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TRACKING AND UNDERSTANDING PARKINSON'S DISEASE PROGRESSION THROUGH THE LENS OF QUANTITATIVE MAGNETIC RESONANCE IMAGING

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ABSTRACT

Parkinson's disease (PD) is an age-related neurodegenerative disorder that typically begins with prodromal non-motor symptoms (such as loss of olfaction and sleep dysfunction) and relatively subtle motor changes (such as reduced arm swing). The current clinical diagnosis is based on the presence of classic motor symptoms such as bradykinesia, rigidity, and tremor at rest. As PD progresses, many patients develop severe motor (including gait and postural changes) and cognitive disability which ultimately lead to the loss of independence. The current treatment of PD is mainly based on management of symptoms, and there is no known therapy that can halt or reverse the progression of neuropathology. As life expectancies increase globally, PD is expected to double in prevalence over the next 25 years. Neuroprotective strategies that can modify the course of the disease, therefore, are critically needed. Past efforts to identify, understand and evaluate potentially neuroprotective treatments for PD, however, have been hindered by the lack of cellular or animal model that exactly mimic human disease, and the lack of objective and reliable *in vivo* markers of PD-related pathology in humans. The goals of this research were to identify structural magnetic resonance imaging (MRI) markers of PD progression and utilize these markers to understand the factors that may influence the progression of PD.

After providing an introduction that underscores the importance of my research goals (*Section I*) and the strengths and limitations of the approaches that I used (versus the current art of the field), I discuss in *Section II* the use of structural magnetic resonance imaging to explore gray matter changes in various stages of PD. Striatal atrophy was found to be related to early changes in PD, and cortical atrophy (particularly loss of cortical folding) was found to be associated with worsening clinical symptoms and longer disease duration. These findings were consistent with the

known pathologic trajectory of PD, where PD-related pathology affects brainstem and nigrostriatal structures well before the time of diagnosis, and Lewy pathology subsequently progresses in an ascending fashion to later involve higher-level cortex structures. In *Section III*, I examine the relationships between cortical gray matter atrophy and changes in subcortical white matter. Poorer subcortical white matter diffusion characteristics were found to be associated with cortical atrophy in PD, and opposite associations were found in control subjects.

These data provided the foundation for *Section IV,* where I utilized these newly described cortical imaging markers, along with changes in cognitive function, to explore the role of plasma cholesterol in PD. The link between plasma low density lipoprotein (LDL) cholesterol and PD had been pioneered by my research mentor's laboratory, and the studies described in this dissertation are the first attempts to associate LDL-cholesterol with specific *in vivo* cortical changes in PD. This relationship, if proven, may be a topic of high public health relevance, given the widespread use of cholesterol-lowering therapies. Higher plasma cholesterol, indeed, was found to be associated with preserved cognitive function and delayed loss of cortical gyrification in PD. Finally, I summarize in *Section V* the clinical and scientific implications of my work and offer future clinical and research directions. Of note, I propose that structural imaging measurements of the cortex are useful as markers of PD progression, and that subcortical white matter changes associated with cortical atrophy are an aspect of PD that may reflect altered neuroplasticity during PD progression. Finally, I show that higher plasma cholesterol is associated with delayed loss of cortical gyrification and slower motor and cognitive decline in PD, supporting the hypothesis drawn from case-control and epidemiological data. Thus, my research has shown how structural

MRI can offer powerful biomarkers to gauge PD progression and also provide clues about how specific factors might influence the course of PD.

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6-OHDA	6-hydroxydopamine
ANCOVA	Analysis of covariance
AD	Axial diffusivity
Apo	Apolipoprotein
C	Control
CoQ10	Co-enzyme Q10
CV	Cardiovascular/cerebrovascular
DOI	Duration of illness
FA	Fractional anisotropy
GM	Gray matter
GP	Globus pallidus
HAM(-D)	Hamilton depression scale
HMG	Hydroxyl-methyl-glutaryl
HY	Hoehn-Yahr
LDL(-c)	Low density lipoprotein (cholesterol)
LDLR	LDL receptor
LEDD	Levodopa daily equivalent dosage
LRP-1	Low-density lipoprotein receptor-related protein 1
MMSE	Mini-Mental Status Exam
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MR(I)	magnetic resonance (imaging)
PD	Parkinson’s disease
PDE/PD-Early	Early-stage Parkinson’s disease
PDL/PD-Late(r)	Late-stage Parkinson’s disease
PDM/PD-Middle	Middle-stage Parkinson’s disease
RBD	Rapid eye movement sleep behavioral disorder
RD	Radial diffusivity
SCWM	Subcortical white matter
SF	Spontaneous flexibility
SS	Set shifting
TIV	Total intracranial volume
UPDRS	Unified Parkinson’s Disease Rating Scale
UPSIT	University of Pittsburgh Smell Identification Test
WM	White matter

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SECTION I: INTRODUCTION TO PARKINSON'S DISEASE & THE NEED FOR BIOMARKERS OF PROGRESSION

Introduction to Parkinson's disease

Parkinson's disease (PD) is an age-related and progressive neurodegenerative disorder that is marked clinically by resting tremor, bradykinesia, and rigidity (1), and pathologically by the loss of nigrostriatal dopaminergic neurons and the presence of Lewy bodies (2). Second only to Alzheimer's disease in prevalence, PD was estimated in 2010 to affect 630,000 people in the United States and cost $14.4 billion per year (3). The pathological hallmark of PD is cell death of the dopaminergic neurons of the substantia nigra pars compacta, where cell losses typically reach greater than 50% by the time of diagnosis (4). Degenerative pathology, however, is widespread throughout the nervous system in PD and is thought to progress from the periphery (i.e vagus and olfactory nerves) to lower brainstem areas (i.e. midbrain areas where nigrostriatal dopaminergic neurons are located), and then involve higher level cortical areas at later disease stages (2, 5-7). Currently, there is no known therapy that can slow down the progression of PD-related brain pathology. Treatment, therefore, is based on management of clinical symptoms and mainly relies on dopaminergic replacement therapy and/or deep brain stimulation to modulate basal ganglia function. PD progresses relentlessly, however, with more than 80% of patients having dementia at 20 years after diagnosis (8). Despite the success of symptomatic treatment, PD remains a devastating disease that has no cure. By the year 2040, the prevalence of PD is expected to double and will post tremendous burden for patients, their family and society (3), there is a critical need to develop neuroprotective therapeutic strategies to halt, slow, or prevent pathologic progression.

Searching for factors that influence the progression of PD

Many potentially neuroprotective factors have been investigated in PD, but only a handful have yielded promising results (9). Based on epidemiology studies, tobacco smoking (see Appendix for supplemental study), caffeine usage, plasma urate, and plasma cholesterol have shown particularly promising associations with lower risk of PD or slower disease progression (10-15). This thesis research focused on plasma cholesterol, since it has shown promising associations with lower risk (10, 16-19) and delayed age of onset (20) of PD especially in high-risk individuals (21), and is easily quantifiable. Furthermore, cholesterol metabolism is able to be modulated pharmacologically and is known to be necessary for synaptogenesis (22, 23). If cholesterol was confirmed to be neuroprotective against PD-related pathology, such findings may have important public health implications and may facilitate the rational design of disease-modifying therapeutics and/or neuroprotective strategies for high-risk groups and PD patients. Although cholesterol has been suggested to have neuroprotective roles in PD, based on epidemiologic and/or postmortem data (24), *in vivo* studies are needed to understand how they might influence the progression of PD.

Past efforts to understand and evaluate potentially neuroprotective treatments for PD have been hindered by the lack of cellular or animal model that mimic the complex nature of human disease and the lack of objective and reliable *in vivo* markers of PD-related pathology in humans. Many potential strategies have been claimed to be neuroprotective in animal studies, but have failed in clinical trials. As discussed below, such “failures” in clinical trials might be due to the lack of objective and reliable biomarkers to gauge PD progression as a benchmark for neuroprotectivity. Another important factor may be that animal models of PD are not currently able to capture the multiple complex genetic and environmental interactions that may underlie PD

etiology (25). In current clinical trials investigating potentially neuroprotective factors, the evaluation of PD severity is based on clinical measurements. Many clinical trials, accordingly, have used measurements such as time to levodopa initiation and/or motor scores to gauge the effects of neuroprotective candidates (9). Such metrics, however, are limited by excessive variability and poor repeatability. The Unified Parkinson's Disease Rating Scale motor examination, for example, is the current standard to evaluate motor severity in PD (26). Despite standardized scoring criteria, the clinical motor assessment has suboptimal inter-rater reliability and is somewhat subjective (27). Similarly, time to levodopa initiation depends upon prescriber bias and the time between actual PD onset and diagnosis is likely to suffer from low precision (28). Another major problem with clinical measurements of disease severity is that they can be affected substantially by symptomatic treatment. Thus, there is a critical needs to have reliable and unbiased *in vivo* markers of neural substrate losses in PD in order to understand the mechanism of factors influencing PD etiology and progression as disease unfold in human, gauge efficacy of neuroprotective therapies in humans in clinical trials.

Limitations of current PD biomarkers

While conversion to PD is a relatively discernable outcome, clinical trials utilizing PD diagnosis as an endpoint are not always practical because of prohibitive cost and time requirements. The ideal measurement of efficacy in neuroprotective trials shall be an objective *in vivo* marker to gauge the extent of neurodegeneration in PD. To date, however, there are no such markers of PD that could be used to estimate progression throughout all disease stages. The premise of this research is that if quantitative imaging can capture the PD-related brain pathology,

then they may be useful to help to understand the effects of candidate neuroprotective factors, such as cholesterol, as PD evolves and progresses in patients.

There are various possible imaging modalities that might be used to study brain changes in PD. Some newer imaging techniques, such as [^{18}F]DOPA-PET or [^{123}I]β-CIT SPECT, may hold promise to estimate nigrostriatal terminal losses in early PD (29). However, such molecular techniques are expensive and might dissociate from real cell losses (30, 31). Whereas nigrostriatal terminal labeling may be useful for PD diagnosis and estimating estimate nigrostriatal terminal cell losses in early PD (29, 30), its utility is likely limited in more widespread neural losses of patients in later-stage disease. In addition, nigrostriatal labeling may be influenced by symptomatic treatment, which particularly targets the nigrostriatal dopaminergic replacement treatment. Thus, it is important to identify and characterize *in vivo* biomarkers that can quantitatively gauge the progression of PD-related neuropathology outside of nigrostriatal and pathway. Markers of PD progression in post-diagnosis stages, in particular, may be useful to estimate diffuse neural cell losses throughout PD progression in neuroprotective trials.

