually had shaking or tremor? 0: Normal: Not at all. I have no shaking or tremor. 1: Slight: Shaking or tremor occurs but does not cause problems with any activities. 2: Mild: Shaking or tremor causes problems with only a few activities. 3: Moderate: Shaking or tremor causes problems with many of my daily activities. 4: Severe: Shaking or tremor causes problems with most or all activities.	☐
2.11 GETTING OUT OF BED, A CAR, OR A DEEP CHAIR Over the past week, have you usually had trouble getting out of bed, a car seat, or a deep chair? 0: Normal: Not at all (no problems). 1: Slight: I am slow or awkward, but I usually can do it on my first try. 2: Mild: I need more than one try to get up or need occasional help. 3: Moderate: I sometimes need help to get up, but most times I can still do it on my own. 4: Severe: I need help most or all of the time.	☐

SCORE

2.12 WALKING AND BALANCE

Over the past week, have you usually had problems with balance and walking?

0: Normal: Not at all (no problems).

1: Slight: I am slightly slow or may drag a leg. I never use a walking aid.

2: Mild: I occasionally use a walking aid, but I do not need any help from another person.

3: Moderate: I usually use a walking aid (cane, walker) to walk safely without falling. However, I do not usually need the support of another person.

4: Severe: I usually use the support of another person to walk safely without falling.

☐

2.13 FREEZING

Over the past week, on your usual day when walking, do you suddenly stop or freeze as if your feet are stuck to the floor.

0: Normal: Not at all (no problems).

1: Slight: I briefly freeze but I can easily start walking again. I do not need help from someone else or a walking aid (cane or walker) because of freezing.

2: Mild: I freeze and have trouble starting to walk again, but I do not need someone's help or a walking aid (cane or walker) because of freezing.

3: Moderate: When I freeze I have a lot of trouble starting to walk again and, because of freezing, I sometimes need to use a walking aid or need someone else's help.

4: Severe: Because of freezing, most or all of the time, I need to use a walking aid or someone's help.

☐

This completes the questionnaire. We may have asked about problems you do not even have, and may have mentioned problems that you may never develop at all. Not all patients develop all these problems, but because they can occur, it is important to ask all the questions to every patient. Thank you for your time and attention in completing this questionnaire.

Part III: Motor Examination

Overview: This portion of the scale assesses the motor signs of PD. In administering Part III of the MDS-UPDRS the examiner should comply with the following guidelines:

At the top of the form, mark whether the patient is on medication for treating the symptoms of Parkinson's disease and, if on levodopa, the time since the last dose.

Also, if the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:

ON is the typical functional state when patients are receiving medication and have a good response.
OFF is the typical functional state when patients have a poor response in spite of taking medications.

The investigator should "rate what you see". Admittedly, concurrent medical problems such as stroke, paralysis, arthritis, contracture, and orthopedic problems such as hip or knee replacement and scoliosis may interfere with individual items in the motor examination. In situations where it is absolutely impossible to test (e.g., amputations, plegia, limb in a cast), use the notation "**UR**" for Unable to Rate. Otherwise, rate the performance of each task as the patient performs in the context of co-morbidities.

All items must have an integer rating (no half points, no missing ratings).

Specific instructions are provided for the testing of each item. These should be followed in all instances. The investigator demonstrates while describing tasks the patient is to perform and rates function immediately thereafter. For Global Spontaneous Movement and Rest Tremor items (3.14 and 3.17), these items have been placed purposefully at the end of the scale because clinical information pertinent to the score will be obtained throughout the entire examination.

At the end of the rating, indicate if dyskinesia (chorea or dystonia) was present at the time of the examination, and if so, whether these movements interfered with the motor examination.

3a Is the patient on medication for treating the symptoms of Parkinson's Disease? ☐ No ☐ Yes

3b If the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:

☐ ON: On is the typical functional state when patients are receiving medication and have a good response.

☐ OFF: Off is the typical functional state when patients have a poor response in spite of taking medications.

3c Is the patient on Levodopa ? ☐ No ☐ Yes

3.C1 If yes, minutes since last levodopa dose: _____

	SCORE
3.1 SPEECH Instructions to examiner: Listen to the patient's free-flowing speech and engage in conversation if necessary. Suggested topics: ask about the patient's work, hobbies, exercise, or how he got to the doctor's office. Evaluate volume, modulation (prosody) and clarity, including slurring, palilalia (repetition of syllables) and tachyphemia (rapid speech, running syllables together). 0: Normal: No speech problems. 1: Slight: Loss of modulation, diction or volume, but still all words easy to understand. 2: Mild: Loss of modulation, diction, or volume, with a few words unclear, but the overall sentences easy to follow. 3: Moderate: Speech is difficult to understand to the point that some, but not most, sentences are poorly understood. 4: Severe: Most speech is difficult to understand or unintelligible.	☐
3.2 FACIAL EXPRESSION Instructions to examiner: Observe the patient sitting at rest for 10 seconds, without talking and also while talking. Observe eye-blink frequency, masked facies or loss of facial expression, spontaneous smiling and parting of lips. 0: Normal: Normal facial expression. 1: Slight: Minimal masked facies manifested only by decreased frequency of blinking. 2: Mild: In addition to decreased eye-blink frequency, Masked facies present in the lower face as well, namely fewer movements around the mouth, such as less spontaneous smiling, but lips not parted. 3: Moderate: Masked facies with lips parted some of the time when the mouth is at rest. 4: Severe: Masked facies with lips parted most of the time when the mouth is at rest.	☐

SCORE

3.3 RIGIDITY

Instructions to examiner: Rigidity is judged on slow passive movement of major joints with the patient in a relaxed position and the examiner manipulating the limbs and neck. First, test without an activation maneuver. Test and rate neck and each limb separately. For arms, test the wrist and elbow joints simultaneously. For legs, test the hip and knee joints simultaneously. If no rigidity is detected, use an activation maneuver such as tapping fingers, fist opening/closing, or heel tapping in a limb not being tested. Explain to the patient to go as limp as possible as you test for rigidity.

0: Normal: No rigidity.

1: Slight: Rigidity only detected with activation maneuver.

2: Mild: Rigidity detected without the activation maneuver, but full range of motion is easily achieved.

3: Moderate: Rigidity detected without the activation maneuver; full range of motion is achieved with effort.

4: Severe: Rigidity detected without the activation maneuver and full range of motion not achieved.

☐ Neck

☐ RUE

☐ LUE

☐ RLE

☐ LLE

3.4 FINGER TAPPING

Instructions to examiner: Each hand is tested separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to tap the index finger on the thumb 10 times as quickly AND as big as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps.

2: Mild: Any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence.

3: Moderate: Any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st tap.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.

☐ R

☐ L

SCORE

3.5 HAND MOVEMENTS

Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to make a tight fist with the arm bent at the elbow so that the palm faces the examiner. Have the patient open the hand 10 times as fully AND as quickly as possible. If the patient fails to make a tight fist or to open the hand fully, remind him/her to do so. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.

0: Normal: No problem.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the task.

2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the task.

3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st open-and-close sequence.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.

☐ R

☐ L

3.6 PRONATION-SUPINATION MOVEMENTS OF HANDS

Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to extend the arm out in front of his/her body with the palms down; then to turn the palm up and down alternately 10 times as fast and as fully as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.

0: Normal: No problems.

1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the sequence.

2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the sequence.

3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing c) the amplitude decrements starting after the 1st supination-pronation sequence.

4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.

☐ R

☐ L

SCORE

3.7 TOE TAPPING

Instructions to examiner: Have the patient sit in a straight-backed chair with arms, both feet on the floor. Test each foot separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the heel on the ground in a comfortable position and then tap the toes 10 times as big and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.

0: Normal:	No problem.
1: Slight:	Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) amplitude decrements near the end of the ten taps.
2: Mild:	Any of the following: a) 3 to 5 interruptions during the tapping movements; b) mild slowing; c) amplitude decrements midway in the task.
3: Moderate:	Any of the following: a) more than 5 interruptions during the tapping movements or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) amplitude decrements after the first tap.
4: Severe:	Cannot or can only barely perform the task because of slowing, interruptions or decrements.

☐ R

☐ L

3.8 LEG AGILITY

Instructions to examiner: Have the patient sit in a straight-backed chair with arms. The patient should have both feet comfortably on the floor. Test each leg separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the foot on the ground in a comfortable position and then raise and stomp the foot on the ground 10 times as high and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.

0: Normal:	No problems.
1: Slight:	Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) amplitude decrements near the end of the task.
2: Mild:	Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowness; c) amplitude decrements midway in the task.
3: Moderate:	Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing in speed; c) amplitude decrements after the first tap.
4: Severe:	Cannot or can only barely perform the task because of slowing, interruptions or decrements.

☐ R

☐ L

	SCORE
3.9 ARISING FROM CHAIR Instructions to examiner: Have the patient sit in a straight-backed chair with arms, with both feet on the floor and sitting back in the chair (if the patient is not too short). Ask the patient to cross his/her arms across the chest and then to stand up. If the patient is not successful, repeat this attempt a maximum up to two more times. If still unsuccessful, allow the patient to move forward in the chair to arise with arms folded across the chest. Allow only one attempt in this situation. If unsuccessful, allow the patient to push off using his/her hands on the arms of the chair. Allow a maximum of three trials of pushing off. If still not successful, assist the patient to arise. After the patient stands up, observe the posture for item 3.13. 0: Normal: No problems. Able to arise quickly without hesitation. 1: Slight: Arising is slower than normal; or may need more than one attempt; or may need to move forward in the chair to arise. No need to use the arms of the chair. 2: Mild: Pushes self up from arms of chair without difficulty. 3: Moderate: Needs to push off, but tends to fall back; or may have to try more than one time using arms of chair, but can get up without help. 4: Severe: Unable to arise without help.	☐
3.10 GAIT Instructions to examiner: Testing gait is best performed by having the patient walking away from and towards the examiner so that both right and left sides of the body can be easily observed simultaneously. The patient should walk at least 10 meters (30 feet), then turn around and return to the examiner. This item measures multiple behaviors: stride amplitude, stride speed, height of foot lift, heel strike during walking, turning, and arm swing, but not freezing. Assess also for "freezing of gait" (next item 3.11) while patient is walking. Observe posture for item 3.13. 0: Normal: No problems. 1: Slight: Independent walking with minor gait impairment. 2: Mild: Independent walking but with substantial gait impairment. 3: Moderate: Requires an assistance device for safe walking (walking stick, walker) but not a person. 4: Severe: Cannot walk at all or only with another person's assistance.	☐

SCORE

3.11 FREEZING OF GAIT

Instructions to examiner: While assessing gait, also assess for the presence of any gait freezing episodes. Observe for start hesitation and stuttering movements especially when turning and reaching the end of the task. To the extent that safety permits, patients may NOT use sensory tricks during the assessment.

0: Normal: No freezing.

1: Slight: Freezes on starting, turning or walking through doorway with a single halt during any of these events, but then continues smoothly without freezing during straight walking.

2: Mild: Freezes on starting, turning or walking through doorway with more than one halt during any of these activities, but continues smoothly without freezing during straight walking.

3: Moderate: Freezes once during straight walking.

4: Severe: Freezes multiple times during straight walking.

3.12 POSTURAL STABILITY

Instructions to examiner: The test examines the response to sudden body displacement produced by a quick, forceful pull on the shoulders while the patient is standing erect with eyes open and feet comfortably apart and parallel to each other. Test retropulsion. Stand behind the patient and instruct the patient on what is about to happen. Explain that s/he is allowed to take a step backwards to avoid falling. There should be a solid wall behind the examiner, at least 1-2 meters away to allow for the observation of the number of retropulsive steps. The first pull is an instructional demonstration and is purposely milder and not rated. The second time the shoulders are pulled briskly and forcefully towards the examiner with enough force to displace the center of gravity so that patient MUST take a step backwards. The examiner needs to be ready to catch the patient, but must stand sufficiently back so as to allow enough room for the patient to take several steps to recover independently. Do not allow the patient to flex the body abnormally forward in anticipation of the pull. Observe for the number of steps backwards or falling. Up to and including two steps for recovery is considered normal, so abnormal ratings begin with three steps. If the patient fails to understand the test, the examiner can repeat the test so that the rating is based on an assessment that the examiner feels reflects the patient's limitations rather than misunderstanding or lack of preparedness. Observe standing posture for item 3.13

0: Normal: No problems: Recovers with one or two steps.