Goals of this research

Structural imaging may offer the promise of gauging cumulative neural losses in PD, rather than transient changes in brain function that could be modulated by symptomatic treatment (32). Magnetic resonance imaging (MRI) is widely available and routinely used in major medical centers, is generally less costly than radioligand techniques, and requires no radiation exposure to human subjects. The first goal of this research, accordingly, was to characterize PD-related structural MRI brain changes throughout different stages of PD. PD-related brain changes are known to be widespread, affecting different brain structures at particular stages of disease (2).

Thus, I utilized a stage-specific approach when characterizing brain changes in PD. The aim of *Sections II* and *III* was to identify and characterize structural brain changes that would progress with disease severity in a relatively stable manner. *Section II* will focus on gray matter, whereas *Section III* will be focusing on white matter and its relationship to gray matter. If such markers could be identified, they may be useful for implementation in clinical trials that test neuroprotective agents. The second goal of this research, accordingly, was to use the brain biomarkers discovered in Sections II and III to understand the factors that may influence PD progression. For this dissertation research, I focused on plasma cholesterol because it can be modified pharmacologically and through diet and exercise and could have important public health implications, especially in developing personalized health strategies in high-risk individuals (21). It is important to consider that the effects of a given neuroprotective factor might not be fully realized until a patient reaches a particular disease stage. I accordingly employed a stage-based approach these investigations because markers that depend upon a specific neural substrate (i.e. cortex) might be affected by pathology until particular disease stages.

SECTION II: STRUCTURAL BRAIN CHANGES IN PD

Preface

While many structural imaging studies have been conducted in PD (33-41), most past studies have been limited by being cross-sectional in nature and lacking investigation of specific disease stages. The overall objective of the research in Section 2, therefore, was to characterize structural brain changes longitudinally throughout various stages of PD progression, with the intent of identifying marker of disease progression. This section begins with a review of the current state of structural imaging research in PD and serves that leads into the experimental studies the subsequent chapters. To characterize the structural brain changes as PD evolves, we conducted a broad initial study that assessed multiple brain regions, and then more targeted studies of specific cortical structural characteristics such as folding. These markers of disease progression will be utilized in the following sections in order to gauge the effects of potentially neuroprotective factors.

Chapter 1: Review: Structural imaging and Parkinson's disease: moving toward quantitative markers of brain tissue pathology

Preface

Many studies have explored structural brain measurements of PD with the hope of finding a metric of cumulative neurodegeneration and to better understand disease pathology. Some brain changes (i.e. putamen atrophy) have been consistently reported in PD, whereas others (i.e. pallidal atrophy) have been reported inconsistently. This review attempts to synthesize and summarize existing literature regarding structural brain changes in PD. The specific emphasis of this chapter is to review how different structural metrics might relate to known pathologic processes, symptomatology, and various PD stages in PD.

[This chapter will be prepared as a review article for publication. I am the first author.]

ABSTRACT

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder. Although the pathological hallmark of PD is cell death of the substantia nigra pars compacta, widespread neurodegenerative changes occur throughout the brain as disease progresses. Post-mortem studies have demonstrated the presence of Lewy pathology, apoptosis, and loss of neurotransmitters and interneurons in both cortical and subcortical regions of the postmortem human brain of PD patients. Many imaging studies have attempted to gauge these PD-related pathology in live human brains using structural measurements to capture the "macroscopic" changes, with the hope of identifying imaging biomarkers of PD progression *in vivo*. Reports of brain atrophy in PD have been inconsistent, however, most likely due to differences in the studied populations (i.e. different disease stages, durations, and/or clinical subtypes), experimental designs (i.e. cross-sectional vs longitudinal), and image analysis methodologies (i.e. automatic vs manual segmentation). This review attempts to summarize the current state of PD structural imaging research, reconcile some of the differences in reported results, and identify challenges and future avenues. I place specific emphasis on structural imaging markers of PD progression, since they will be critical for quantifying the efficacy *in vivo* of potentially neuroprotective agents in future clinical trials.

Introduction to structural imaging in Parkinson's disease

Parkinson's disease (PD) is an age-related neurodegenerative disorder marked clinically by resting tremor, bradykinesia, and rigidity. PD diagnosis is primarily treated based on clinical symptoms and physical examination. Currently, there are no objective biomarkers that can be used for PD diagnosis or gauging disease severity. Thus, there is a need for markers that can follow different clinical trajectories and have different treatments (42, 43).

There are, however, even more compelling reasons to investigate brain structure in PD. First, there is an urgent need for neuroprotective agents that can slow or reverse the trajectory of PD-related neurodegeneration. The majority of trials investigating neuroprotective agents, however, have utilized clinical criteria (i.e. motor scores and time to levodopa initiation) as endpoints (9). Such clinical measurements are known to have poor inter-rater reliability and are subject to rater bias (27, 28). Thus, structural imaging has the potential to provide objective *in vivo* measurements of neurodegeneration. Second, structural imaging may provide insights regarding fundamental pathologic processes in PD, leading to better scientific understanding of the disease process.

Structural MRI may capture "macroscopic" changes associated with PD and provide such biomarkers. Reports of atrophy in PD, however, have been inconsistent and the locations of structural change have been extremely variable. This might suggest that structural imaging currently has little diagnostic utility at the individual patient level, but another interpretation is that structural methodologies have been crude in the past, necessitating further technical advancement to be useful clinically. There are remarkable differences in the methods used for anatomic definitions across studies (i.e. manual tracing, automatic segmentation, voxel-based techniques). These differences in methodologies and experimental designs may account for much of these heterogeneity in results reported.

In addition to MRI technology, it is also important to point out the choice of subjects included in each study may also contribute to inconsistencies in reported findings. For example, while many previous studies focused on the simple differentiation of PD and Control subjects, there was overall a lack of focus on clinical heterogeneity in subtypes, disease duration, and stages. Indeed, our study (Chapters II and III) indicated that the spatial pattern of gray matter atrophy undergoing seems to correspond with particular clinical subtypes and disease stages (40, 41, 44-46). The purpose of this review is to summarize the current state of structural MRI research in PD, identify knowledge gaps, and provide guidance for future research endeavors for MRI-based biomarker research.

Basal ganglia

The basal ganglia have been a main target of interest in PD for several reasons. First, the putamen and caudate are directly downstream from the substantia nigra, which is the primary site of pathology in PD (47). Degeneration of nigrostriatal terminals results in lower dopamine levels in the striatum, which has been shown to be associated with reduced striatal spine density in a dose-dependent manner (48, 49). Second, the traditional staging model by Braak et al. describes basal ganglia involvement relatively early in the disease, whereas cortical (especially neocortical) involvement is thought to occur later in disease. Third, cognitive decline is well documented in PD (50), even in earlier stages, and the striatum (via cortico-striatal connections) and hippocampus have been known to play important role in cognition in PD (51, 52). Thus, there is an interest in utilizing these structures as markers of PD progression throughout several stages of disease. Thus, we will discuss the MRI finding BG structures, and their pathological and clinical implications.

Putamen

Nigrostriatal losses occur rapidly in early PD, with more than 50% of putamen dopamine being lost before clinical diagnosis of disease (4). The putamen is a principle target of nigrostriatal projections that degenerate in PD (4). Thus, there has been particular interest in using measurements of putamen structure to detect PD onset and perhaps differentiate PD from other parkinsonisms (53, 54). Indeed, putamen atrophy has been reported widely in PD and in a variety of disease stages (35, 46, 55-63), but not necessarily in all studies (64-70). Because the putamen becomes depleted of dopamine well before clinical disease diagnosis (29), several studies have hypothesized that putamen atrophy would be diagnostically useful for PD and/or measuring progression. While reduced putamen volume and/or atrophied shape are commonly reported in the PD literature, it is likely that a substantial amount of putamen atrophy, similar to nigrostriatal dopaminergic losses, occurs prior to disease onset. Ellmore et al. in 2010, for example, reported that subjects having rapid eye movement sleep behavioral disorder (RBD), a possible pre-PD phase, had lower putamen volumes than control subjects (60), Geng et al., in 2006, reported similar findings among early-stage PD subjects (59). In a recent shape analysis, Sterling et al., in 2013, reported atrophy of the posterior putamen in early PD, localized with the known preferential pattern of dopamine depletion in PD (51). Most recently, Lewis et al., in 2015, (see detail in *Chapter 2*) showed that putamen volume is lower in the earliest post-diagnosis stages of PD compared to control subjects, and undergoes accelerated atrophy in the early stages of PD (41). A similar observation of accelerated atrophy in early PD was observed by Tessa et al., in 2014, (71). However, in the study by Lewis et al., in 2015, putamen atrophy reached a floor after roughly five years of disease, despite the fact that subjects continued to age. Taken together, these results suggest that putamen volume and/or shape could be useful not only in observing PD-related pathology near the time of disease onset, but also in high-risk groups (such as rapid eye movement

sleep behavioral disorder). However, it may not be the ideal measurement to gauge PD-related pathology progression in the later stages of disease.

Caudate

Compared to the putamen, the caudate undergoes a slightly delayed loss of dopamine in early PD, as nigrostriatal projections to the putamen degenerate preferentially. Thus, one might expect a slightly longer post-diagnosis course of atrophy in the caudate, which might make caudate volume more suitable to monitor post-diagnosis progression. Consistent with the analysis, a number of studies have reported no significant differences in caudate volume between PD and control subjects, particularly in early-state disease (72, 73), although some of those studies were likely limited by small sample size (59, 60, 64, 65). Interestingly, two recent shape analyses have shown that the greatest extent of PD-related atrophy occurs in the head of the caudate, where dopamine losses are known to be most severe in the caudate (51, 74). In addition, here have been reports of accelerated caudate atrophy in early-stage PD, followed by a plateau (41, 71), which might indicate that the notion that the greatest amounts of striatal atrophy occur in the earlier stages of disease. Thus, similar to putamen atrophy, caudate atrophy might not be the best metric to gauge progression in the later stages of PD.

Several studies have reported lower caudate volume in PD compared to control subjects, particularly in cognitively impaired or demented subjects (62, 63, 75). These findings are consistent with the role of caudate in cognition (76). In addition to cognitively impaired and/or demented PD subjects (62, 74, 77), lower caudate volume has been noted in PD subjects having postural instability, poorer phonemic fluency (75, 78), and poorer motor scores (79).

Substantia nigra, Nucleus accumbens and globus pallidus

Fewer studies have reported atrophy of the substantial nigra, nucleus accumbens or globus pallidus in PD. This is likely due to several reasons. First, these structures can be difficult to define

precisely on T1-weighted MRI. The nucleus accumbens, for example, is continuous with the putamen and caudate. The globus pallidus, on the other hand, is a mixture of white and gray matter. Like the substantia nigra, it has two distinct functional components (interna and externa) downstream from the striatum (80). To investigate these structures, it is necessary to have extremely consistent and reliable imaging segmentation techniques, which are not widely available for the substantia nigra, nucleus accumbens, or globus pallidus. Second, these structures are relatively small, so there is likely to be more measurement error due to partial volume effects.

Some evidence suggests that the substantia nigra volume may be decreased in PD. Previous studies might have been limited by the difficulty of delineating precise boundaries of the substantia nigra using routine structural imaging techniques (59, 81, 82). The substantia nigral accounts for only 0.5-0.6% of total brain volume, and partial volume effects can blur structural boundaries. More recent studies suggest that multimodal imaging may facilitate more precise detection of substantia nigra atrophy, even in early in PD (83). It is important to note, however, that at least 50% of nigral cells may be lost before PD diagnosis, and this could potentially limit the utility of substantia nigra volume in tracking PD progression (4). For the nucleus accumbens, there was a trend lower volume reported by Tinaz et al., in 2011, (35). Similarly, Carriere et al., in 2014, reported an inverse correlation between nucleus accumbens volume and apathy scores in PD, possibly representing limbic dysfunction (84). Studies investigating pallidal atrophy in PD have been roughly split between reporting atrophy (55, 66, 69), particularly with impaired cognition and more advanced disease stages (61, 62, 74, 77), and absence of atrophy (41, 59, 60, 64, 65, 72, 73). Taken together, these reports suggest that the extent of pallidal atrophy is relatively weak and/or that there is a lack of reliable segmentation techniques to define pallidal anatomy.

Hippocampus & amygdala

Hippocampal structural has been a topic of considerable interest in PD because of its well-documented role in memory decline and dementia (85). Indeed, many studies have demonstrated an association between hippocampal atrophy and PD with dementia (46, 62, 77, 79, 86-95), cognitive impairment (45, 46, 62, 96, 97), and visual hallucinations (94). Within PD, hippocampal atrophy has been associated consistently with impaired memory (45, 46, 88, 96, 98). It has also been reported that hippocampal structural changes may be useful to predict future cognitive decline in PD (45). In line with the notion that hippocampal structure is associated primarily with cognition, several studies have reported a lack of hippocampal atrophy in PD subjects without substantial cognitive impairment or dementia (67, 72, 74, 99, 100).

Atrophy of the amygdala, similarly, been associated with dementia (62, 89, 92), cognitive impairment (46, 62), poor memory performance (93), and depression in PD (99). Amygdala atrophy has been suggested to occur in several stages of PD, and results have been relatively inconsistent. Ibarretxe-Bilbao et al., for example, demonstrated lower amygdala volumes and accelerated atrophy in early-stage PD (37, 101), which is in agreement with post-mortem data suggesting amygdala pathology relatively early in disease (102). Morgen et al., in 2011, reported amygdala atrophy among PD subjects without dementia, also in agreement with this notion (103). A potential technical challenge in studying both hippocampus and amygdala structures is that it is difficult to obtain accurate structural measurements from automatic segmentation tools (104). Adding to this challenge is the relatively ambiguous boundary between the hippocampus and amygdala, which can appear as a continuous structure with standard structural magnetic resonance imaging modalities, and the multiple nuclei and neural substrate types within each structure. Thus, to fully leverage available the wealth of available structural imaging data that has been collected over many years, more accurate and precise segmentation techniques will be needed.

Thalamus

The thalamus is the master relay station for brain structures, and it is intimately relevant to PD because it connects basal ganglia to cortical regions. Although several studies have investigated thalamic structure in PD, few have reported significant atrophy, possibly suggesting that structural changes are mild or spatially focused in the thalamus. Lisanby et al., in 1993, and Nagano-Saito et al., in 2005, for example, reported lower thalamus volumes in PD compared to controls (55, 66, 77). On the contrary, some studies have found no differences in thalamus volume between PD and control (56, 67). Interestingly, Messina et al., in 2011, reported lower thalamic volumes in atypical parkinsonism compared to PD, suggesting that thalamic structure might be useful for narrowing the differential diagnosis of parkinsonian patients (67). McKeown et al., in 2008, furthermore, utilized shape analysis to investigate thalamic structure, reporting atrophy via shape analysis but no volume differences between PD and controls (105). Thus, shape analysis might be more sensitive to detecting focused degeneration in specific thalamic nuclei. Indeed, the thalamus is a large structure and more refined imaging and segmentation techniques may be needed to investigate changes in specific nuclei (106).

Cortex

Cortical thickness

Cortical structure has been a topic of considerable interest in PD imaging research, possibly because it might serve as a marker of cognitive decline. Indeed, cortical thinning in Alzheimer's disease, a more common neurodegenerative disease, is well documented (107). However, past studies have yielded inconsistent results regarding the presence of cortical thinning at various stages of PD. In cognitively normal and early PD subjects, past studies have reported minimal or no evidence suggesting cortical thinning (40, 79, 108, 109). Lyoo et al., in 2011, reported weak correlations with disease duration after adjusting for age and cognitive scores, although no control

group was included for comparison (110). One small study of 16 PD and 15 control subjects, however, did report fast rates of cortical thinning in early PD, but still no differences in cortical thickness between PD and control subjects (37). There is more substantial evidence, however, to suggest that lower cortical thickness may be related to cognitive decline or impairment (33, 38, 40, 79, 109, 111), worsening motor scores (33, 110), and visual hallucinations in PD (33). Pereira et al., in 2014, for example, demonstrated that PD subjects having mild cognitive impairment at an average 6 months post-diagnosis had cognitive domain specific patterns of cortical thinning (38). Segura et al., in 2014, demonstrated similar findings of a posterior pattern of atrophy in PD with mild cognitive impairment, although the disease duration was approximately 6.4 years (40). A recent study by Mak et al., in 2015, suggested that while PD subjects with normal cognition have relatively minimal cortical thinning, PD subjects with cognitive impairment faster cortical thinning compared to PD subjects with normal cognition (112). Finally, there is a well-documented relationship between lower cortical thickness and PD-related dementia (33, 79, 109). Combining cortical thickness with hippocampal volume, Zarei et al., in 2013, reported 80% accuracy in identifying PD patients with dementia (79).

The results of cortical thickness studies are in agreement with the documented pathologic trajectory of Lewy pathology, which is thought to involve cortical structures at a later stage than many subcortical structures (2). However, it should be noted that even in later stage PD, there is little evidence of cortical thinning if cognition is not impaired. Hwang et al., in 2013, for example, reported thinner cortices in PD dementia patients compared to PD with normal cognition, despite the PD patients with dementia having shorter disease durations. Thus, cortical thinning, as a marker of disease progression seems difficult to separate from dementia, which is a consequence of the natural progression of PD (52). Interestingly, Lewy pathology in PD is known to occur in layer-

specific patterns in the cortex, preferentially affecting certain cortical layers depending upon the brain region. It seems possible that cortical thinning could represent involvement of additional cortical layers as disease progresses, although this is speculative. To understand the exact mechanisms, future post-mortem studies will need to determine the direct relationships between cortical thickness and pathologic changes that are known to occur in PD cortices [layer-specific distribution of Lewy pathology (2), lower levels of neurotransmitters and tyrosine hydroxylase immunoreactive interneurons (6, 7), and increased apoptotic signaling (5)] and differentiate these changes from Alzheimer's pathology.

Cortical gyrification

Most studies of cortical structure in PD have utilized cortical thickness as the sole metric to gauge cortical atrophy. However, it is important to consider the layered structure of the cortex and columnar organization. Acknowledging these structural intricacies, several recent studies (see detail in *Chapter 3*) have focused upon the folding structure (gyrification indices) of the cortex in PD. Historically, gyrification index offered a method to quantify cortical folding of a two-dimensional brain slice, defined as the ratio of cortical surface over an outer perimeter (113). Local gyrification index is a newer, image-based metric to quantify gyrification in three dimensions (114). One study to investigate cortical folding in PD reported relatively diffuse reductions in a PD cohort having disease duration of approximately 4 years, compared to control subjects (34). Another recent study found no differences in cortical gyrification between PD and control subjects, but reported inverse correlations between cortical gyrification in several frontal and parietal areas and a composite score of disease severity that included Hoehn-Yahr stage, cognitive impairment, and disease duration (79). In this particular study, it was unclear whether dementia (known to affect cortical structure) had contributed to the lower gyrification indices seem in more advanced-stages PD subjects. Recently, however, our group published (*Chapter 3*) a combined exploration

and validation study of PD subjects who had no dementia at baseline. The results suggested that PD subjects having disease duration less than five years undergo accelerated loss of gyrification over time, although they did not have significantly lower gyrification compared to controls. Subjects having disease duration greater than five years showed significantly lower gyrification indices in several key areas (supramarginal, inferior parietal, superior frontal, precentral, and postcentral) (44). Taken together, these results suggest that PD subjects undergo loss of cortical folding as disease progresses. Future studies should focus on developing and implementing more sophisticated methods of quantifying cortical folding, which may be useful to quantify cortical disease progression in PD.

Challenges in structural imaging and the future

Despite the promise of structural imaging assisting macroscopic changes associated with PD and tracking disease progression at various stages of PD, there are several major challenges that currently hinder practical implementation in clinical studies. One such problem is the lack of automatic methods that can precisely define the boundaries of gray matter structures. The effect sizes of gray matter atrophy are often very small (roughly a few percent loss is typical in PD). This makes it critical to reduce measurement error in the delineation of gray matter structures. Unfortunately, the most widely used segmentation techniques are still prone to low precision and systematic bias (115), while manual segmentation is extremely time consuming and prone similar issues of rater and left-right bias (116). Precise and unbiased definition of structures is particularly important for shape analysis studies, where there is lost power if correction for multiple comparisons is utilized. Thus, the techniques used to control false positives in shape analysis must also be optimized for power. Finally, automatic segmentation methods have been shown to have the potential for bias in neurodegenerative diseases (115). Thus, there is a need to develop new

technologies that can define the precise boundaries of gray matter structure in an unbiased manner. Such technologies would benefit structural imaging not only in PD research, but a wide range of neurodegenerative diseases. If these issues can be addressed and accuracy improved substantially, then structural imaging might have a particularly useful role in monitoring PD progression and predicting outcomes at the individual level.