1: Slight: 3-5 steps, but subject recovers unaided.

2: Mild: More than 5 steps, but subject recovers unaided.

3: Moderate: Stands safely, but with absence of postural response; falls if not caught by examiner.

4: Severe: Very unstable, tends to lose balance spontaneously or with just a gentle pull on the shoulders.

	SCORE
3.13 POSTURE Instructions to examiner: Posture is assessed with the patient standing erect after arising from a chair, during walking, and while being tested for postural reflexes. If you notice poor posture, tell the patient to stand up straight and see if the posture improves (see option 2 below). Rate the worst posture seen in these three observation points. Observe for flexion and side-to-side leaning. 0: Normal: No problems. 1: Slight: Not quite erect, but posture could be normal for older person. 2: Mild: Definite flexion, scoliosis or leaning to one side, but patient can correct posture to normal posture when asked to do so. 3: Moderate: Stooped posture, scoliosis or leaning to one side that cannot be corrected volitionally to a normal posture by the patient. 4: Severe: Flexion, scoliosis or leaning with extreme abnormality of posture.	☐
3.14 GLOBAL SPONTANEITY OF MOVEMENT (BODY BRADYKINESIA) Instructions to examiner: This global rating combines all observations on slowness, hesitancy, and small amplitude and poverty of movement in general, including a reduction of gesturing and of crossing the legs. This assessment is based on the examiner's global impression after observing for spontaneous gestures while sitting, and the nature of arising and walking. 0: Normal: No problems. 1: Slight: Slight global slowness and poverty of spontaneous movements. 2: Mild: Mild global slowness and poverty of spontaneous movements. 3: Moderate: Moderate global slowness and poverty of spontaneous movements. 4: Severe: Severe global slowness and poverty of spontaneous movements.	☐
3.15 POSTURAL TREMOR OF THE HANDS Instructions to examiner: All tremor, including re-emergent rest tremor, that is present in this posture is to be included in this rating. Rate each hand separately. Rate the highest amplitude seen. Instruct the patient to stretch the arms out in front of the body with palms down. The wrist should be straight and the fingers comfortably separated so that they do not touch each other. Observe this posture for 10 seconds. 0: Normal: No tremor. 1: Slight: Tremor is present but less than 1 cm in amplitude. 2: Mild: Tremor is at least 1 but less than 3 cm in amplitude. 3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude. 4: Severe: Tremor is at least 10 cm in amplitude.	☐ R ☐ L

SCORE

3.16 KINETIC TREMOR OF THE HANDS

Instructions to examiner: This is tested by the finger-to-nose maneuver. With the arm starting from the outstretched position, have the patient perform at least three finger-to-nose maneuvers with each hand reaching as far as possible to touch the examiner's finger. The finger-to-nose maneuver should be performed slowly enough not to hide any tremor that could occur with very fast arm movements. Repeat with the other hand, rating each hand separately. The tremor can be present throughout the movement or as the tremor reaches either target (nose or finger). Rate the highest amplitude seen.

0: Normal:	No tremor.	
1: Slight:	Tremor is present but less than 1 cm in amplitude.	R
2: Mild:	Tremor is at least 1 but less than 3 cm in amplitude.	
3: Moderate:	Tremor is at least 3 but less than 10 cm in amplitude.	
4: Severe:	Tremor is at least 10 cm in amplitude.	L

3.17 REST TREMOR AMPLITUDE

Instructions to examiner: This and the next item have been placed purposefully at the end of the examination to allow the rater to gather observations on rest tremor that may appear at any time during the exam, including when quietly sitting, during walking and during activities when some body parts are moving but others are at rest. Score the maximum amplitude that is seen at any time as the final score. Rate only the amplitude and not the persistence or the intermittency of the tremor.
As part of this rating, the patient should sit quietly in a chair with the hands placed on the arms of the chair (not in the lap) and the feet comfortably supported on the floor for 10 seconds with no other directives. Rest tremor is assessed separately for all four limbs and also for the lip/jaw. Rate only the maximum amplitude that is seen at any time as the final rating.

Extremity ratings

0: Normal:	No tremor.	RUE
1: Slight:	≤ 1 cm in maximal amplitude.	
2: Mild:	> 1 cm but < 3 cm in maximal amplitude.	LUE
3: Moderate:	3 - 10 cm in maximal amplitude.	
4: Severe:	> 10 cm in maximal amplitude.	RLE

Lip/Jaw ratings

0: Normal:	No tremor.	LLE
1: Slight:	≤ 1 cm in maximal amplitude.	
2: Mild:	> 1 cm but ≤ 2 cm in maximal amplitude.	Lip/Jaw
3: Moderate:	> 2 cm but ≤ 3 cm in maximal amplitude.	
4: Severe:	> 3 cm in maximal amplitude.	

3.18 CONSTANCY OF REST TREMOR

SCORE

Instructions to examiner: This item receives one rating for all rest tremor and focuses on the constancy of rest tremor during the examination period when different body parts are variously at rest. It is rated purposefully at the end of the examination so that several minutes of information can be coalesced into the rating.

0: Normal: No tremor.

1: Slight: Tremor at rest is present ≤ 25% of the entire examination period.

2: Mild: Tremor at rest is present 26-50% of the entire examination period.

3: Moderate: Tremor at rest is present 51-75% of the entire examination period.

4: Severe: Tremor at rest is present > 75% of the entire examination period.

☐

DYSKINESIA IMPACT ON PART III RATINGS

A. Were dyskinesias (chorea or dystonia) present during examination? ☐ No ☐ Yes

B. If yes, did these movements interfere with your ratings? ☐ No ☐ Yes

HOEHN AND YAHR STAGE

0: Asymptomatic.

1: Unilateral involvement only.

2: Bilateral involvement without impairment of balance.

3: Mild to moderate involvement; some postural instability but physically independent; needs assistance to recover from pull test.

4: Severe disability; still able to walk or stand unassisted.

5: Wheelchair bound or bedridden unless aided.

☐

Part IV: Motor Complications

Overview and Instructions: In this section, the rater uses historical and objective information to assess two motor complications, dyskinesias and motor fluctuations that include OFF-state dystonia. Use all information from patient, caregiver, and the examination to answer the six questions that summarize function over the past week including today. As in the other sections, rate using only integers (no half points allowed) and leave no missing ratings. If the item cannot be rated, place UR for Unable to Rate. You will need to choose some answers based on percentages, and therefore you will need to establish how many hours generally are awake hours and use this figure as the denominator for "OFF" time and dyskinesias. For "OFF dystonia", the total "Off" time will be the denominator. Operational definitions for examiner's use.

Dyskinesias: Involuntary random movements
Words that patients often recognize for dyskinesias include "irregular jerking", "wiggling", "twitching". It is essential to stress to the patient the difference between dyskinesias and tremor, a common error when patients are assessing dyskinesias.

Dystonia: contorted posture, often with a twisting component:
Words that patients often recognize for dystonia include "spasms", "cramps", "posture".

Motor fluctuation: Variable response to medication:
Words that patients often recognize for motor fluctuation include "wearing out", "wearing off", "roller-coaster effect", "on-off", "uneven medication effects".

OFF: Typical functional state when patients have a poor response in spite of taking mediation or the typical functional response when patients are on NO treatment for parkinsonism. Words that patients often recognize include "low time", "bad time", "shaking time", "slow time", "time when my medications don't work."

ON: Typical functional state when patients are receiving medication and have a good response:
Words that patients often recognize include "good time", "walking time", "time when my medications work."

A. DYSKINESIAS [exclusive of OFF-state dystonia]

SCORE

4.1 TIME SPENT WITH DYSKINESIAS

Instructions to examiner: Determine the hours in the usual waking day and then the hours of dyskinesias. Calculate the percentage. If the patient has dyskinesias in the office, you can point them out as a reference to ensure that patients and caregivers understand what they are rating. You may also use your own acting skills to enact the dyskinetic movements you have seen in the patient before or show them dyskinetic movements typical of other patients. Exclude from this question early morning and nighttime painful dystonia.

Instructions to patient [and caregiver]: Over the past week, how many hours do you usually sleep on a daily basis, including nighttime sleep and daytime napping? Alright, if you sleep ___ hrs, you are awake ____ hrs. Out of those awake hours, how many hours in total do you have wiggling, twitching or jerking movements? Do not count the times when you have tremor, which is a regular back and forth shaking or times when you have painful foot cramps or spasms in the early morning or at nighttime. I will ask about those later. Concentrate only on these types of wiggling, jerking and irregular movements. Add up all the time during the waking day when these usually occur. How many hours ____ (use this number for your calculations).

0: Normal:	No dyskinesias.
1: Slight:	≤ 25% of waking day.
2: Mild:	26 - 50% of waking day.
3: Moderate:	51 - 75% of waking day.
4: Severe:	> 75% of waking day.

1. Total Hours Awake: _____
2. Total Hours with Dyskinesia: _____
3. % Dyskinesia = ((2/1)*100): _____

4.2 FUNCTIONAL IMPACT OF DYSKINESIAS

SCORE

Instructions to examiner: Determine the degree to which dyskinesias impact on the patient's daily function in terms of activities and social interactions. Use the patient's and caregiver's response to your question and your own observations during the office visit to arrive at the best answer.

Instructions to patient [and caregiver]: Over the past week, did you usually have trouble doing things or being with people when these jerking movements occurred? Did they stop you from doing things or from being with people?

0: Normal:	No dyskinesias or no impact by dyskinesias on activities or social interactions.
1: Slight:	Dyskinesias impact on a few activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods.
2: Mild:	Dyskinesias impact on many activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods.
3: Moderate:	Dyskinesias impact on activities to the point that the patient usually does not perform some activities or does not usually participate in some social activities during dyskinetic episodes.
4: Severe:	Dyskinesias impact on function to the point that the patient usually does not perform most activities or participate in most social interactions during dyskinetic episodes.

B. MOTOR FLUCTUATIONS

4.3 TIME SPENT IN THE OFF STATE

Instructions to examiner: Use the number of waking hours derived from 4.1 and determine the hours spent in the "OFF" state. Calculate the percentage. If the patient has an OFF period in the office, you can point to this state as a reference. You may also use your knowledge of the patient to describe a typical OFF period. Additionally you may use your own acting skills to enact an OFF period you have seen in the patient before or show them OFF function typical of other patients. Mark down the typical number of OFF hours, because you will need this number for completing 4.6.

Instructions to patient [and caregiver]: Some patients with Parkinson's disease have a good effect from their medications throughout their awake hours and we call that "ON" time. Other patients take their medications but still have some hours of low time, bad time, slow time or shaking time. Doctors call these low periods "OFF" time. Over the past week, you told me before that you are general awake ____ hrs each day. Out of these awake hours, how many hours in total do you usually have this type of low level or OFF function ____ (use this number for your calculations).

0: Normal:	No OFF time.
1: Slight:	≤ 25% of waking day.
2: Mild:	26 - 50% of waking day.
3: Moderate:	51 - 75% of waking day.
4: Severe:	> 75% of waking day.

1. Total Hours Awake: _____
2. Total Hours OFF: _____
3. % OFF = ((2/1)*100): _____

SCORE

4.4 FUNCTIONAL IMPACT OF FLUCTUATIONS

Instructions to examiner: Determine the degree to which motor fluctuations impact on the patient's daily function in terms of activities and social interactions. This question concentrates on the difference between the ON state and the OFF state. If the patient has no OFF time, the rating must be 0, but if patients have very mild fluctuations, it is still possible to be rated 0 on this item if no impact on activities occurs. Use the patient's and caregiver's response to your question and your own observations during the office visit to arrive at the best answer.

Instructions to patient [and caregiver]: Think about when those low or "OFF" periods have occurred over the past week. Do you usually have more problems doing things or being with people than compared to the rest of the day when you feel your medications working? Are there some things you usually do during a good period that you have trouble with or stop doing during a low period?

0: Normal: No fluctuations or No impact by fluctuations on performance of activities or social interactions.