Chapter 2: The pattern of gray matter atrophy in Parkinson's disease differs in cortical and subcortical regions

Preface

In the initial experiment, we explored and characterized structural changes in several brain areas. The basal ganglia were of particular interest in these initial investigations because these structures (esp. putamen and caudate) are directly downstream from the primary site of pathology. Furthermore, a previous study had shown that the distribution of striatal atrophy in PD was highly co-localized with the spatial areas most severely affected by dopamine depletion (51). These findings, taken together with cellular studies (48, 49), results suggested that deafferentation could result in permanent neural losses and that brain atrophy might be useful as a surrogate to gauge the severity of PD-related brain changes. We also included cortex structure in the initial exploration of structural brain changes in PD, since the cortex is known to be affected by Lewy pathology at later stages (2). While many previous studies had utilized cortical thickness as the sole or primary metric to gauge PD-related cortex atrophy (33, 35, 37-39, 79, 109, 110), the exploration study utilized cortical volume (the combined property of thickness and surface area) to permit the detection of either thickness and/or surface area changes in PD. In contrast to healthy aging, where cortical thinning thought to be the primary structural change (117), the main structural changes associated with PD-related neurodegeneration were unknown. Furthermore, most previous studies had focused on cross-sectional differences between PD and control. The longitudinal analyses were conducted to understand how brain structure evolves dynamically in PD.

[This chapter is published (see citation below) and represents my contribution to an unfolding story of how the trajectories of structural imaging markers evolve throughout PD progression. My roles in this work were to help to conceptualize and execute the data analyses, interpret results, perform the literature search, and write the manuscript. Although I am not the first author, this chapter is included in the main section of the dissertation because

it is vital to understand the story and initial study that led me to focus on cortical markers of PD progression, which were then explored in the following chapters where I am first author.]

Lewis, M. M., Du, G., Lee, E. Y., Nasralah, Z., Sterling, N. W., Zhang, L., Wagner, D., Kong, L., Troster A.L., Styner, M., . Eslinger, P. J., Mailman, R.B., Huang, X. (2015). The pattern of gray matter atrophy in Parkinson's disease differs in cortical and subcortical regions. Journal of neurology, 1-8.

Abstract

Background: Cortical and subcortical gray matter (GM) atrophy may progress differently during the course of Parkinson's disease (PD). We delineated and compared the longitudinal pattern of these PD-related changes.

Methods: Structural MRIs and clinical measures were obtained from 76 PD with different disease durations and 70 Controls at baseline, 18- and 36-months. Both cortical and subcortical (putamen, caudate, and globus pallidus) GM volumes were obtained, compared, and associated with PD clinical measures at baseline. Their volumes and rates of change also were compared among Controls, PDs, and PD subgroups based on duration of illness [≤1 year (PD_E), 1-5 years (PD_M), and >5 years (PD_L)].

Results: Compared to Controls, PD subjects displayed smaller cortical GM and striatal (putamen, caudate, ps≤0.001), volumes at baseline. Cortical GM volumes were negatively associated with disease duration at baseline, whereas striatal volumes were not. PD subjects demonstrated accelerated volume loss in cortical GM (p=0.006), putamen (p=0.034), and caudate (p=0.008) compared to Controls. Subgroup analyses demonstrated that accelerated cortical atrophy reached statistical significance in PD subjects with duration of illness 1-5 years (PD_M, ps<0.001) and the trend of accelerated atrophy seemed to persist until later stages, whereas striatal atrophy occurred in PD subjects with PD_E (p=0.021 for putamen, p=0.005 for caudate) and PD_M (p=0.002 for putamen, p=0.001 for caudate) that significantly slowed down in PD_L (ps for PD_L vs PD_E or PD_M: <0.01).

Conclusions: The pattern of GM loss in PD differs in cortical and subcortical regions, with striatal atrophy occurring earlier and extra-striatal cortical atrophy later.

Introduction

Parkinson's disease (PD) affects both motor and non-motor functions. Postmortem studies have shown focused pathology in the substantia nigra of the basal ganglia, as well as more diffuse Lewy pathology in extranigral brain regions (2). Postmortem histologic studies are limited by the inability to capture dynamic changes as the disease unfolds in live patients. The exact pathoetiology and course of PD-related cell loss and its progression, however, remain unclear.

MR volumetric imaging can gauge *in vivo* macroscopic atrophy and assess both nigrostriatal and extranigral changes longitudinally. Extensive studies have demonstrated both cortical and subcortical gray matter (GM) atrophy in PD with both cross-sectional (62, 118, 119) and longitudinal designs (37, 112, 120) although some studies report no significant longitudinal volume changes in PD (121, 122).

It is well-known that PD progression is not uniform over the disease course. Converging anatomical (123), biochemical (31), and clinical (124) evidence suggest the rate of motor and nigrostriatal pathological progression in PD is most rapid within the first five years of the diagnosis and then slows down or even plateaus, whereas extra-nigrostriatal and non-motor symptoms continue to progress during the remaining course of the disease (125). Thus, GM changes in nigrostriatal and extra-nigrostriatal brain regions may evolve differently during the course of PD. This study investigated simultaneously cortical and subcortical GM changes and compared their atrophic patterns during the course of PD.

Methods

Subjects

A total of 76 PD and 70 control subjects (Controls; Table 2.1) were included in the study. PD subjects were recruited from a tertiary movement disorders clinic and Controls were recruited from the spouse population of the clinic or via IRB-approved recruitment materials posted in the local community.

All subjects gave informed consent and were free of major/unstable medical issues such as liver, kidney, or thyroid abnormality, and deficiency of vitamin B_{12}, or any cerebrovascular disease or neurological condition (other than PD). PD diagnosis was confirmed according to published criteria (126). Disease duration was obtained from subject history with onset defined as the first diagnosis by a medical professional. The study was conducted in accordance with the principles of the Declaration of Helsinki and reviewed and approved by the Penn State Hershey Institutional Review Board.

At baseline, subjects were screened using the Mini Mental Status Exam (MMSE) and only those with MMSE≥24 were enrolled. Olfactory function was evaluated using the University of Pennsylvania Smell Identification Test (UPSIT) (127) and depression assessed using the Hamilton depression scale (128). Imaging and clinical data were captured at baseline, 18-, and 36-month follow-up visits, with 18- and 36-month follow-up visits occurring on average 19.3±3.1 and 39.3±4.8 months after the baseline visit, respectively.

Motor function assessments

Unified PD Rating Scale part III-motor scores (UPDRS-III) (129) and Hoehn & Yahr (HY) stages were obtained for each PD subject in a practically defined "off" state after withholding PD medications overnight (~12 hr; (130)). The levodopa-equivalent daily dose (LEDD) was calculated according to published criteria (131).

MRI data acquisition

All subjects were scanned using a 3.0 Tesla MR Scanner (Trio, Siemens Magnetom, Erlangen, Germany, with an 8-channel phased array head coil) at baseline, 18-, and 36-months. To avoid systematic bias, PD and Controls were scanned in an intermixed fashion throughout the longitudinal study. A magnetization-prepared rapid acquisition gradient echo sequence was used to obtain T1-weighted images with TR/TE=1540/2.34, FOV=256 mm x 256 mm, matrix=256 x 256, slice thickness=1 mm

(with no gap), slice number=176. Each MRI was inspected and deemed free of any significant structural abnormalities (tumor or vascular malformations).

Image processing and analysis

T1-weighted images were processed automatically using the longitudinal stream of FreeSurfer (version 5.1.0). This processing pipeline utilizes longitudinal image data from each time point to create an unbiased, within-subject template via a nonlinear surface-based registration procedure. Within-subject templates were used to initialize image processing (skull stripping, Talairach transforms, atlas registration, spherical surface maps, and parcellation) for subject scans at each visit. The final volumes of putamen, caudate, and globus pallidus are the sum of the left and right sides since there were no significant differences between sides (data not shown). The FreeSurfer longitudinal pipeline generated an average total intracranial volume (TIV, i.e., the sum of GM, WM, and CSF) across visits for each subject. Each region for individual subjects then was normalized by this TIV to calculate a percentage of TIV measurement that was included in the statistical analysis and presented in the results.

Statistical analysis

Gender, age, and education were compared using Fisher's exact test or two-tailed Student’s t-test as appropriate. Raw UPSIT scores were compared between groups using analysis of covariance (ANCOVA) with adjustment for age and gender. Clinical measures were compared using two-sample t-tests (Controls and PDs) or analysis of variance (Controls and PD subgroups). The association between baseline volumes and clinical measures was assessed in PD subjects using Pearson’s correlation.

For group and subgroup comparisons, baseline, 18-, and 36-month volumes were entered into a linear mixed-effects model to estimate baseline volume differences and the rate of GM atrophy for each region of interest (ROI). Age and gender also were entered as predictors in the mixed-effect models because of their known effects on brain volume and rate of brain atrophy (132). Volume (normalized by

TIV) at each visit was entered as the dependent variable and time elapsed since baseline visit was entered as the time variable. We assumed that the mean volume change was a linear function of time with intercept depending on linear and quadratic terms of baseline age (centered at 60 years), gender, and group (PD vs. Controls), and slope depending on the linear terms of age, gender, and group. A random intercept was included in the mixed-effects models to account for within-subject correlations of observations.

In order to evaluate the pattern of GM atrophy during the course of PD, the annual rate of change in both cortical and striatal regions was graphed (Figure 2.1) based on disease duration. In an effort to understand the stage-dependent volume changes in PD, PD subjects were sub-divided into three subgroups based upon the number of years since diagnosis [PD_E ($\leq$1yr), PD_M (1-5yr), PD_L (>5yr)], as we have done previously (133). All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Demographic and study characteristics

PD and Control subjects did not differ significantly in gender or education at any visit (Table 2.1). Overall, PD subjects showed significantly higher depression and lower UPSIT scores compared to Controls. PD subjects also were older than Controls at each time point.

At baseline, PD subjects displayed smaller total GM ($p<0.001$), cortical GM ($p<0.001$), putamen ($p=0.001$), and caudate ($p=0.001$) volumes compared to Controls (Table 2.2), with no significant difference observed in the globus pallidus. All three PD subgroups (PD_E, PD_M, and PD_L subjects) had decreased total GM, cortical GM, and putamen volumes compared to Controls. PD_M and PD_L subgroups demonstrated decreased caudate, with PD_E showing a trend ($p=0.058$). Within PD subgroups, there were no significant baseline volume differences.