1: Slight: Fluctuations impact on a few activities, but during OFF, the patient usually performs all activities and participates in all social interactions that typically occur during the ON state.

2: Mild: Fluctuations impact many activities, but during OFF, the patient still usually performs all activities and participates in all social interactions that typically occur during the ON state.

3: Moderate: Fluctuations impact on the performance of activities during OFF to the point that the patient usually does not perform some activities or participate in some social interactions that are performed during ON periods.

4: Severe: Fluctuations impact on function to the point that, during OFF, the patient usually does not perform most activities or participate in most social interactions that are performed during ON periods.

4.5 COMPLEXITY OF MOTOR FLUCTUATIONS

Instructions to examiner: Determine the usual predictability of OFF function whether due to dose, time of day, food intake or other factors. Use the information provided by the patients and caregiver and supplement with your own observations. You will ask if the patient can count on them always coming at a special time, mostly coming at a special time (in which case you will probe further to separate slight from mild), only sometimes coming at a special time or are they totally unpredictable? Narrowing down the percentage will allow you to find the correct answer.

Instructions to patient [and caregiver]: For some patients, the low or "OFF" periods happen at certain times during day or when they do activities like eating or exercising. Over the past week, do you usually know when your low periods will occur? In other words, do your low periods always come at a certain time? Do they mostly come at a certain time? Do they only sometimes come at a certain time? Are your low periods totally unpredictable?"

0: Normal: No motor fluctuations.

1: Slight: OFF times are predictable all or almost all of the time (> 75%).

2: Mild: OFF times are predictable most of the time (51-75%).

3: Moderate: OFF times are predictable some of the time (26-50%).

4: Severe: OFF episodes are rarely predictable (≤ 25%).

C. "OFF" DYSTONIA

4.6 PAINFUL OFF-STATE DYSTONIA

Instructions to examiner: For patients who have motor fluctuations, determine what proportion of the OFF episodes usually includes painful dystonia? You have already determined the number of hours of "OFF" time (4.3). Of these hours, determine how many are associated with dystonia and calculate the percentage. If there is no OFF time, mark 0.

Instructions to patient [and caregiver]: In one of the questions I asked earlier, you said you generally have ____ hours of low or "OFF" time when your Parkinson's disease is under poor control. During these low or "OFF" periods, do you usually have painful cramps or spasms? Out of the total ____ hrs of this low time, if you add up all the time in a day when these painful cramps come, how many hours would this make?

0: Normal: No dystonia OR NO OFF TIME.

1: Slight: ≤ 25% of time in OFF state.

2: Mild: 26-50% of time in OFF state.

3: Moderate: 51-75% of time in OFF state.

4: Severe: > 75% of time in OFF state.

1. Total Hours Off: _____
2. Total Off Hours w/Dystonia: _____
3. % Off Dystonia = ((2/1)*100): _____

Summary statement to patient: READ TO PATIENT

This completes my rating of your Parkinson's disease. I know the questions and tasks have taken several minutes, but I wanted to be complete and cover all possibilities. In doing so, I may have asked about problems you do not even have, and I may have mentioned problems that you may never develop at all. Not all patients develop all these problems, but because they can occur, it is important to ask all the questions to every patient. Thank you for your time and attention in completing this scale with me.

Patient Name or Subject ID	Site ID	Assessment Date (mm-dd-yyyy)	Investigator's Initials
_____	___ ___ ___	____ - ____ - ____	___ ___ ___

MDS UPDRS Score Sheet

1.A	Source of information	☐ Patient ☐ Caregiver ☐ Patient + Caregiver	3.3b	Rigidity– RUE	
			3.3c	Rigidity– LUE	
Part I			3.3d	Rigidity– RLE	
1.1	Cognitive impairment		3.3e	Rigidity– LLE	
1.2	Hallucinations and psychosis		3.4a	Finger tapping– Right hand	
1.3	Depressed mood		3.4b	Finger tapping– Left hand	
1.4	Anxious mood		3.5a	Hand movements– Right hand	
1.5	Apathy		3.5b	Hand movements– Left hand	
1.6	Features of DDS		3.6a	Pronation- supination movements– Right hand	
1.6a	Who is filling out questionnaire	☐ Patient ☐ Caregiver ☐ Patient + Caregiver	3.6b	Pronation- supination movements– Left hand	
			3.7a	Toe tapping– Right foot	
1.7	Sleep problems		3.7b	Toe tapping– Left foot	
1.8	Daytime sleepiness		3.8a	Leg agility– Right leg	
1.9	Pain and other sensations		3.8b	Leg agility– Left leg	
1.10	Urinary problems		3.9	Arising from chair	
1.11	Constipation problems		3.10	Gait	
1.12	Light headedness on standing		3.11	Freezing of gait	
1.13	Fatigue		3.12	Postural stability	
Part II			3.13	Posture	
2.1	Speech		3.14	Global spontaneity of movement	
2.2	Saliva and drooling		3.15a	Postural tremor– Right hand	
2.3	Chewing and swallowing		3.15b	Postural tremor– Left hand	
2.4	Eating tasks		3.16a	Kinetic tremor– Right hand	
2.5	Dressing		3.16b	Kinetic tremor– Left hand	
2.6	Hygiene		3.17a	Rest tremor amplitude– RUE	
2.7	Handwriting		3.17b	Rest tremor amplitude– LUE	
2.8	Doing hobbies and other activities		3.17c	Rest tremor amplitude– RLE	
2.9	Turning in bed		3.17d	Rest tremor amplitude– LLE	
2.10	Tremor		3.17e	Rest tremor amplitude– Lip/jaw	
2.11	Getting out of bed		3.18	Constancy of rest	
2.12	Walking and balance			Were dyskinesias present?	☐ No ☐ Yes
2.13	Freezing			Did these movements interfere with ratings?	☐ No ☐ Yes
3a	Is the patient on medication?	☐ No ☐ Yes		Hoehn and Yahr Stage	
3b	Patient's clinical state	☐ Off ☐ On	**Part IV**		
3c	Is the patient on Levodopa?	☐ No ☐ Yes	4.1	Time spent with dyskinesias	
3.C1	If yes, minutes since last dose:		4.2	Functional impact of dyskinesias	
Part III			4.3	Time spent in the OFF state	
3.1	Speech		4.4	Functional impact of fluctuations	
3.2	Facial expression		4.5	Complexity of motor fluctuations	
3.3a	Rigidity– Neck		4.6	Painful OFF-state dystonia	

Hamilton Anxiety Rating Scale (HAM-A)

Reference: **Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol 1959; 32:50–55.**

Rating Clinician-rated

Administration time 10–15 minutes

Main purpose To assess the severity of symptoms of anxiety

Population Adults, adolescents and children

Commentary

The HAM-A was one of the first rating scales developed to measure the severity of anxiety symptoms, and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Although the HAM-A remains widely used as an outcome measure in clinical trials, it has been criticized for its sometimes poor ability to discriminate between anxiolytic and antidepressant effects, and somatic anxiety versus somatic side effects. The HAM-A does not provide any standardized probe questions. Despite this, the reported levels of inter-rater reliability for the scale appear to be acceptable.

Scoring

Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0–56, where <17 indicates mild severity, 18–24 mild to moderate severity and 25–30 moderate to severe.

Versions

The scale has been translated into: Cantonese for China, French and Spanish. An IVR version of the scale is available from Healthcare Technology Systems.

Additional references

Maier W, Buller R, Philipp M, Heuser I. The Hamilton Anxiety Scale: reliability, validity and sensitivity to change in anxiety and depressive disorders. J Affect Disord 1988;14(1):61–8.

Borkovec T and Costello E. Efficacy of applied relaxation and cognitive behavioral therapy in the treatment of generalized anxiety disorder. J Clin Consult Psychol 1993; 61(4):611–19

Address for correspondence

The HAM-A is in the public domain.

Hamilton Anxiety Rating Scale (HAM-A)

Below is a list of phrases that describe certain feeling that people have. Rate the patients by finding the answer which best describes the extent to which he/she has these conditions. Select one of the five responses for each of the fourteen questions.

0 = Not present, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very severe.

1 Anxious mood [0] [1] [2] [3] [4]

Worries, anticipation of the worst, fearful anticipation, irritability.

2 Tension [0] [1] [2] [3] [4]

Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax.

3 Fears [0] [1] [2] [3] [4]

Of dark, of strangers, of being left alone, of animals, of traffic, of crowds.

4 Insomnia [0] [1] [2] [3] [4]

Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors.

5 Intellectual [0] [1] [2] [3] [4]

Difficulty in concentration, poor memory.

6 Depressed mood [0] [1] [2] [3] [4]

Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing.

7 Somatic (muscular) [0] [1] [2] [3] [4]

Pains and aches, twitching, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone.

8 Somatic (sensory) [0] [1] [2] [3] [4]

Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation.

9 Cardiovascular symptoms [0] [1] [2] [3] [4]

Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, missing beat.

10 Respiratory symptoms [0] [1] [2] [3] [4]

Pressure or constriction in chest, choking feelings, sighing, dyspnea.

11 Gastrointestinal symptoms [0] [1] [2] [3] [4]

Difficulty in swallowing, wind abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation.

12 Genitourinary symptoms [0] [1] [2] [3] [4]

Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence.

13 Autonomic symptoms [0] [1] [2] [3] [4]

Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair.

14 Behavior at interview [0] [1] [2] [3] [4]

Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.

Patient Name:_____ **Date**:_____

Hamilton Rating Scale for Depression (17-items)

Instructions: For each item select the "cue" which best characterizes the patient during the past week.

1. **Depressed Mood**
(sadness, hopeless, helpless, worthless)
0 Absent
1 These feeling states indicated only on questioning
2 These feeling states spontaneously reported verbally
3 Communicates feeling states nonverbally, i.e., through facial expression, posture, voice and tendency to weep
4 Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and nonverbal communication

2. **Feelings of Guilt**
0 Absent
1 Self-reproach, feels he has let people down
2 Ideas of guilt or rumination over past errors or sinful deeds
3 Present illness is a punishment. Delusions of guilt
4 Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

3. **Suicide**
0 Absent
1 Feels life is not worth living
2 Wishes he were dead or any thoughts of possible death to self
3 Suicide ideas or gesture
4 Attempts at suicide (any serious attempt rates 4)

4. **Insomnia - Early**
0 No difficulty falling asleep
1 Complains of occasional difficulty falling asleep i.e., more than ½ hour
2 Complains of nightly difficulty falling asleep

5. **Insomnia - Middle**
0 No difficulty
1 Patient complains of being restless and disturbed during the night
2 Waking during the night – any getting out of bed rates 2 (except for purposes of voiding)

6. **Insomnia - Late**
0 No difficulty
1 Waking in early hours of the morning but goes back to sleep
2 Unable to fall asleep again if gets out of bed

7. **Work and Activities**
0 No difficulty
1 Thoughts and feelings of incapacity, fatigue or weakness related to activities; work or hobbies
2 Loss of interest in activity; hobbies or work – either directly reported by patient, or indirect in listlessness, indecision and vacillation (feels he has to push self to work or activities)
3 Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores.
4 Stopped working because of present illness. In hospital, rate 4 if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted.

8. **Retardation**
(slowness of thought and speech; impaired ability to concentrate; decreased motor activity)
0 Normal speech and thought
1 Slight retardation at interview
2 Obvious retardation at interview
3 Interview difficult
4 Complete stupor

9. **Agitation**
0 None
1 "Playing with" hand, hair, etc.
2 Hand-wringing, nail-biting, biting of lips

10. **Anxiety - Psychic**
0 No difficulty
1 Subjective tension and irritability
2 Worrying about minor matters
3 Apprehensive attitude apparent in face or speech
4 Fears expressed without questioning

11. **Anxiety - Somatic**
0 Absent
1 Mild
2 Moderate
3 Severe
4 Incapacitating
Physiological concomitants of anxiety such as:
Gastrointestinal - dry mouth, wind, indigestion, diarrhea, cramps, belching
Cardiovascular – palpitations, headaches
Respiratory - hyperventilation, sighing
Urinary frequency
Sweating

12. **Somatic Symptoms - Gastrointestinal**
0 None
1 Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
2 Difficulty eating without staff urging. Requests or requires laxatives or medications for bowels or medication for G.I. symptoms.