From baseline, the dropout rate at 18-months was 18.5% for PD and 12.9% for Controls, respectively. Between the 18- and 36-month visits, the dropout rate for PDs and Controls was 19.4% and 7.2%, respectively. There were no significant differences between subjects remaining in the study and those who dropped out (data not shown).

Relationship of baseline volume and clinical measures in PD

Total and cortical GM volumes, but not striatum (caudate and putamen), were associated negatively with disease duration in PD subjects (Table 2.3). Cortical GM volume also was correlated with UPDRS-III, UPSIT and MoCA scores, whereas caudate volume was correlated only with LEDD.

Longitudinal volume changes in PD and PD subgroups

Compared to Controls, PD subjects overall demonstrated significantly faster volume loss in total GM (p=0.002), cortical GM (p=0.006), putamen (p=0.034), and caudate (p=0.008; Table 2.4).

Visual inspection of Figure 2.1 indicates that whereas the most dynamic changes occurred within five years of diagnosis, the annual rate of volume loss remained at the rate of ~1% in total and cortical GM, even 15+ years following diagnosis. Statistically, cortical GM annual volume changes were significantly accelerated only in PD_M subjects (p=0.001), and there were no significant differences between PD_M and the other subgroups.

Conversely, annual volume changes in striatum (putamen and caudate) were accelerated only in PD_E and PD_M subjects compared to Controls (Table 2.4) but not in PD_L. Indeed, annual volume changes appeared to plateau in these regions (hover ~0) 5-10 years after diagnosis (Figure 2.1), with significant differences comparing PD_E and PD_M to PD_L subjects.

Discussion

The current study replicated past results indicating that GM atrophy occurs in PD. Most importantly, the current study provides the first *in vivo* evidence that cortical and subcortical GM have a different pattern and rate of atrophy during the course of PD. The results may reconcile past controversial findings relating GM changes associated with PD and guide the choice of more sensitive stage-dependent biomarkers for PD progression.

Cortical gray matter loss and its clinical and pathological implications

Previous imaging studies have reported mixed results in overall GM volumes in PD (53, 62, 122). Several recent studies demonstrated (75, 112, 134), yet others failed to find (135, 136), reduced cortical volume in PD. The exact reason for these discrepancies is not known but different methodologies (region of interest vs. voxel-based) may be one possible contributory factor. The current study employed a ROI-based and longitudinal design that allowed us to perform focused hypothesis testing. The finding of significant differences in both atrophy at baseline and rates of change longitudinally strengthens the argument for accelerated atrophy occurring in PD.

In our study, cortical GM volume was associated with MoCA and UPSIT scores. These results suggest that GM volume may reflect extra-nigral changes associated with PD. The findings are consistent with a recent report indicating that GM atrophy is associated with cognitive impairment and decline in PD (137) and with other studies linking GM changes to cognitive performance (118, 119). Moreover, they are congruent with reduced olfactory function in PD being related to reduced volume in piriform and orbitofrontal cortices (138).

It is well established that the striatum makes significant connections with cortical areas through striato-thalamo-cortical pathways and reciprocal, topographic projections via cortico-striatal circuits (139). Our finding of a significant association between cortical GM changes and UPDRS-III scores is

supportive of the hypothesis that cortical GM changes also may capture at least part of nigrostriatal pathologies. Future studies interrogating the relationship between detailed motor/non-motor dysfunctions and refined cortical regions of interest in PD are warranted, and may yield knowledge on the neuropathological underpinning of different PD-related symptomologies.

As expected, cortical GM volumes were related to disease duration. Visual inspection of the time course of GM volume changes and PD subgroup analyses suggested that the accelerated cortical GM seems to be most significant one year after PD diagnosis, and the accelerated rate seems to maintain thereafter at 1% per year (Figure S1). This finding is consistent with Braak staging, which demonstrated that diffuse cortical involvement of PD pathology probably does not occur until later disease stages (2). Cortical GM changes may be useful to gauge pathological changes in later-stage patients.

Striatal volume loss and its clinical and pathological implications

The striatum (caudate and putamen) composes the primary input stage of the basal ganglia and receives direct projections from dopamine neurons of the substantia nigra, whereas the globus pallidus is regulated indirectly by the substantia nigra pars compacta via the striatum (139). Basal ganglia structures may undergo structural changes due to nigrostriatal denervation. In this study, PD subjects (and subgroups) displayed putamen and caudate atrophy, with no significant change in the globus pallidus, compared to Controls. These results are consistent with several prior studies (137, 140) but in contrast to a recent study that reported no change in basal ganglia volumes in cognitively-intact PD subjects (112). The current data, however, support the hypothesis that nigral denervation affects the structure of immediate downstream targets. Consistent with this, the current study also found that caudate volume is significantly associated with LEDD, a measurement reflecting striatal dopamine deficits. In addition, positive correlations also were found between putamen volume and MoCA scores. These results underscore the function of striatal structures in cognition (141).

Interestingly, striatal atrophy was not correlated with duration of illness. These results are consistent with previous clinical and radioligand studies that have shown dopamine cell loss in PD may not be linear throughout PD progression (142, 143). In order to understand the potential stage dependent GM changes, we subdivided PD group to three subgroups based on the following rationale: 1) The upper limit of disease duration of one year was chosen to define a group of PD_E subjects that had not received extended treatment. 2) PD_L was defined as those PD subjects having at least five years of disease duration because nigrostriatal terminal labeling is known to reach a floor after approximately five years (29), although there are some data suggesting that nigral cell death may continue (30). 3) Clinically, dyskinesias, cognitive decline, and dopamine-non-responsive symptoms tend to be more prominent after the first five years ("honeymoon" phase) (144). 4) Lastly, these subgroup categorizations also happen to yield relatively balanced subgroup sample sizes, which is powerful for equivalence testing (29, 142, 145). Consistent with our expectations, the highest rates of striatal atrophy were observed in patients during the first five years of disease (PD_E and PD_M) and then plateaued thereafter (Figure S1). These temporal relations of our study lend further support to the hypothesis that basal ganglia atrophy may be the consequence of nigrostriatal dopamine denervation, rather than an independent pathology (such as new spreading Lewy pathology). We recognize that despite our rationale, the selection of subgroups based upon disease duration might seem arbitrary, so we repeated our analyses using HY stage, yielding similar results (data not presented). Together, our results support the notion that cortical and subcortical GM atrophy have different patterns and are stage-dependent.

Limitations

Despite a number of strengths, the study has several limitations. The number of subjects included in the study was moderately large for a longitudinal imaging study, but small compared to PD-related epidemiological studies. Moreover, because most PD subjects included in the study were within ~10 years of diagnosis, we may not have captured disease changes that may arise in very late stages of

disease. The subcortical volume analysis was focused on striatal and pallidal regions, and thus may have missed important changes associated with PD in other brain areas. In addition, cortical GM was not parcellated into refined regions of interests to interrogate their individual trajectories or clinical implications.

Summary

In summary, the current study not only confirmed GM atrophy in PD, but demonstrated different patterns of cortical and subcortical atrophy during PD. Further studies are warranted to investigate the potential of using striatal atrophy to gauge early nigrostriatal denervation-related changes and cortical GM volumes to monitor later, global pathological processes and their clinical implications in PD.

Ethical Standards

The study was approved by the Penn State Hershey Institutional Review Board and conducted in accordance with the principles of the Declaration of Helsinki. All subjects gave their informed consent prior to their inclusion in the study.

Conflict of Interests

The authors declare that they have no conflict of interest.

Tables

(see below)

Table 2.1. *Demographic and clinical characteristics of participants at baseline, 18 months, and 36 months.*

	Control	**PD**	**PD$_E$**	**PD$_M$**	**PD$_L$**	**P-values[1]**	**P-values[2]**
Baseline							
N Subjects (F,M)	70 (36, 34)	76 (29, 47)	22 (11, 11)	27 (10, 17)	27 (8, 19)	0.134*	0.207*
Age (y)	59.8 ± 7.7	63.3 ± 8.4	61.5 ± 9.8	61.4 ± 6.9	66.6 ± 7.8	***0.011***	***0.008***
Education (y)	16.6 ± 2.8	15.9 ± 2.7	16.1 ± 2.4	15.8 ± 2.4	15.9 ± 3.3	0.135	0.163
MMSE	29.5 ± 0.9	29.1 ± 1.1	29.3 ± 1.2	29.1 ± 1.3	29.0 ± 0.9	0.121	0.124
HAM-D	3.8 ± 2.5	7.7 ± 4.5	7.3 ± 4.4	7.2 ± 3.7	8.4 ± 5.3	***<0.0001***	***<0.0001***
UPSIT	34.1 ± 5.3	21.3 ± 7.4	23.9 ± 7.6	20.6 ± 8.3	20.1 ± 5.5	***<0.0001***	***<0.0001***
Clinical Measures							
Disease duration (y)	na	4.9 ± 5.5	0.4 ± 0.3	2.5 ± 1.2	11.0 ± 5.0	-	***0.0001***
LEDD (mg)	na	557 ± 458	247 ± 203	474 ± 361	893 ± 484	-	***<0.0001***
UPDRS-III	na	22.5 ± 14.3	13.1 ± 7.2	22.6 ± 11.1	31.2 ± 17.3	-	***0.002***
HY Stage	na	1.8 ± 0.7	1.4 ± 0.6	1.7 ± 0.7	2.2 ± 0.6	-	***<0.0001***
18 months							
N Subjects (F,M)	61 (31, 30)	62 (27, 35)	18 (10, 8)	21 (10, 11)	23 (7, 16)	0.472*	0.329*
Age (y)	61.3 ± 7.8	64.9 ± 8.4	62.7 ± 9.4	63.2 ± 7.1	68.1 ± 8.0	***0.017***	***0.002***
Education (y)	16.5 ± 2.9	15.8 ± 2.7	16.2 ± 2.4	15.6 ± 2.3	15.7 ± 3.4	0.140	0.112
Clinical Measures							
Disease duration (y)	na	6.6 ± 5.5	2.1 ± 0.4	4.1 ± 1.2	12.3 ± 5.1	-	***<0.0001***
LEDD (mg)	na	767 ± 491	444 ± 223	709 ± 438	1043 ± 528	-	***0.0001***
UPDRS-III	na	26.3 ± 19.7	14.8 ± 7.1	26.4 ± 21.3	37.8 ± 20.6	-	***<0.0001***
HY Stage	na	2.2 ± 1.0	1.6 ± 0.7	2.2 ± 0.9	2.6 ± 1.1	-	***<0.0001***
36 months							
N Subjects (F,M)	56 (30, 26)	50 (24, 26)	16 (8, 8)	18 (9, 9)	16 (7, 9)	0.697*	0.956*
Age (y)	63.0 ± 7.8	66.4 ± 7.9	66.7 ± 9.5	64.3 ± 6.2	68.6 ± 7.6	***0.027***	***0.024***
Education (y)	16.4 ± 2.8	15.7 ± 2.7	16.3 ± 2.5	15.3 ± 2.3	15.5 ± 3.3	0.206	0.139
Clinical Measures							
Disease duration (y)	na	7.8 ± 5.3	3.7 ± 0.4	5.6 ± 1.2	14.5 ± 4.1	-	***0.008***
LEDD (mg)	na	911 ± 604	685 ± 250	745 ± 509	1304 ± 738	-	***0.009***
UPDRS-III	na	32.7 ± 18.6	22.0 ± 9.0	33.6 ± 19.9	46.8 ± 18.1	-	***<0.0001***
HY Stage	na	1.9 ± 0.6	1.7 ± 0.7	2.0 ± 0.5	2.1 ± 0.8	-	***<0.0001***

Measurements presented as mean ± SD unless otherwise indicated. [1]P-values for comparisons between all PD and Control subjects using two-sample t-tests. [2]P-values of ANOVA across PD subgroups (and Controls as appropriate). [*]P-values obtained using Fisher's exact test.