13. **Somatic Symptoms - General**
0 None
1 Heaviness in limbs, back or head, backaches, headache, muscle aches, loss of energy and fatigability
2 Any clear-cut symptom rates 2

14. **Genital Symptoms**
0 Absent
1 Mild
2 Severe
0 Not ascertained
Symptoms such as: loss of libido, menstrual disturbances

15. **Hypochondriasis**
0 Not present
1 Self-absorption (bodily)
2 Preoccupation with health
3 Frequent complaints, requests for help, etc.
4 Hypochondriacal delusions

16. **Loss of Weight**
A. When Rating by History:
0 No weight loss
1 Probable weight loss associated with present illness
2 Definite (according to patient) weight loss

B. On Weekly Ratings by Ward Psychiatrist, When Actual Changes are Measured:
0 Less than 1 lb. weight loss in week
1 Greater than 1 lb. weight loss in week
2 Greater than 2 lb. weight loss in week

17. **Insight**
0 Acknowledges being depressed and ill
1 Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2 Denies being ill at all

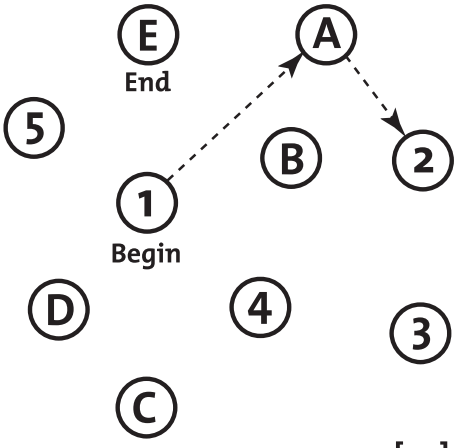
Total Score:

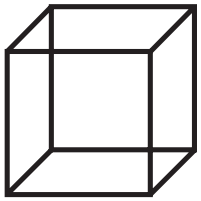
Citation: Hamilton M: A rating scale for depression. Journal of Neurology, Neurosurgery and Psychiatry 23:56-62, 1960

MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE





Copy cube

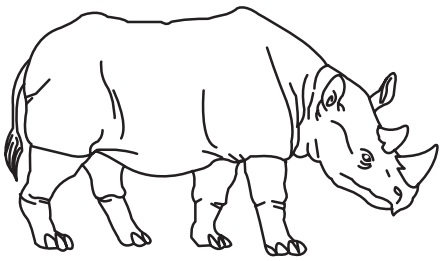
[ ] [ ]

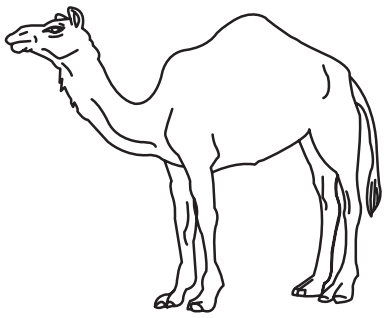
Draw CLOCK (Ten past eleven)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING





[ ] [ ] [ ]

___/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

___/3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

___/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED
		[ ]	[ ]	[ ]	[ ]	[ ]
Optional	Category cue					
	Multiple choice cue					

Points for UNCUED recall only

___/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

___/6

 Version November 7, 2004

www.mocatest.org

Normal ≥ 26 / 30

TOTAL ___/30

Add 1 point if ≤ 12 yr edu

UNIFIED PARKINSON'S DISEASE RATING SCALE

I. MENTATION, BEHAVIOR AND MOOD

1. Intellectual Impairment
0 = None.
1 = Mild. Consistent forgetfulness with partial recollection of events and no other difficulties.
2 = Moderate memory loss, with disorientation and moderate difficulty handling complex problems. Mild but definite impairment of function at home with need of occasional prompting.
3 = Severe memory loss with disorientation for time and often to place. Severe impairment in handling problems.
4 = Severe memory loss with orientation preserved to person only. Unable to make judgements or solve problems. Requires much help with personal care. Cannot be left alone at all.

2. Thought Disorder (Due to dementia or drug intoxication)
0 = None.
1 = Vivid dreaming.
2 = "Benign" hallucinations with insight retained.
3 = Occasional to frequent hallucinations or delusions; without insight; could interfere with daily activities.
4 = Persistent hallucinations, delusions, or florrid psychosis. Not able to care for self.

3. Depression
1 = Periods of sadness or guilt greater than normal, never sustained for days or weeks.
2 = Sustained depression (1 week or more).
3 = Sustained depression with vegetative symptoms (insomnia, anorexia, weight loss, loss of interest).
4 = Sustained depression with vegetative symptoms and suicidal thoughts or intent.

4. Motivation/Initiative
0 = Normal.
1 = Less assertive than usual; more passive.
2 = Loss of initiative or disinterest in elective (nonroutine) activities.
3 = Loss of initiative or disinterest in day to day (routine) activities.
4 = Withdrawn, complete loss of motivation.

II. ACTIVITIES OF DAILY LIVING (for both "on" and "off")

5. Speech
0 = Normal.
1 = Mildly affected. No difficulty being understood.
2 = Moderately affected. Sometimes asked to repeat statements.
3 = Severely affected. Frequently asked to repeat statements.
4 = Unintelligible most of the time.

6. Salivation
0 = Normal.
1 = Slight but definite excess of saliva in mouth; may have nighttime drooling.
2 = Moderately excessive saliva; may have minimal drooling.
3 = Marked excess of saliva with some drooling.
4 = Marked drooling, requires constant tissue or handkerchief.

7. Swallowing
0 = Normal.
1 = Rare choking.
2 = Occasional choking.
3 = Requires soft food.
4 = Requires NG tube or gastrotomy feeding.

8. Handwriting
0 = Normal.
1 = Slightly slow or small.
2 = Moderately slow or small; all words are legible.
3 = Severely affected; not all words are legible.
4 = The majority of words are not legible.

9. Cutting food and handling utensils
0 = Normal.
1 = Somewhat slow and clumsy, but no help needed.
2 = Can cut most foods, although clumsy and slow; some help needed.
3 = Food must be cut by someone, but can still feed slowly.
4 = Needs to be fed.

10. Dressing
0 = Normal.
1 = Somewhat slow, but no help needed.
2 = Occasional assistance with buttoning, getting arms in sleeves.
3 = Considerable help required, but can do some things alone.
4 = Helpless.

11. Hygiene
0 = Normal.
1 = Somewhat slow, but no help needed.
2 = Needs help to shower or bathe; or very slow in hygienic care.
3 = Requires assistance for washing, brushing teeth, combing hair, going to bathroom.
4 = Foley catheter or other mechanical aids.

12. Turning in bed and adjusting bed clothes
0 = Normal.
1 = Somewhat slow and clumsy, but no help needed.
2 = Can turn alone or adjust sheets, but with great difficulty.
3 = Can initiate, but not turn or adjust sheets alone.
4 = Helpless.

13. Falling (unrelated to freezing)
0 = None.
1 = Rare falling.
2 = Occasionally falls, less than once per day.
3 = Falls an average of once daily.
4 = Falls more than once daily.

14. Freezing when walking
0 = None.
1 = Rare freezing when walking; may have starthesitation.
2 = Occasional freezing when walking.
3 = Frequent freezing. Occasionally falls from freezing.
4 = Frequent falls from freezing.

15. Walking
0 = Normal.
1 = Mild difficulty. May not swing arms or may tend to drag leg.
2 = Moderate difficulty, but requires little or no assistance.
3 = Severe disturbance of walking, requiring assistance.
4 = Cannot walk at all, even with assistance.

16. Tremor (Symptomatic complaint of tremor in any part of body.)
0 = Absent.
1 = Slight and infrequently present.
2 = Moderate; bothersome to patient.
3 = Severe; interferes with many activities.
4 = Marked; interferes with most activities.

17. Sensory complaints related to parkinsonism
0 = None.
1 = Occasionally has numbness, tingling, or mild aching.
2 = Frequently has numbness, tingling, or aching; not distressing.
3 = Frequent painful sensations.
4 = Excruciating pain.

III. MOTOR EXAMINATION

18. Speech
0 = Normal.
1 = Slight loss of expression, diction and/or volume.
2 = Monotone, slurred but understandable; moderately impaired.
3 = Marked impairment, difficult to understand.
4 = Unintelligible.

19. Facial Expression
0 = Normal.
1 = Minimal hypomimia, could be normal "Poker Face".
2 = Slight but definitely abnormal diminution of facial expression
3 = Moderate hypomimia; lips parted some of the time.
4 = Masked or fixed facies with severe or complete loss of facial expression; lips parted 1/4 inch or more.

20. Tremor at rest (head, upper and lower extremities)
0 = Absent.
1 = Slight and infrequently present.
2 = Mild in amplitude and persistent. Or moderate in amplitude, but only intermittently present.
3 = Moderate in amplitude and present most of the time.
4 = Marked in amplitude and present most of the time.

21. Action or Postural Tremor of hands
0 = Absent.
1 = Slight; present with action.
2 = Moderate in amplitude, present with action.
3 = Moderate in amplitude with posture holding as well as action.
4 = Marked in amplitude; interferes with feeding.

22. Rigidity (Judged on passive movement of major joints with patient relaxed in sitting position. Cogwheeling to be ignored.)
0 = Absent.
1 = Slight or detectable only when activated by mirror or other movements.
2 = Mild to moderate.
3 = Marked, but full range of motion easily achieved.
4 = Severe, range of motion achieved with difficulty.

23. Finger Taps (Patient taps thumb with index finger in rapid succession.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

24. Hand Movements (Patient opens and closes hands in rapid succesion.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

25. Rapid Alternating Movements of Hands (Pronation-supination movements of hands, vertically and horizontally, with as large an amplitude as possible, both hands simultaneously.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

26. Leg Agility (Patient taps heel on the ground in rapid succession picking up entire leg. Amplitude should be at least 3 inches.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

27. Arising from Chair (Patient attempts to rise from a straightbacked chair, with arms folded across chest.)
0 = Normal.
1 = Slow; or may need more than one attempt.
2 = Pushes self up from arms of seat.
3 = Tends to fall back and may have to try more than one time, but can get up without help.
4 = Unable to arise without help.

28. Posture
0 = Normal erect.
1 = Not quite erect, slightly stooped posture; could be normal for older person.
2 = Moderately stooped posture, definitely abnormal; can be slightly leaning to one side.
3 = Severely stooped posture with kyphosis; can be moderately leaning to one side.
4 = Marked flexion with extreme abnormality of posture.

29. Gait
0 = Normal.
1 = Walks slowly, may shuffle with short steps, but no festination (hastening steps) or propulsion.
2 = Walks with difficulty, but requires little or no assistance; may have some festination, short steps, or propulsion.
3 = Severe disturbance of gait, requiring assistance.
4 = Cannot walk at all, even with assistance.

30. Postural Stability (Response to sudden, strong posterior displacement produced by pull on shoulders while patient erect with eyes open and feet slightly apart. Patient is prepared.)
0 = Normal.
1 = Retropulsion, but recovers unaided.
2 = Absence of postural response; would fall if not caught by examiner.
3 = Very unstable, tends to lose balance spontaneously.
4 = Unable to stand without assistance.

31. Body Bradykinesia and Hypokinesia (Combining slowness, hesitancy, decreased armswing, small amplitude, and poverty of movement in general.)
0 = None.
1 = Minimal slowness, giving movement a deliberate character; could be normal for some persons. Possibly reduced amplitude.
2 = Mild degree of slowness and poverty of movement which is definitely abnormal. Alternatively, some reduced amplitude.
3 = Moderate slowness, poverty or small amplitude of movement.
4 = Marked slowness, poverty or small amplitude of movement.

IV. COMPLICATIONS OF THERAPY (In the past week)

A. DYSKINESIAS

32. Duration: What proportion of the waking day are dyskinesias present? (Historical information.)
0 = None
1 = 1-25% of day.
2 = 26-50% of day.
3 = 51-75% of day.
4 = 76-100% of day.

33. Disability: How disabling are the dyskinesias? (Historical information; may be modified by office examination.)
0 = Not disabling.
1 = Mildly disabling.
2 = Moderately disabling.
3 = Severely disabling.
4 = Completely disabled.