Table 2.2. *Baseline volume estimate data of Controls, PDs, and PD subgroups.*

	Total GM	**Cortical GM**	**Putamen**	**Caudate**	**GP**
Baseline Volumes of PD and its subgroups (mean ±SE)					
Control, N=70	414.8±2.6	305.3±2.1	3.254±0.044	2.370±0.030	0.890±0.019
PD, N=76	402.5±2.8	294.5±2.2	3.029±0.047	2.248±0.032	0.908±0.020
PD_E, N=22	400.6±4.5	292.6±3.6	2.978±0.076	2.268±0.030	0.930±0.033
PD_M, N=27	405.1±3.8	296.8±3.0	3.068±0.064	2.257±0.043	0.900±0.027
PD_L, N=27	400.3±4.4	292.6±3.5	3.020±0.074	2.214±0.050	0.899±0.032
P-values for group comparisons					
Control vs. PD	**<0.001**	**<0.001**	**0.001**	**0.001**	0.450
Control vs. PD_E	**0.004**	**0.001**	**0.001**	0.058	0.245
Control vs. PD_M	**0.032**	**0.018**	**0.014**	**0.024**	0.781
Control vs. PD_L	**0.002**	**0.001**	**0.004**	**0.003**	0.809
PD_E vs. PD_M	0.409	0.349	0.340	0.868	0.443
PD_E vs. PD_L	0.959	0.999	0.656	0.394	0.433
PD_M vs. PD_L	0.364	0.332	0.596	0.477	0.986

The volume estimate for each subject was expressed relative to baseline total intracranial volume (TIV) for that subject, with units per mille. Baseline volumes were compared among groups using longitudinal mixed-effects analysis (see Methods for details). P values are bolded for those reaching statistical significance.

***Table 2.3**. Correlations between volume and clinical measures in PD subjects at baseline.*

	Disease duration		**UPDRS-III**		**LEDD**		**MoCA**		**HamD**		**UPSIT**	
	r	***p-value***	***r***	***p-value***	***r***	***p-value***	***r***	***p-value***	***r***	***p-value***	***r***	***p-value***
Total GM	**-0.304**	**0.008**	-0.219	0.057	-0.193	0.095	**0.370**	**0.001**	-0.038	0.751	0.221	0.066
Cortical GM	**-0.316**	**0.005**	**-0.231**	**0.045**	-0.207	0.072	**0.384**	**<0.001**	-0.022	0.853	**0.242**	**0.044**
Putamen	-0.157	0.177	-0.074	0.550	-0.070	0.550	**0.280**	**0.016**	0.004	0.973	-0.134	0.267
Caudate	-0.156	0.177	-0.103	0.374	**-0.304**	**0.008**	0.213	0.069	-0.029	0.807	0.016	0.895
Globus pallidus	-0.001	0.993	-0.020	0.861	-0.053	0.648	0.004	0.976	-0.125	0.296	-0.081	0.503

Values represent the Pearson's correlation coefficient (r), with the associated p-value to the right.
Bold text represents significant correlations.

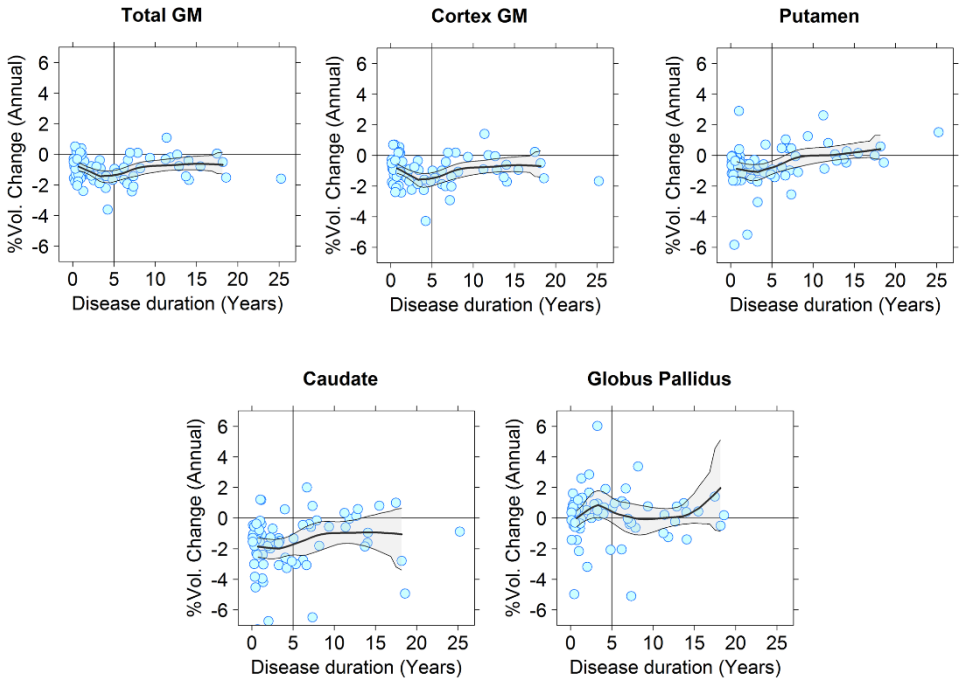
***Table 2.4**. Annual rates of change estimate data over 36 months in Controls, PDs, and PD subgroups.*

	Total GM	**Cortical GM**	**Putamen**	**Caudate**	**GP**
Estimates of annual rates of volume changes (mean±SE):					
Control, N=70	-2.26±0.35	-1.81±0.30	-0.015±0.004	-0.021±0.005	0.001±0.002
PD, N=76	-3.71±0.38	-2.86±0.32	-0.027±0.005	-0.037±0.005	0.002±0.002
PD_E, N=22	-3.12±0.61	-2.33±0.52	-0.032±0.007	-0.044±0.008	-0.003±0.003
PD_M, N=27	-4.55±0.53	-3.48±0.45	-0.037±0.006	-0.045±0.007	0.003±0.003
PD_L, N=27	-3.02±0.58	-2.44±0.50	-0.008±0.007	-0.019±0.007	0.004±0.003
P values of group comparisons:					
Control vs. PD	**0.002**	**0.006**	**0.034**	**0.008**	0.802
Control vs. PD_E	0.136	0.278	**0.021**	**0.005**	0.205
Control vs. PD_M	**<0.001**	**0.001**	**0.002**	**0.001**	0.434
Control vs. PD_L	0.166	0.186	0.443	0.852	0.374
PD_E vs. PD_M	0.064	0.081	0.618	0.864	0.097
PD_E vs. PD_L	0.896	0.870	**0.010**	**0.011**	0.074
PD_M vs. PD_L	**0.042**	0.101	**0.002**	**0.006**	0.918

Control, PD, and PD subgroup annual rates of change estimates for each group or subgroup. The annual volume change for each subject was expressed relative to total intracranial volume (TIV) for that subject, with units per mille. Annual rates of change were compared among groups using longitudinal mixed-effects analysis (see Methods for details), with decreasing numbers of subjects at the 18- and 36 month visits (see Table 11 for subject sample sizes at the different time points). P values are listed for the relevant comparisons, with those reaching statistical significance bolded.

Figures

Figure 2.1 *Annual rates of change in PD subjects were plotted based on disease duration for each region of interest. The solid black line in each graph represents the rolling average and the shaded areas the confidence intervals.*



Chapter 3: Stage-dependent loss of cortical gyrification as Parkinson's disease "unfolds"

Preface

In *Chapter 2*, cortical atrophy was found to associate with higher disease duration, and lower motor, cognitive, and olfactory functions. Whereas to striatal atrophy (putamen and caudate) was more rapid in the earliest stages of PD and reached a floor after approximately five years, cortex volume seemed to decline more steadily throughout all disease stages (Figure 2.1). These findings parallel data showing that 60-80% of striatal dopamine depleted at disease diagnosis (4). Cortical structures, particular of the neocortex, are thought to be affected by PD-related pathology at a later time in PD progression (2). Thus, the overall hypothesis of *Chapter 2* was that cortical structure would serve as a metric by which PD progression could be measured, particularly in the later stages.

Most previous studies utilized cortical thickness to evaluate cortical atrophy in PD, yielding relatively inconsistent results (33, 35, 37-39, 79, 109, 110). While cortical thickness has been correlated with cognitive impairment and/or dementia in PD (38, 40, 79), other aspects of cortical structure have been relatively unexplored. In *Chapter 2* we utilized cortical volume (the product of thickness and surface area) in order to assess general cortical atrophy. In this chapter, I investigated cortical folding, which had been relatively uncharacterized in PD. The focus of this study was to investigate how cortical gyrification (a measure of cortical folding) related to disease progression.

[This chapter is accepted to be published in *Neurology*. I am the first author.]

Sterling N.W., Wang M., Zhang L., Lee E.Y., Du G., Lewis M.M., Huang X. Stage-dependent loss of cortical gyrification as Parkinson's disease "unfolds." Neurology. In press, accepted Oct 15 2015.

Abstract

Objectives: Nigrostriatal terminal losses are known to progress most rapidly in early-stage Parkinson's disease (PD) and then plateau, whereas cortical pathology continues and may provide better markers of PD progression in later stages. We investigated cortical gyrification indices in patients with different durations of PD, since cortical folding may capture complex processes involving transverse forces of neuronal sheets or underlying axonal connectivity.