34. Painful Dyskinesias: How painful are the dyskinesias?
0 = No painful dyskinesias.
1 = Slight.
2 = Moderate.
3 = Severe.
4 = Marked.

35. Presence of Early Morning Dystonia (Historical information.)
0 = No
1 = Yes

B. CLINICAL FLUCTUATIONS

36. Are "off" periods predictable?
0 = No
1 = Yes

37. Are "off" periods unpredictable?
0 = No
1 = Yes

38. Do "off" periods come on suddenly, within a few seconds?
0 = No
1 = Yes

39. What proportion of the waking day is the patient "off" on average?
0 = None
1 = 1-25% of day.
2 = 26-50% of day.
3 = 51-75% of day.
4 = 76-100% of day.

C. OTHER COMPLICATIONS

40. Does the patient have anorexia, nausea, or vomiting?
0 = No
1 = Yes

41. Any sleep disturbances, such as insomnia or hypersomnolence?
0 = No
1 = Yes

42. Does the patient have symptomatic orthostasis?
(Record the patient's blood pressure, height and weight on the scoring form)
0 = No
1 = Yes

V. MODIFIED HOEHN AND YAHR STAGING

STAGE 0 = No signs of disease.
STAGE 1 = Unilateral disease.
STAGE 1.5 = Unilateral plus axial involvement.
STAGE 2 = Bilateral disease, without impairment of balance.
STAGE 2.5 = Mild bilateral disease, with recovery on pull test.
STAGE 3 = Mild to moderate bilateral disease; some postural instability; physically independent.
STAGE 4 = Severe disability; still able to walk or stand unassisted.
STAGE 5 = Wheelchair bound or bedridden unless aided.

VI. SCHWAB AND ENGLAND ACTIVITIES OF DAILY LIVING SCALE

100% = Completely independent. Able to do all chores without slowness, difficulty or impairment. Essentially normal. Unaware of any difficulty.
90% = Completely independent. Able to do all chores with some degree of slowness, difficulty and impairment. Might take twice as long. Beginning to be aware of difficulty.
80% = Completely independent in most chores. Takes twice as long. Conscious of difficulty and slowness.
70% = Not completely independent. More difficulty with some chores. Three to four times as long in some. Must spend a large part of the day with chores.
60% = Some dependency. Can do most chores, but exceedingly slowly and with much effort. Errors; some impossible.
50% = More dependent. Help with half, slower, etc. Difficulty with everything.
40% = Very dependent. Can assist with all chores, but few alone.
30% = With effort, now and then does a few chores alone or begins alone. Much help needed.
20% = Nothing alone. Can be a slight help with some chores. Severe invalid.
10% = Totally dependent, helpless. Complete invalid.
0% = Vegetative functions such as swallowing, bladder and bowel functions are not functioning. Bedridden.

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.

0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive
3 I believe that I look ugly.

15.

0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.

0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20.

0 I am no more worried about my health than usual.
1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think of anything else.

21.

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

http://www.med.navy.mil/sites/NMCP2/PatientServices/SleepClinicLab/Documents/Beck_Depression_Inventory.pdf

V: References

Aarsland, D., Andersen, K., Larsen, J. P., Lolk, A., & Kragh-Sorensen, P. (2003). Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol, 60*(3), 387-392.

Aarsland, D., & Kramberger, M. G. (2015). Neuropsychiatric Symptoms in Parkinson's Disease. *J Parkinsons Dis, 5*(3), 659-667. doi:10.3233/JPD-150604

Aarsland, D., & Kurz, M. W. (2010). The epidemiology of dementia associated with Parkinson disease. *J Neurol Sci, 289*(1-2), 18-22. doi:10.1016/j.jns.2009.08.034

Aarsland, D., Larsen, J. P., Tandberg, E., & Laake, K. (2000). Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. *J Am Geriatr Soc, 48*(8), 938-942.

Aarsland, D., Marsh, L., & Schrag, A. (2009). Neuropsychiatric symptoms in Parkinson's disease. *Mov Disord, 24*(15), 2175-2186. doi:10.1002/mds.22589

Aasly, J. O., Shi, M., Sossi, V., Stewart, T., Johansen, K. K., Wszolek, Z. K., . . . Zhang, J. (2012). Cerebrospinal fluid amyloid beta and tau in LRRK2 mutation carriers. *Neurology, 78*(1), 55-61. doi:10.1212/WNL.0b013e31823ed101

Ascherio, A., LeWitt, P. A., Xu, K., Eberly, S., Watts, A., Matson, W. R., . . . Schwarzschild, M. A. (2009). Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch Neurol, 66*(12), 1460-1468. doi:10.1001/archneurol.2009.247

Backstrom, D. C., Eriksson Domellof, M., Linder, J., Olsson, B., Ohrfelt, A., Trupp, M., . . . Forsgren, L. (2015). Cerebrospinal Fluid Patterns and the Risk of Future Dementia in Early, Incident Parkinson Disease. *JAMA Neurol, 72*(10), 1175-1182. doi:10.1001/jamaneurol.2015.1449

Beach, T. G., Adler, C. H., Sue, L. I., Vedders, L., Lue, L., White Iii, C. L., . . . Walker, D. G. (2010). Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. *Acta Neuropathol, 119*(6), 689-702. doi:10.1007/s00401-010-0664-3

Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. (2001). *Clin Pharmacol Ther, 69*(3), 89-95. doi:10.1067/mcp.2001.113989

Biundo, R., Weis, L., & Antonini, A. (2016). Cognitive decline in Parkinson's disease: the complex picture. *NPJ Parkinsons Dis, 2*, 16018. doi:10.1038/npjparkd.2016.18

Bolognesi, B., Kumita, J. R., Barros, T. P., Esbjorner, E. K., Luheshi, L. M., Crowther, D. C., . . . Yerbury, J. J. (2010). ANS binding reveals common features of cytotoxic amyloid species. *ACS Chem Biol, 5*(8), 735-740. doi:10.1021/cb1001203

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., & Del Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol, 112*(4), 389-404. doi:10.1007/s00401-006-0127-z

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging, 24*(2), 197-211.

Brahmachari, S., Ge, P., Lee, S. H., Kim, D., Karuppagounder, S. S., Kumar, M., . . . Ko, H. S. (2016). Activation of tyrosine kinase c-Abl contributes to alpha-synuclein-induced neurodegeneration. *J Clin Invest, 126*(8), 2970-2988. doi:10.1172/jci85456

Breteler, M. M. (2000). Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. *Neurobiol Aging, 21*(2), 153-160.

Campioni, S., Mannini, B., Zampagni, M., Pensalfini, A., Parrini, C., Evangelisti, E., . . . Chiti, F. (2010). A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol, 6*(2), 140-147. doi:10.1038/nchembio.283

Cersosimo, M. G., & Benarroch, E. E. (2012). Autonomic involvement in Parkinson's disease: pathology, pathophysiology, clinical features and possible peripheral biomarkers. *J Neurol Sci, 313*(1-2), 57-63. doi:10.1016/j.jns.2011.09.030

Chaudhuri, K. R., & Schapira, A. H. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol, 8*(5), 464-474. doi:10.1016/s1474-4422(09)70068-7

Chen-Plotkin, A. S., Albin, R., Alcalay, R., Babcock, D., Bajaj, V., Bowman, D., . . . Zhang, J. (2018). Finding useful biomarkers for Parkinson's disease. *Sci Transl Med, 10*(454). doi:10.1126/scitranslmed.aam6003

Cholerton, B. A., Zabetian, C. P., Quinn, J. F., Chung, K. A., Peterson, A., Espay, A. J., . . . Leverenz, J. B. (2013). Pacific Northwest Udall Center of excellence clinical consortium: study design and baseline cohort characteristics. *J Parkinsons Dis, 3*(2), 205-214. doi:10.3233/JPD-130189

ClinicalTrials.gov. Retrieved from (https://clinicaltrials.gov/ct2/show/NCT03205488?term=nilotinib+parkinsons&cond=Parkinson+Disease&rank=2

ClinicalTrials.gov. Retrieved from https://clinicaltrials.gov/ct2/show/NCT01983358?cond=parp1+inhibitor&rank=5

ClinicalTrials.gov. Retrieved from https://clinicaltrials.gov/ct2/show/NCT02500901?cond=parp1+inhibitor&draw=1&rank=6

ClinicalTrials.gov. Retrieved from https://clinicaltrials.gov/ct2/show/NCT01560754?term=nicotine&cond=Parkinson+Disease&rank=1

ClinicalTrials.gov. Retrieved from https://clinicaltrials.gov/ct2/show/NCT02782481?term=subcutaneous+levodopa&cond=Parkinson+Disease&draw=1&rank=3

Cummings, J., Isaacson, S., Mills, R., Williams, H., Chi-Burris, K., Corbett, A., . . . Ballard, C. (2014). Pimavanserin for patients with Parkinson's disease psychosis: a randomised, placebo-controlled phase 3 trial. *Lancet, 383*(9916), 533-540. doi:10.1016/s0140-6736(13)62106-6

Danzer, K. M., Haasen, D., Karow, A. R., Moussaud, S., Habeck, M., Giese, A., . . . Kostka, M. (2007). Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci, 27*(34), 9220-9232. doi:10.1523/jneurosci.2617-07.2007

Dietrichs, E., & Odin, P. (2017). Algorithms for the treatment of motor problems in Parkinson's disease. *Acta Neurol Scand*. doi:10.1111/ane.12733

Ding, H., Dhima, K., Lockhart, K. C., Locascio, J. J., Hoesing, A. N., Duong, K., . . . Scherzer, C. R. (2013). Unrecognized vitamin D3 deficiency is common in

Parkinson disease: Harvard Biomarker Study. *Neurology, 81*(17), 1531-1537. doi:10.1212/WNL.0b013e3182a95818

Dissanayaka, N. N. W., Lawson, R. A., Yarnall, A. J., Duncan, G. W., Breen, D. P., Khoo, T. K., . . . group, I.-P. s. (2017). Anxiety is associated with cognitive impairment in newly-diagnosed Parkinson's disease. *Parkinsonism Relat Disord, 36*, 63-68. doi:10.1016/j.parkreldis.2017.01.001

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., . . . Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology, 68*(5), 384-386. doi:10.1212/01.wnl.0000247740.47667.03

Ebersbach, G., Grust, U., Ebersbach, A., Wegner, B., Gandor, F., & Kuhn, A. A. (2015). Amplitude-oriented exercise in Parkinson's disease: a randomized study comparing LSVT-BIG and a short training protocol. *J Neural Transm (Vienna), 122*(2), 253-256. doi:10.1007/s00702-014-1245-8

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Cooper, L. J., Fullwood, N. J., Gibson, M. J., . . . Allsop, D. (2003). Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *Faseb j, 17*(13), 1945-1947. doi:10.1096/fj.03-0098fje

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Curran, M. D., Gibson, M. J., Court, J. A., . . . Allsop, D. (2006). Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *Faseb j, 20*(3), 419-425. doi:10.1096/fj.03-1449com

Emre, M., Aarsland, D., Brown, R., Burn, D. J., Duyckaerts, C., Mizuno, Y., . . . Dubois, B. (2007). Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord, 22*(12), 1689-1707; quiz 1837. doi:10.1002/mds.21507

Fasano, A., Daniele, A., & Albanese, A. (2012). Treatment of motor and non-motor features of Parkinson's disease with deep brain stimulation. *Lancet Neurol, 11*(5), 429-442. doi:10.1016/s1474-4422(12)70049-2

Giannantoni, A., Rossi, A., Mearini, E., Del Zingaro, M., Porena, M., & Berardelli, A. (2009). Botulinum toxin A for overactive bladder and detrusor muscle overactivity in patients with Parkinson's disease and multiple system atrophy. *J Urol, 182*(4), 1453-1457. doi:10.1016/j.juro.2009.06.023

Giasson, B. I., Forman, M. S., Higuchi, M., Golbe, L. I., Graves, C. L., Kotzbauer, P. T., . . . Lee, V. M. (2003). Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science, 300*(5619), 636-640. doi:10.1126/science.1082324

Golde, T. E., Borchelt, D. R., Giasson, B. I., & Lewis, J. (2013). Thinking laterally about neurodegenerative proteinopathies. *J Clin Invest, 123*(5), 1847-1855. doi:10.1172/jci66029

Goldman, S. M. (2014). Environmental toxins and Parkinson's disease. *Annu Rev Pharmacol Toxicol, 54*, 141-164. doi:10.1146/annurev-pharmtox-011613-135937

Goodwin, V. A., Richards, S. H., Taylor, R. S., Taylor, A. H., & Campbell, J. L. (2008). The effectiveness of exercise interventions for people with Parkinson's disease: a systematic review and meta-analysis. *Mov Disord, 23*(5), 631-640. doi:10.1002/mds.21922

Gottesman, R. F., Albert, M. S., Alonso, A., Coker, L. H., Coresh, J., Davis, S. M., . . . Knopman, D. S. (2017). Associations Between Midlife Vascular Risk Factors and 25-Year Incident Dementia in the Atherosclerosis Risk in Communities (ARIC) Cohort. *JAMA Neurol, 74*(10), 1246-1254. doi:10.1001/jamaneurol.2017.1658

Gottesman, R. F., Schneider, A. L., Zhou, Y., Coresh, J., Green, E., Gupta, N., . . . Mosley, T. H. (2017). Association Between Midlife Vascular Risk Factors and Estimated Brain Amyloid Deposition. *Jama, 317*(14), 1443-1450. doi:10.1001/jama.2017.3090

Guo, J. L., Covell, D. J., Daniels, J. P., Iba, M., Stieber, A., Zhang, B., . . . Lee, V. M. (2013). Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. *Cell, 154*(1), 103-117. doi:10.1016/j.cell.2013.05.057

Guo, J. L., & Lee, V. M. (2014). Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat Med, 20*(2), 130-138. doi:10.1038/nm.3457

Gwinn, K., David, K. K., Swanson-Fischer, C., Albin, R., Hillaire-Clarke, C. S., Sieber, B. A., . . . Rosenthal, L. S. (2017). Parkinson's disease biomarkers: perspective from the NINDS Parkinson's Disease Biomarkers Program. *Biomark Med, 11*(6), 451-473. doi:10.2217/bmm-2016-0370

Hall, S., Ohrfelt, A., Constantinescu, R., Andreasson, U., Surova, Y., Bostrom, F., . . . Hansson, O. (2012). Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. *Arch Neurol, 69*(11), 1445-1452. doi:10.1001/archneurol.2012.1654

Hamilton, M. (1959). The assessment of anxiety states by rating. *Br J Med Psychol, 32*(1), 50-55.

Hamza, T. H., Chen, H., Hill-Burns, E. M., Rhodes, S. L., Montimurro, J., Kay, D. M., . . . Payami, H. (2011). Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. *PLoS Genet, 7*(8), e1002237. doi:10.1371/journal.pgen.1002237

Hanai, S., Kanai, M., Ohashi, S., Okamoto, K., Yamada, M., Takahashi, H., & Miwa, M. (2004). Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in Drosophila melanogaster. *Proc Natl Acad Sci U S A, 101*(1), 82-86. doi:10.1073/pnas.2237114100

Hebron, M. L., Lonskaya, I., & Moussa, C. E. (2013). Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of alpha-synuclein in Parkinson's disease models. *Hum Mol Genet, 22*(16), 3315-3328. doi:10.1093/hmg/ddt192

Hebron, M. L., Lonskaya, I., Olopade, P., Selby, S. T., Pagan, F., & Moussa, C. E. (2014). Tyrosine Kinase Inhibition Regulates Early Systemic Immune Changes and Modulates the Neuroimmune Response in alpha-Synucleinopathy. *J Clin Cell Immunol, 5*, 259. doi:10.4172/2155-9899.1000259

Hely, M. A., Reid, W. G., Adena, M. A., Halliday, G. M., & Morris, J. G. (2008). The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov Disord, 23*(6), 837-844. doi:10.1002/mds.21956

Hong, Z., Shi, M., Chung, K. A., Quinn, J. F., Peskind, E. R., Galasko, D., . . . Zhang, J. (2010). DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain, 133*(Pt 3), 713-726. doi:10.1093/brain/awq008

Hughes, A. J., Daniel, S. E., Kilford, L., & Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry, 55*(3), 181-184.

Imam, S. Z., Trickler, W., Kimura, S., Binienda, Z. K., Paule, M. G., Slikker, W., Jr., . . . Ali, S. F. (2013). Neuroprotective efficacy of a new brain-penetrating C-Abl inhibitor in a murine Parkinson's disease model. *PLoS One, 8*(5), e65129. doi:10.1371/journal.pone.0065129

Imam, S. Z., Zhou, Q., Yamamoto, A., Valente, A. J., Ali, S. F., Bains, M., . . . Li, S. (2011). Novel regulation of parkin function through c-Abl-mediated tyrosine phosphorylation: implications for Parkinson's disease. *J Neurosci, 31*(1), 157-163. doi:10.1523/jneurosci.1833-10.2011

Inglis, K. J., Chereau, D., Brigham, E. F., Chiou, S. S., Schobel, S., Frigon, N. L., . . . Anderson, J. P. (2009). Polo-like kinase 2 (PLK2) phosphorylates alpha-synuclein at serine 129 in central nervous system. *J Biol Chem, 284*(5), 2598-2602. doi:10.1074/jbc.C800206200

Irwin, D. J., Grossman, M., Weintraub, D., Hurtig, H. I., Duda, J. E., Xie, S. X., . . . Trojanowski, J. Q. (2017). Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol, 16*(1), 55-65. doi:10.1016/S1474-4422(16)30291-5

Irwin, D. J., Lee, V. M., & Trojanowski, J. Q. (2013). Parkinson's disease dementia: convergence of alpha-synuclein, tau and amyloid-beta pathologies. *Nat Rev Neurosci, 14*(9), 626-636. doi:10.1038/nrn3549

Irwin, D. J., White, M. T., Toledo, J. B., Xie, S. X., Robinson, J. L., Van Deerlin, V., . . . Trojanowski, J. Q. (2012). Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol, 72*(4), 587-598. doi:10.1002/ana.23659

Iwanaga, K., Wakabayashi, K., Yoshimoto, M., Tomita, I., Satoh, H., Takashima, H., . . . Takahashi, H. (1999). Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. *Neurology, 52*(6), 1269-1271.

Jang, A., Lee, H. J., Suk, J. E., Jung, J. W., Kim, K. P., & Lee, S. J. (2010). Non-classical exocytosis of alpha-synuclein is sensitive to folding states and promoted under stress conditions. *J Neurochem, 113*(5), 1263-1274. doi:10.1111/j.1471-4159.2010.06695.x

Jankovic, J. (2008). Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry, 79*(4), 368-376. doi:10.1136/jnnp.2007.131045

Jones, D. R., Delenclos, M., Baine, A. T., DeTure, M., Murray, M. E., Dickson, D. W., & McLean, P. J. (2015). Transmission of Soluble and Insoluble alpha-Synuclein to Mice. *J Neuropathol Exp Neurol, 74*(12), 1158-1169. doi:10.1097/nen.0000000000000262

Kam, T. I., Mao, X., Park, H., Chou, S. C., Karuppagounder, S. S., Umanah, G. E., . . . Dawson, V. L. (2018). Poly(ADP-ribose) drives pathologic alpha-synuclein neurodegeneration in Parkinson's disease. *Science, 362*(6414). doi:10.1126/science.aat8407

Kamel, F., Tanner, C., Umbach, D., Hoppin, J., Alavanja, M., Blair, A., . . . Sandler, D. (2007). Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J Epidemiol, 165*(4), 364-374. doi:10.1093/aje/kwk024

Kang, J. H. (2016). Cerebrospinal Fluid Amyloid beta1-42, Tau, and Alpha-Synuclein Predict the Heterogeneous Progression of Cognitive Dysfunction in Parkinson's Disease. *J Mov Disord, 9*(2), 89-96. doi:10.14802/jmd.16017

Kang, J. H., Mollenhauer, B., Coffey, C. S., Toledo, J. B., Weintraub, D., Galasko, D. R., . . . Parkinson's Progression Marker, I. (2016). CSF biomarkers associated with disease heterogeneity in early Parkinson's disease: the Parkinson's Progression Markers Initiative study. *Acta Neuropathol, 131*(6), 935-949. doi:10.1007/s00401-016-1552-2

Karuppagounder, S. S., Brahmachari, S., Lee, Y., Dawson, V. L., Dawson, T. M., & Ko, H. S. (2014). The c-Abl inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. *Sci Rep, 4*, 4874. doi:10.1038/srep04874

Kaushik, S., Massey, A. C., Mizushima, N., & Cuervo, A. M. (2008). Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. *Mol Biol Cell, 19*(5), 2179-2192. doi:10.1091/mbc.E07-11-1155

Kayed, R., Sokolov, Y., Edmonds, B., McIntire, T. M., Milton, S. C., Hall, J. E., & Glabe, C. G. (2004). Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J Biol Chem, 279*(45), 46363-46366. doi:10.1074/jbc.C400260200

Khalaf, O., Fauvet, B., Oueslati, A., Dikiy, I., Mahul-Mellier, A. L., Ruggeri, F. S., . . . Lashuel, H. A. (2014). The H50Q mutation enhances alpha-synuclein aggregation, secretion, and toxicity. *J Biol Chem, 289*(32), 21856-21876. doi:10.1074/jbc.M114.553297

Khan, N. L., Graham, E., Critchley, P., Schrag, A. E., Wood, N. W., Lees, A. J., . . . Quinn, N. (2003). Parkin disease: a phenotypic study of a large case series. *Brain, 126*(Pt 6), 1279-1292.

Ko, H. S., Kim, S. W., Sriram, S. R., Dawson, V. L., & Dawson, T. M. (2006). Identification of far upstream element-binding protein-1 as an authentic Parkin substrate. *J Biol Chem, 281*(24), 16193-16196. doi:10.1074/jbc.C600041200

Ko, H. S., Lee, Y., Shin, J. H., Karuppagounder, S. S., Gadad, B. S., Koleske, A. J., . . . Dawson, T. M. (2010). Phosphorylation by the c-Abl protein tyrosine kinase inhibits parkin's ubiquitination and protective function. *Proc Natl Acad Sci U S A, 107*(38), 16691-16696. doi:10.1073/pnas.1006083107

Koga, H., Martinez-Vicente, M., Macian, F., Verkhusha, V. V., & Cuervo, A. M. (2011). A photoconvertible fluorescent reporter to track chaperone-mediated autophagy. *Nat Commun, 2*, 386. doi:10.1038/ncomms1393

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B., & Olanow, C. W. (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med, 14*(5), 504-506. doi:10.1038/nm1747

Kordower, J. H., Chu, Y., Hauser, R. A., Olanow, C. W., & Freeman, T. B. (2008). Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. *Mov Disord, 23*(16), 2303-2306. doi:10.1002/mds.22369

Lang, A. E., & Espay, A. J. (2018). Disease Modification in Parkinson's Disease: Current Approaches, Challenges, and Future Considerations. *Mov Disord, 33*(5), 660-677. doi:10.1002/mds.27360

Lee, H. J., Baek, S. M., Ho, D. H., Suk, J. E., Cho, E. D., & Lee, S. J. (2011). Dopamine promotes formation and secretion of non-fibrillar alpha-synuclein oligomers. *Exp Mol Med, 43*(4), 216-222. doi:10.3858/emm.2011.43.4.026

Lee, H. J., Cho, E. D., Lee, K. W., Kim, J. H., Cho, S. G., & Lee, S. J. (2013). Autophagic failure promotes the exocytosis and intercellular transfer of alpha-synuclein. *Exp Mol Med, 45*, e22. doi:10.1038/emm.2013.45

Lee, H. J., Patel, S., & Lee, S. J. (2005). Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci, 25*(25), 6016-6024. doi:10.1523/jneurosci.0692-05.2005

Lee, J. S., & Lee, S. J. (2016). Mechanism of Anti-alpha-Synuclein Immunotherapy. *J Mov Disord, 9*(1), 14-19. doi:10.14802/jmd.15059

Lee, Y., Karuppagounder, S. S., Shin, J. H., Lee, Y. I., Ko, H. S., Swing, D., . . . Dawson, T. M. (2013). Parthanatos mediates AIMP2-activated age-dependent dopaminergic neuronal loss. *Nat Neurosci, 16*(10), 1392-1400. doi:10.1038/nn.3500

LeWitt, P. A., Hauser, R. A., Grosset, D. G., Stocchi, F., Saint-Hilaire, M. H., Ellenbogen, A., . . . Kieburtz, K. D. (2016). A randomized trial of inhaled levodopa (CVT-301) for motor fluctuations in Parkinson's disease. *Mov Disord, 31*(9), 1356-1365. doi:10.1002/mds.26611

Li, F., Harmer, P., Fitzgerald, K., Eckstrom, E., Stock, R., Galver, J., . . . Batya, S. S. (2012). Tai chi and postural stability in patients with Parkinson's disease. *N Engl J Med, 366*(6), 511-519. doi:10.1056/NEJMoa1107911

Li, G., Xiong, K., Korff, A., Pan, C., Quinn, J. F., Galasko, D. R., . . . Zhang, J. (2015). Increased CSF E-Selectin in Clinical Alzheimer's Disease without Altered CSF Abeta42 and Tau. *J Alzheimers Dis, 47*(4), 883-887. doi:10.3233/JAD-150420

Li, J., Uversky, V. N., & Fink, A. L. (2001). Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human alpha-synuclein. *Biochemistry, 40*(38), 11604-11613.