Methods: Longitudinal cohort structural MR images were obtained at baseline, 18, and 36 months from 70 non-demented PD and 70 Control subjects. Cortical local gyrification index (LGI) was compared between Controls and PD subgroups based upon duration of illness (DOI, <1yr [PD_E, n=17], 1-5yr [PD_M, n=19], >5yr [PD_L, n=24]) and adjusted using false discovery rate (FDR). Associations between LGI and clinical measurements were assessed using multiple linear regression. Areas having significantly reduced LGI also were analyzed using baseline data from a newly established cohort (PD n=87, Control n=66) to validate our findings.

Results: In the longitudinal cohort, PD_L had significantly reduced overall gyrification, and bilaterally in the inferior parietal, postcentral, precentral, superior frontal, and supramarginal areas compared to Controls ($p<0.05$). Longitudinally, loss of gyrification was accelerated in PD_M subjects, compared to Controls. LGI showed robust correlations with DOI and also was correlated with PD-related clinical measurements. Similar results were obtained in the validation sample.

Conclusions: Loss of cortical gyrification may accelerate within the first few years after PD diagnosis, and become particularly prominent in later stages. Thus, it may provide a metric for monitoring progression *in vivo*.

Introduction

In Parkinson's disease (PD), degeneration of dopamine terminal is thought to progress rapidly within the first few years after diagnosis and then plateau (29). Thus, in more advanced stages of disease, non-nigrostriatal brain changes may serve as better markers of PD progression. Evidence suggests that widespread pathologic changes occur in the cortex, including apoptotic signaling, Lewy pathology, reduction in other neurotransmitters, and interneuron loss (5-7). It is, however, unclear how cell death relates to the pattern of cortical Lewy pathology, and whether cortical changes can be used to gauge PD progression (2).

Lewy pathology has been documented in specific cortical layers (i.e., preferentially in deep layers of high-order sensory association areas) (2, 146). Some previous imaging studies have demonstrated decreased cortical thickness in PD (79, 109), but reported results have been inconsistent and have not shown robust associations with disease progression in the absence of dementia. These inconsistencies may be attributable to several factors. First, cortical thickness may be less sensitive in areas where cortex pathology is not transmural. Second, thickness measurements may not reflect more complex changes of cortex surface architecture (147). Thus, the distinction between structural metrics is important because they may reflect different aspects of cortical neurodegeneration. In the current study, we aimed to characterize changes in cortical gyrification during PD progression by studying subjects having different durations of illness. We hypothesized that reductions in gyrification would follow the spatiotemporal distribution described in studies of Lewy pathology (2, 146).

Methods

Longitudinal cohort subjects

PD (n=70) and Controls (n=70) with an MMSE score ≥26 were selected from a large cohort study based on matching for baseline age distribution, gender ratio, and number of follow-up visits (Table 3.1) (148, 149). PD patients were recruited from a tertiary movement disorders clinic, and Controls from spouses and the local community. PD diagnosis was confirmed according to published criteria (150). All subjects were free of major and acute medical issues or neurological disorders other than PD. All brain images were inspected and deemed free of any major structural abnormalities. Hamilton Depression Rating Scale scores (HAM) were obtained at each visit (128). PD subgroups were assigned for comparisons to Controls based upon duration of illness (DOI), defined as the number of years since diagnosis in the same fashion that we had done previously [PD_E (<1yr), PD_M (1-5yr), PD_L (>5yr)] (151).

Validation study subjects

A validation study was conducted using the baseline data from a newly established cohort under the NIH PD Biomarkers Program (NCT01888185) (152). Subjects were recruited in a similar manner as the longitudinal cohort subjects, except there were more advanced-stage patients. The original population of validation subjects included 104 PD and 71 Controls. Subjects having signs of dementia were excluded using the MMSE score cutoff described above.

Standard protocol approvals, registrations, and patient consents

Written informed consent was obtained for all subjects, in accordance with the Declaration of Helsinki. The research study protocol was approved by the Penn State Hershey Institutional Review Board.

Clinical evaluation

Unified PD Rating Scale (UPDRS) motor scores and Hoehn-Yahr (HY) stages were obtained for PD in the "on-medication" state at each visit. Longitudinal cohort UPDRS motor scores were recorded using the original UPDRS (26). Validation study UPDRS motor scores were recorded using the revised UPDRS (129). Levodopa-equivalent daily dose (LEDD) was calculated according to published criteria (153).

MRI data acquisition & analysis

All subjects were scanned using a 3.0 Tesla MR Scanner (Trio, Siemens Magnetom, Erlangen, Germany, with an 8-channel phased array head coil) at baseline, 18, and 36 months. A magnetization-prepared rapid acquisition gradient echo sequence was used to obtain T1-weighted images with TR/TE = 1540/2.34, FOV = 256 mm x 256 mm, matrix = 256 x 256, slice thickness = 1 mm (with no gap), slice number = 176.

T1-weighted images were processed automatically using FreeSurfer (version 5.1.0) (154). The longitudinal pipeline was utilized to process longitudinal cohort images by first creating unbiased within-subject templates. The within-subject templates were then used to initialize image processing (skull stripping, Talairach transforms, atlas registration, spherical surface maps) for scans at each visit (155, 156).

Local gyrification index (LGI) was used as a measurement of cortical folding. Historically, LGI was defined as the ratio of cortical surface over the outer contour (perimeter) of a two-dimensional brain section (113). LGI offers a method to quantify gyrification as it varies across the surface of a three-dimensional cortical mesh (~150,000 vertices) (114). For each vertex v_i, a circular region of interest was defined on the mesh surface having radius 25mm and center vertex v_i. Outer and pial surface areas (A_O, A_P) were computed as the sum of surface areas assigned to vertices that

fell within the region of interest. LGI was defined as the ratio of A_P/A_O. The final computation of LGI at each vertex was calculated by inverse weighting based upon distance. Thus, the LGI computed for each vertex v_i contains information from both the center vertex v_i and vertices that are nearby. However, this is to be expected, since gyrification is aimed to represent the combined structural properties of neighboring gyri and the sulci between them (114).

Statistical analysis

Gender and age were compared between PD and Controls using Fisher's exact test and two-sample Student's t-test, respectively. ANOVA was used to assess differences among PD subgroups and/or Controls. We performed longitudinal analyses of cortical structure at the vertex level using a validated framework (spatiotemporal linear mixed effects model) that leverages covariance among neighboring vertices, and can yield increases in statistical power while also providing good control of false positive rate (157). Briefly, each hemisphere was divided into ~ 30,000 regions of homogenous covariance from ~150,000 vertices. Fast expectation maximization iterations were applied to obtain more accurate parameter estimates, which were averaged within each region (158). Hypothesis testing utilized the Satterthwaite-based approximation of a scaled F-statistic. P-values were adjusted using an expected false discovery rate of 0.05 (159, 160).

The final mixed effects model used for group comparisons included the following: linear and quadratic terms of age at baseline, gender, years elapsed since baseline, years of education, HAM at each visit, intracranial volume (ICV), the terms for PD stages, the respective interaction terms for PD stages and years elapsed, the term for interaction between age at baseline and years elapsed, and the term for interaction between gender and years elapsed. ICV was included as a covariate because it was associated with overall LGI ($p<0.0001$).

General linear hypothesis testing using the F-statistic was utilized to conduct group and subgroup comparisons (161). Overall LGI was defined as the average of LGI across all cortical vertices. Regional and overall gyrification indices were analyzed using R version 3.1.1 (162).

The relationships between clinical measurements and cortical structural measures among PD subjects at baseline were assessed 1) descriptively using locally weighted regression and 95% bootstrapped confidence regions and 2) quantitatively using multiple linear regression for each variable of interest independently (covariates included age, gender, education years, ICV, and HAM as appropriate).

The validation study was conducted in cortical areas that were found to be significantly associated with advanced PD stage in the longitudinal cohort. For this analysis, we utilized the bilateral regional means of LGI for the respective cortical areas. The general linear model was used to conduct these validation analyses and included the following variables: linear and quadratic terms of age at baseline, gender, years of education, HAM, ICV, and the terms for PD stages. Outliers having extremely low gyrification values (2 standard deviations < sample mean) were excluded from the study (1 Control, 2 PD_M, 2 PD_L in validation sample).

Results

Demographic and characteristics of study subjects

In the longitudinal cohort, PD and Controls were not significantly different in age or gender frequencies at any visit (Table 3.1). Controls had more years of education than PD, but education did not correlate with any cortical metrics in either the PD or Controls. From the baseline visit, the 18 month dropout rate was 20.0% and 17.1% for PD and Controls, respectively, and 14.3% and 10.3%, respectively, from 18 to 36 months. The total number of visits did not differ between PD and Controls (p=0.210). The demographic characteristics of those who dropped out did not differ

between PDs and Controls. PD demonstrated the expected progression of symptoms as reflected by increased UPDRS-III scores and LEDD. PD had increased depression scores compared to Controls ($p<0.0001$). Among PD, disease stage and DOI were significantly associated with depression scores at all visits ($p<0.0001$).

In the validation study sample, PD subgroups and Controls were relatively similar in age, gender frequencies, and education, but PD had trend-level lower MMSE scores compared to Controls ($p=0.055$).

Longitudinal cohort analysis of cortical gyrification

We first investigated LGI differences between PD and Controls using vertex-level analyses. Compared to Controls, PD overall had reduced LGI in the left inferior parietal, superior frontal, frontal pole, and rostral anterior cingulate, and the right inferior parietal, precentral, rostral middle frontal, and fusiform areas ($p<0.05$). There were no significant differences in LGI between completed PD subjects and PD subjects lost to follow-up ($p\geq0.20$).

At baseline and 36-month visits, LGI was not significantly reduced in any region in PD_E or PD_M subgroups versus controls. There were substantial differences in LGI between PD_L and Controls, however, at baseline bilaterally in overall LGI, inferior parietal, postcentral, precentral, superior frontal, and supramarginal areas ($p<0.05$) (Figure 3.1, Table 3.2). At the 36-month visit, these bilateral differences persisted and also extended to include bilaterally the transverse temporal, fusiform, inferior temporal, and pars orbitalis regions ($p<0.05$). Comparisons of LGI between PD_L and Controls at the 18-month visit revealed patterns of reduced LGI that were intermediate between baseline and 36-month visits. Figure 3.4 depicts the longitudinal trajectories of LGI in cortical regions where LGI was significantly reduced in PD_L.