Love, S., & Miners, J. S. (2016). Cerebrovascular disease in ageing and Alzheimer's disease. *Acta Neuropathol, 131*(5), 645-658. doi:10.1007/s00401-015-1522-0

Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q., & Lee, V. M. (2012). Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science, 338*(6109), 949-953. doi:10.1126/science.1227157

Ma, C., Liu, Y., Neumann, S., & Gao, X. (2017). Nicotine from cigarette smoking and diet and Parkinson disease: a review. *Transl Neurodegener, 6*, 18. doi:10.1186/s40035-017-0090-8

Mahul-Mellier, A. L., Fauvet, B., Gysbers, A., Dikiy, I., Oueslati, A., Georgeon, S., . . . Lashuel, H. A. (2014). c-Abl phosphorylates alpha-synuclein and regulates its degradation: implication for alpha-synuclein clearance and contribution to the pathogenesis of Parkinson's disease. *Hum Mol Genet, 23*(11), 2858-2879. doi:10.1093/hmg/ddt674

Mandir, A. S., Przedborski, S., Jackson-Lewis, V., Wang, Z. Q., Simbulan-Rosenthal, C. M., Smulson, M. E., . . . Dawson, T. M. (1999). Poly(ADP-ribose) polymerase

activation mediates 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. *Proc Natl Acad Sci U S A, 96*(10), 5774-5779.

Martinez-Fernandez, R., Schmitt, E., Martinez-Martin, P., & Krack, P. (2016). The hidden sister of motor fluctuations in Parkinson's disease: A review on nonmotor fluctuations. *Mov Disord, 31*(8), 1080-1094. doi:10.1002/mds.26731

Martire, S., Mosca, L., & d'Erme, M. (2015). PARP-1 involvement in neurodegeneration: A focus on Alzheimer's and Parkinson's diseases. *Mech Ageing Dev, 146-148*, 53-64. doi:10.1016/j.mad.2015.04.001

Massey, A. C., Kaushik, S., Sovak, G., Kiffin, R., & Cuervo, A. M. (2006). Consequences of the selective blockage of chaperone-mediated autophagy. *Proc Natl Acad Sci U S A, 103*(15), 5805-5810. doi:10.1073/pnas.0507436103

Masters, J. M., Noyce, A. J., Warner, T. T., Giovannoni, G., & Proctor, G. B. (2015). Elevated salivary protein in Parkinson's disease and salivary DJ-1 as a potential marker of disease severity. *Parkinsonism Relat Disord, 21*(10), 1251-1255. doi:10.1016/j.parkreldis.2015.07.021

McKeith, I. G., Boeve, B. F., Dickson, D. W., Halliday, G., Taylor, J. P., Weintraub, D., . . . Kosaka, K. (2017). Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. *Neurology, 89*(1), 88-100. doi:10.1212/WNL.0000000000004058

Miller, D. W., Hague, S. M., Clarimon, J., Baptista, M., Gwinn-Hardy, K., Cookson, M. R., & Singleton, A. B. (2004). Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology, 62*(10), 1835-1838.

Mills, K. A., Mari, Z., Bakker, C., Johnson, V., Pontone, G. M., Pantelyat, A., . . . Rosenthal, L. S. (2016). Gait function and locus coeruleus Lewy body pathology in 51 Parkinson's disease patients. *Parkinsonism Relat Disord, 33*, 102-106. doi:10.1016/j.parkreldis.2016.09.024

Minguez-Castellanos, A., Chamorro, C. E., Escamilla-Sevilla, F., Ortega-Moreno, A., Rebollo, A. C., Gomez-Rio, M., . . . Munoz, D. G. (2007). Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders?: a cohort study. *Neurology, 68*(23), 2012-2018. doi:10.1212/01.wnl.0000264429.59379.d9

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., . . . Berg, L. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology, 41*(4), 479-486.

Mohammadi, D. (2013). The Harvard Biomarker Study's big plan. *Lancet Neurol, 12*(8), 739-740. doi:10.1016/s1474-4422(13)70155-8

Mollenhauer, B., El-Agnaf, O. M., Marcus, K., Trenkwalder, C., & Schlossmacher, M. G. (2010). Quantification of alpha-synuclein in cerebrospinal fluid as a biomarker candidate: review of the literature and considerations for future studies. *Biomark Med, 4*(5), 683-699. doi:10.2217/bmm.10.90

Montine, T. J., Shi, M., Quinn, J. F., Peskind, E. R., Craft, S., Ginghina, C., . . . Zhang, J. (2010). CSF Abeta(42) and tau in Parkinson's disease with cognitive impairment. *Mov Disord, 25*(15), 2682-2685. doi:10.1002/mds.23287

Morales, R., Duran-Aniotz, C., Diaz-Espinoza, R., Camacho, M. V., & Soto, C. (2012). Protein misfolding cyclic amplification of infectious prions. *Nat Protoc, 7*(7), 1397-1409. doi:10.1038/nprot.2012.067

Narhi, L., Wood, S. J., Steavenson, S., Jiang, Y., Wu, G. M., Anafi, D., . . . Citron, M. (1999). Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation. *J Biol Chem, 274*(14), 9843-9846.

Olanow, C. W., Obeso, J. A., & Stocchi, F. (2006). Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Nat Clin Pract Neurol, 2*(7), 382-392. doi:10.1038/ncpneuro0222

Osterberg, V. R., Spinelli, K. J., Weston, L. J., Luk, K. C., Woltjer, R. L., & Unni, V. K. (2015). Progressive aggregation of alpha-synuclein and selective degeneration of lewy inclusion-bearing neurons in a mouse model of parkinsonism. *Cell Rep, 10*(8), 1252-1260. doi:10.1016/j.celrep.2015.01.060

Oueslati, A., Schneider, B. L., Aebischer, P., & Lashuel, H. A. (2013). Polo-like kinase 2 regulates selective autophagic alpha-synuclein clearance and suppresses its toxicity in vivo. *Proc Natl Acad Sci U S A, 110*(41), E3945-3954. doi:10.1073/pnas.1309991110

Pachana, N. A., Egan, S. J., Laidlaw, K., Dissanayaka, N., Byrne, G. J., Brockman, S., . . . Starkstein, S. (2013). Clinical issues in the treatment of anxiety and depression in older adults with Parkinson's disease. *Mov Disord, 28*(14), 1930-1934. doi:10.1002/mds.25689

Pagan, F., Hebron, M., Valadez, E. H., Torres-Yaghi, Y., Huang, X., Mills, R. R., . . . Moussa, C. (2016). Nilotinib Effects in Parkinson's disease and Dementia with Lewy bodies. *J Parkinsons Dis, 6*(3), 503-517. doi:10.3233/jpd-160867

Periquet, M., Fulga, T., Myllykangas, L., Schlossmacher, M. G., & Feany, M. B. (2007). Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. *J Neurosci, 27*(12), 3338-3346. doi:10.1523/jneurosci.0285-07.2007

Pontes-Neto, O. M., Auriel, E., & Greenberg, S. M. (2012). Advances in our Understanding of the Pathophysiology, Detection and Management of Cerebral Amyloid Angiopathy. *Eur Neurol Rev, 7*(2), 134-139.

Pontone, G. M., Bakker, C. C., Chen, S., Mari, Z., Marsh, L., Rabins, P. V., . . . Bassett, S. S. (2016). The longitudinal impact of depression on disability in Parkinson disease. *Int J Geriatr Psychiatry, 31*(5), 458-465. doi:10.1002/gps.4350

Pontone, G. M., Williams, J. R., Anderson, K. E., Chase, G., Goldstein, S. A., Grill, S., . . . Marsh, L. (2009). Prevalence of anxiety disorders and anxiety subtypes in patients with Parkinson's disease. *Mov Disord, 24*(9), 1333-1338. doi:10.1002/mds.22611

Popat, R. A., Van Den Eeden, S. K., Tanner, C. M., Kamel, F., Umbach, D. M., Marder, K., . . . Nelson, L. M. (2011). Coffee, ADORA2A, and CYP1A2: the caffeine connection in Parkinson's disease. *Eur J Neurol, 18*(5), 756-765. doi:10.1111/j.1468-1331.2011.03353.x

Postuma, R. B., Poewe, W., Litvan, I., Lewis, S., Lang, A. E., Halliday, G., . . . Berg, D. (2018). Validation of the MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord, 33*(10), 1601-1608. doi:10.1002/mds.27362

Pouclet, H., Lebouvier, T., Coron, E., des Varannes, S. B., Rouaud, T., Roy, M., . . . Derkinderen, P. (2012). A comparison between rectal and colonic biopsies to detect Lewy pathology in Parkinson's disease. *Neurobiol Dis, 45*(1), 305-309. doi:10.1016/j.nbd.2011.08.014

PPMI. Retrieved from http://www.ppmi-info.org

Qiang, J. K., Wong, Y. C., Siderowf, A., Hurtig, H. I., Xie, S. X., Lee, V. M., . . . Chen-Plotkin, A. S. (2013). Plasma apolipoprotein A1 as a biomarker for Parkinson disease. *Ann Neurol, 74*(1), 119-127. doi:10.1002/ana.23872

Rajabally, Y. A., & Martey, J. (2011). Neuropathy in Parkinson disease: prevalence and determinants. *Neurology, 77*(22), 1947-1950. doi:10.1212/WNL.0b013e31823a0ee4

Rascol, O., Brooks, D. J., Korczyn, A. D., De Deyn, P. P., Clarke, C. E., & Lang, A. E. (2000). A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. *N Engl J Med, 342*(20), 1484-1491. doi:10.1056/nejm200005183422004

Ritz, B., Lee, P. C., Lassen, C. F., & Arah, O. A. (2014). Parkinson disease and smoking revisited: ease of quitting is an early sign of the disease. *Neurology, 83*(16), 1396-1402. doi:10.1212/wnl.0000000000000879

Rosenthal, L. S., Drake, D., Alcalay, R. N., Babcock, D., Bowman, F. D., Chen-Plotkin, A., . . . Gwinn, K. (2016). The NINDS Parkinson's disease biomarkers program. *Mov Disord, 31*(6), 915-923. doi:10.1002/mds.26438

Ryan, B. J., Hoek, S., Fon, E. A., & Wade-Martins, R. (2015). Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends Biochem Sci, 40*(4), 200-210. doi:10.1016/j.tibs.2015.02.003

Saa, P., Castilla, J., & Soto, C. (2006). Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. *J Biol Chem, 281*(46), 35245-35252. doi:10.1074/jbc.M603964200

Saborio, G. P., Permanne, B., & Soto, C. (2001). Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature, 411*(6839), 810-813. doi:10.1038/35081095

Salvadores, N., Shahnawaz, M., Scarpini, E., Tagliavini, F., & Soto, C. (2014). Detection of misfolded Abeta oligomers for sensitive biochemical diagnosis of Alzheimer's disease. *Cell Rep, 7*(1), 261-268. doi:10.1016/j.celrep.2014.02.031

Sanchez-Ferro, A., Rabano, A., Catalan, M. J., Rodriguez-Valcarcel, F. C., Fernandez Diez, S., Herreros-Rodriguez, J., . . . Molina-Arjona, J. A. (2015). In vivo gastric detection of alpha-synuclein inclusions in Parkinson's disease. *Mov Disord, 30*(4), 517-524. doi:10.1002/mds.25988

Scherzer, C. R., Eklund, A. C., Morse, L. J., Liao, Z., Locascio, J. J., Fefer, D., . . . Gullans, S. R. (2007). Molecular markers of early Parkinson's disease based on gene expression in blood. *Proc Natl Acad Sci U S A, 104*(3), 955-960. doi:10.1073/pnas.0610204104

Schrag, A., Siddiqui, U. F., Anastasiou, Z., Weintraub, D., & Schott, J. M. (2017). Clinical variables and biomarkers in prediction of cognitive impairment in patients with

newly diagnosed Parkinson's disease: a cohort study. *Lancet Neurol, 16*(1), 66-75. doi:10.1016/S1474-4422(16)30328-3

Schwartz, R. S., Halliday, G. M., Soh, D., Cordato, D. J., & Kril, J. J. (2018). Impact of small vessel disease on severity of motor and cognitive impairment in Parkinson's disease. *J Clin Neurosci, 58*, 70-74. doi:10.1016/j.jocn.2018.10.029

Scott, L., Dawson, V. L., & Dawson, T. M. (2017). Trumping neurodegeneration: Targeting common pathways regulated by autosomal recessive Parkinson's disease genes. *Exp Neurol, 298*(Pt B), 191-201. doi:10.1016/j.expneurol.2017.04.008

Seppi, K., Weintraub, D., Coelho, M., Perez-Lloret, S., Fox, S. H., Katzenschlager, R., . . . Sampaio, C. (2011). The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for the non-motor symptoms of Parkinson's disease. *Mov Disord, 26 Suppl 3*, S42-80. doi:10.1002/mds.23884

Shahnawaz, M., Tokuda, T., Waragai, M., Mendez, N., Ishii, R., Trenkwalder, C., . . . Soto, C. (2017). Development of a Biochemical Diagnosis of Parkinson Disease by Detection of alpha-Synuclein Misfolded Aggregates in Cerebrospinal Fluid. *JAMA Neurol, 74*(2), 163-172. doi:10.1001/jamaneurol.2016.4547

Shi, M., Bradner, J., Hancock, A. M., Chung, K. A., Quinn, J. F., Peskind, E. R., . . . Zhang, J. (2011). Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Ann Neurol, 69*(3), 570-580. doi:10.1002/ana.22311

Shi, M., Furay, A. R., Sossi, V., Aasly, J. O., Armaly, J., Wang, Y., . . . Zhang, J. (2012). DJ-1 and alphaSYN in LRRK2 CSF do not correlate with striatal dopaminergic function. *Neurobiol Aging, 33*(4), 836.e835-837. doi:10.1016/j.neurobiolaging.2011.09.015

Shi, M., Liu, C., Cook, T. J., Bullock, K. M., Zhao, Y., Ginghina, C., . . . Zhang, J. (2014). Plasma exosomal alpha-synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta Neuropathol, 128*(5), 639-650. doi:10.1007/s00401-014-1314-y

Shi, M., Zabetian, C. P., Hancock, A. M., Ginghina, C., Hong, Z., Yearout, D., . . . Zhang, J. (2010). Significance and confounders of peripheral DJ-1 and alpha-synuclein in Parkinson's disease. *Neurosci Lett, 480*(1), 78-82. doi:10.1016/j.neulet.2010.06.009

Shin, J. H., Ko, H. S., Kang, H., Lee, Y., Lee, Y. I., Pletinkova, O., . . . Dawson, T. M. (2011). PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell, 144*(5), 689-702. doi:10.1016/j.cell.2011.02.010

Simonsen, A. H., Kuiperij, B., El-Agnaf, O. M., Engelborghs, S., Herukka, S. K., Parnetti, L., . . . Mollenhauer, B. (2016). The utility of alpha-synuclein as biofluid marker in neurodegenerative diseases: a systematic review of the literature. *Biomark Med, 10*(1), 19-34. doi:10.2217/bmm.14.105

Soh, S. E., Morris, M. E., & McGinley, J. L. (2011). Determinants of health-related quality of life in Parkinson's disease: a systematic review. *Parkinsonism Relat Disord, 17*(1), 1-9. doi:10.1016/j.parkreldis.2010.08.012

Stewart, T., Liu, C., Ginghina, C., Cain, K. C., Auinger, P., Cholerton, B., . . . Zhang, J. (2014). Cerebrospinal fluid alpha-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort. *Am J Pathol, 184*(4), 966-975. doi:10.1016/j.ajpath.2013.12.007

Stojkovic, T., Stefanova, E., Soldatovic, I., Markovic, V., Stankovic, I., Petrovic, I., . . . Kostic, V. (2018). Exploring the relationship between motor impairment, vascular burden and cognition in Parkinson's disease. *J Neurol, 265*(6), 1320-1327. doi:10.1007/s00415-018-8838-3

Sturkenboom, I. H., Graff, M. J., Borm, G. F., Veenhuizen, Y., Bloem, B. R., Munneke, M., & Nijhuis-van der Sanden, M. W. (2013). The impact of occupational therapy in Parkinson's disease: a randomized controlled feasibility study. *Clin Rehabil, 27*(2), 99-112. doi:10.1177/0269215512448382

Sun, X., Majumder, P., Shioya, H., Wu, F., Kumar, S., Weichselbaum, R., . . . Kufe, D. (2000). Activation of the cytoplasmic c-Abl tyrosine kinase by reactive oxygen species. *J Biol Chem, 275*(23), 17237-17240. doi:10.1074/jbc.C000099200

Surmeier, D. J., & Sulzer, D. (2013). The pathology roadmap in Parkinson disease. *Prion, 7*(1), 85-91. doi:10.4161/pri.23582

Svenningsson, P., Westman, E., Ballard, C., & Aarsland, D. (2012). Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment. *Lancet Neurol, 11*(8), 697-707. doi:10.1016/S1474-4422(12)70152-7

Swanson, C. R., Li, K., Unger, T. L., Gallagher, M. D., Van Deerlin, V. M., Agarwal, P., . . . Chen-Plotkin, A. S. (2015). Lower plasma apolipoprotein A1 levels are found in Parkinson's disease and associate with apolipoprotein A1 genotype. *Mov Disord, 30*(6), 805-812. doi:10.1002/mds.26022

Tanabe, A., Yamamura, Y., Kasahara, J., Morigaki, R., Kaji, R., & Goto, S. (2014). A novel tyrosine kinase inhibitor AMN107 (nilotinib) normalizes striatal motor

behaviors in a mouse model of Parkinson's disease. *Front Cell Neurosci, 8*, 50. doi:10.3389/fncel.2014.00050

Tanner, C. M., Ross, G. W., Jewell, S. A., Hauser, R. A., Jankovic, J., Factor, S. A., . . . Langston, J. W. (2009). Occupation and risk of parkinsonism: a multicenter case-control study. *Arch Neurol, 66*(9), 1106-1113. doi:10.1001/archneurol.2009.195

Tolosa, E., Marti, M. J., Valldeoriola, F., & Molinuevo, J. L. (1998). History of levodopa and dopamine agonists in Parkinson's disease treatment. *Neurology, 50*(6 Suppl 6), S2-10; discussion S44-18.

Tomlinson, C. L., Stowe, R., Patel, S., Rick, C., Gray, R., & Clarke, C. E. (2010). Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord, 25*(15), 2649-2653. doi:10.1002/mds.23429

Virag, L. (2005). Structure and function of poly(ADP-ribose) polymerase-1: role in oxidative stress-related pathologies. *Curr Vasc Pharmacol, 3*(3), 209-214.

Volpicelli-Daley, L. A., Luk, K. C., Patel, T. P., Tanik, S. A., Riddle, D. M., Stieber, A., . . . Lee, V. M. (2011). Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron, 72*(1), 57-71. doi:10.1016/j.neuron.2011.08.033

Vytal, K. E., Cornwell, B. R., Letkiewicz, A. M., Arkin, N. E., & Grillon, C. (2013). The complex interaction between anxiety and cognition: insight from spatial and verbal working memory. *Front Hum Neurosci, 7*, 93. doi:10.3389/fnhum.2013.00093

Wang, J. Y., Wu, J. N., Cherng, T. L., Hoffer, B. J., Chen, H. H., Borlongan, C. V., & Wang, Y. (2001). Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats. *Brain Res, 904*(1), 67-75.

Wang, Y., Shi, M., Chung, K. A., Zabetian, C. P., Leverenz, J. B., Berg, D., . . . Zhang, J. (2012). Phosphorylated alpha-synuclein in Parkinson's disease. *Sci Transl Med, 4*(121), 121ra120. doi:10.1126/scitranslmed.3002566

Weintraub, D., Koester, J., Potenza, M. N., Siderowf, A. D., Stacy, M., Voon, V., . . . Lang, A. E. (2010). Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Arch Neurol, 67*(5), 589-595. doi:10.1001/archneurol.2010.65

Winner, B., Jappelli, R., Maji, S. K., Desplats, P. A., Boyer, L., Aigner, S., . . . Riek, R. (2011). In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci U S A, 108*(10), 4194-4199. doi:10.1073/pnas.1100976108

Winslow, A. R., Chen, C. W., Corrochano, S., Acevedo-Arozena, A., Gordon, D. E., Peden, A. A., . . . Rubinsztein, D. C. (2010). alpha-Synuclein impairs macroautophagy: implications for Parkinson's disease. *J Cell Biol, 190*(6), 1023-1037. doi:10.1083/jcb.201003122

Xin, W., Emadi, S., Williams, S., Liu, Q., Schulz, P., He, P., . . . Sierks, M. R. (2015). Toxic Oligomeric Alpha-Synuclein Variants Present in Human Parkinson's Disease Brains Are Differentially Generated in Mammalian Cell Models. *Biomolecules, 5*(3), 1634-1651. doi:10.3390/biom5031634

Yu, Y. J., & Watts, R. J. (2013). Developing therapeutic antibodies for neurodegenerative disease. *Neurotherapeutics, 10*(3), 459-472. doi:10.1007/s13311-013-0187-4

Zange, L., Noack, C., Hahn, K., Stenzel, W., & Lipp, A. (2015). Phosphorylated alpha-synuclein in skin nerve fibres differentiates Parkinson's disease from multiple system atrophy. *Brain, 138*(Pt 8), 2310-2321. doi:10.1093/brain/awv138

Zhang, J., Pieper, A., & Snyder, S. H. (1995). Poly(ADP-ribose) synthetase activation: an early indicator of neurotoxic DNA damage. *J Neurochem, 65*(3), 1411-1414.

Curriculum Vitae

Liana Rosenthal was born on April 23, 1978 in Kansas City, Missouri. She received her undergraduate degree from Duke University in 2000 and medical degree from Johns Hopkins in 2006. She completed her residency in Neurology at Johns Hopkins in 2010 and fellowship at the Johns Hopkins Parkinson's Disease and Movement Disorders Center in 2012. She is currently an Assistant Professor of Neurology at Johns Hopkins University. She serves as the Director of the Clinical Core of the Morris K. Udall Center of Excellence for Parkinson's Disease Research and Director of the Ataxia Center. Her research focuses on the identification of biomarkers for Parkinson's disease and Parkinson's disease-related cognitive impairment with a growing interest in the identification of biomarkers for other neurodegenerative diseases including ataxia.

She has served as co-chair of the Steering Committee of the NINDS Parkinson's Disease biomarker program and is currently co-chair of the Parkinson's Study Group biomarker working group. She is also a member of the National Ataxia Foundation Medical Advisory Board and is on the planning committee for the 2020 Ataxia Investigator's Meeting. Together her efforts seek to apply translational and clinical science methodologies to basic science research discoveries, thus bringing to patients innovative and eventually disease modifying therapies.