We next compared LGI among PD subgroups (Figure 3.1). PD_L had reduced LGI at baseline in the pre- and post-central and superior frontal areas bilaterally; the left inferior parietal, pars orbitalis, superior temporal banks, lateral orbitofrontal, and rostral middle frontal; and right caudal middle frontal areas compared to combined PD_E and PD_M subgroups ($p<0.05$). At the 36-month visit, there were significant differences in LGI between PD_E/PD_M and PD_L in the right precentral and postcentral areas ($p<0.05$).

Longitudinal analysis revealed accelerated overall LGI loss in the PD_M subgroup ($p<0.001$) and non-significant accelerated loss in PD_E ($p=0.056$) compared to Controls. Loss of LGI also was accelerated in the postcentral, precentral, superior frontal, and supramarginal areas among PD_M ($p<0.05$) compared to Controls, and non-significant accelerated loss was present in the inferior parietal area ($p=0.055$) (Table 3.2).

Several clinical measurements were correlated with LGI. DOI was correlated negatively with overall LGI and regional LGI of the postcentral, precentral, superior frontal, and supramarginial areas ($p<0.05$). LEDD was correlated negatively with LGI in all of the aforementioned areas and UPDRS showed correlations with LGI of the inferior parietal area ($p=0.026$) (Figure 3.2).

Validation study sample analysis of cortical gyrification

For the validation study, we utilized bilateral averages of LGI in regions that were shown to be reduced bilaterally in the longitudinal cohort study. Compared to Controls, PD_L demonstrated significantly lower overall LGI and in the inferior parietal, postcentral, precentral, superior frontal areas ($p<0.05$), although the LGI differences in the supramarginal area did not reach statistical significance ($p=0.054$) (Table 3.2).

DOI was correlated with overall LGI and regional LGI of the postcentral, precentral, superior frontal, and supramarginial areas ($p<0.05$), and the correlation for the inferior parietal area did not reach statistical significance ($p=0.052$) (Figure 3.3). UPDRS-III scores were correlated negatively with overall LGI, and with regional LGI of the inferior parietal, postcentral, precentral, superior frontal, and supramarginal areas ($p<0.05$).

Discussion

This study demonstrated that reduced cortical gyrification is related to disease progression in non-demented PD subjects. Losses of gyrification accelerated early after diagnosis, and became prominent in later stages of disease, suggesting that measurements of cortical folding may be useful for monitoring disease progression. Interestingly, we found that the loss of gyrification was particularly prominent in the neocortical regions that are thought to be relatively spared from Lewy pathology in PD (2, 163). In contrast, we did not observe altered gyrification in areas known to be more heavily affected by Lewy pathology (i.e. brain base and temporal areas). Taken together, these findings raise the possibility that cortical folding abnormalities may reflect pathologic processes not attributable solely to Lewy pathology.

Although recent data provided initial evidence that gyrification of the cortex is reduced in PD (average DOI ~ 3.9 years) (34), the association between cortical folding and disease progression remained unclear. Indeed, another recent study reported no differences between PD and Controls, although there were some areas of correlations between a composite measure of disease progression that included dementia in PD and cortical gyrification of the left middle frontal, superior parietal, superior frontal, supramarginal, lateral occipital, inferior parietal, and right superior frontal and superior parietal areas (79). Whereas these findings may have been attributable to the inclusion of dementia (mean MMSE = 18.3 in the PD dementia group), our study excluded dementia at baseline.

Subjects with PD_E or PD_M did not have reduced gyrification at baseline, but demonstrated accelerated loss of gyrification longitudinally. PD_L had prominently reduced gyrification bilaterally in several cortical areas despite lack of dementia (MMSE ~ 28-29). Disease duration, LEDD, and motor scores were also associated with reduced overall LGI in PD. Together, these findings suggest that accelerated loss of cortical folding occurs shortly after PD diagnosis and may be associated with disease progression prior to the occurrence of dementia. Accordingly, metrics of cortical folding may provide sensitive measurements to gauge ongoing cortical neurodegeneration as PD progresses.

Previous studies have reported findings of widespread cortical pathology in PD, including reduced levels of neurotransmitters and tyrosine hydroxylase immunoreactive interneurons (6, 7), increased apoptotic signaling (5), and Lewy pathology (2). Neocortical areas, however, have been shown to be relatively spared from Lewy pathology (2). The current study demonstrated that the loss of gyrification is especially prominent in the precentral and postcentral areas in PD, suggesting that cortical gyrification might not reflect Lewy pathology directly as we hypothesized. These differences may be explained by several possibilities. First, Lewy pathology may not be correlated with cell death equally throughout all cortical regions. Second, Lewy pathology is known to occur in distinct cortical layers in PD, preferentially in deeper layers in the neocortical areas and in superficial layers of the mesocortex (2). This differential pattern of pathology may contribute to the gyrification losses observed in this study. Few details, however, are available regarding the exact layer-specific pattern of Lewy pathology, warranting further investigation (2). Third, changes in underlying white matter could result in altered cortical folding (164).

To understand the stage-dependent changes of gyrification in PD, we sub-divided PD patients into three subgroups as we have done previously (165). The PD_E upper limit of DOI (one year) was chosen to define PD subjects who had not received extended treatment. PD_L was defined

as PD subjects having at least five years of disease duration for several reasons. For example, nigrostriatal terminal labeling has been suggested to reach a floor after approximately five years (29), although some data suggest that nigral cell death continues (30), and dopamine levels certainly decline throughout disease progression (31). Clinically, dyskinesias, cognitive decline, and dopamine-non-responsive symptoms tend to be more prominent after the first five years ("honeymoon" phase) (144, 166). These subgroup categorizations provided balanced subgroup sample sizes that are powerful for equivalence testing (29, 142, 145). Interestingly, the LGI continued to decline after five years (Figure 3.3). We also repeated our analyses using HY stage, with similar results (Table 3.e-1). There were also inverse correlations between gyrification indices and clinical measurements (LEDD and UPDRS-III, Figures 2 and 3). Together, our results support the notion that gyrification is stage-dependent and associated with PD progression.

The current study had several limitations. Although the overall sample size is large, the sample size for PD subgroup analysis was relatively small. Larger sample sizes and longer follow up of various disease staging categories will be needed to generalize these findings. In addition, as is common in longitudinal studies, there was significant dropout in both the PD and Control groups. The total number of visits, however, did not differ between these groups. There also may be considerable variability in clinical severity and disease duration (27). We, however, repeated all of our analyses using HY staging and found similar results (Figure 3.4).

The PD_L group also had a relatively high male-to-female ratio. Most relationships between LGI and disease duration persisted when male and female PD subjects were analyzed separately (Table 3.e-1). Although the p-values in Table 3.e-1 may give the impression of discordant results among females, the directions of the correlations were the same for all regions regardless of gender or cohort. Furthermore, we found no significant differences in overall, inferior parietal, postcentral,

precentral, superior frontal, or supramarginal LGI between male and female Controls, and gender had no effect on the rate of LGI change in Controls. Nevertheless, to minimize any potential confounding effect, we included gender in our statistical model. We also utilized "on-medication" motor scores because some subjects could not tolerate "off-medication" assessment. Of note, the scores obtained in the practically defined "OFF-medication" state may not represent true "off-medication" symptoms, since some drugs may not completely wash out. "On-medication" scores may be more representative of the levodopa-unresponsive components of patient symptoms, which may be more closely related to cortical findings. Finally, the study was validated using the baseline data from another newly established cohort, and the longitudinal data is not yet available. Although PPMI has longitudinal data, this cohort only includes PD subjects in very early stages. Independent validation of longitudinal trajectories in advanced stage PD is needed.

In summary, this study demonstrated that cortical gyrification is reduced among non-demented PD subjects, and is associated with measurements of PD progression. Loss of cortical gyrification may be accelerated shortly after PD diagnosis and becomes prominent in later stages. These findings suggest that folding metrics may be informative for quantifying cortical changes throughout PD progression. Moreover, the finding of reduced gyrification in areas known to be spared from Lewy pathology is unexpected, raising the possibility that cortical folding abnormalities reflect processes not attributable solely to Lewy pathology in PD.

Acknowledgements

We would like to express our gratitude to all of the participants who volunteered for this study. We acknowledge the MRI technical support of Mr. Jeffery Vesek, and the assistance of study coordinators Ms. Eleanore Hernandez, Ms. Brittany Jones, Ms. Melissa Santos, and Ms. Raghda Clayiff.

Table 3.1. Demographic and clinical properties of study participants. Measurements presented as mean ± SD unless otherwise indicated.

	Longitudinal Cohort Subjects						
	Control	**PD Overall**	**PD_E**	**PD_M**	**PD_L**	**P-values[1]**	**P-values[2]**
Baseline							
N Subjects (F,M)	70 (35, 35)	70 (30, 40)	17 (12, 5)	29 (9, 20)	24 (9, 15)	0.498*	***0.050****
Age (y)	61.3 ± 6.72	62.4 ± 8.00	60.9 ± 9.11	61.3 ± 7.03	65.0 ± 7.90	0.395	0.136
Education Years	16.9 ± 2.6	15.8 ± 2.6	15.8 ± 2.4	15.5 ± 2.7	16.0 ± 3.1	***0.017***	0.099
MMSE	29.4 ± 0.85	29.5 ± 0.85	29.2 ± 1.10	29.1 ± 1.31	29.2 ± 0.83	0.416	0.169
HAM	3.9 ± 2.4	7.5 ± 4.1	6.8 ± 2.9	7.1 ± 4.5	8.3 ± 4.2	***<0.0001***	***<0.0001***
DOI (y)	-	4.60 ± 4.92	0.48 ± 0.29	2.31 ± 1.18	10.3 ± 4.21	-	***<0.0001***
LEDD (mg)	-	738 ± 504	310 ± 211	624 ± 401	1097 ± 481	-	***<0.0001***
UPDRS-III	-	17.8 ± 11.0	11.4 ± 8.2	19.8 ± 10.4	20.4 ± 11.9	-	***0.135***
HY Stage	-	1.7 ± 0.70	1.4 ± 0.63	1.5 ± 0.69	2.2 ± 0.60	-	***0.002***
36 months							
N Subjects (F,M)	52 (27, 25)	48 (26, 22)	10 (7, 3)	23 (8, 15)	15 (7, 8)	0.556	0.295
Age (y)	64.7 ± 6.68	67.0 ± 7.9	67.9 ± 7.18	66.3 ± 5.99	68.5 ± 7.88	0.100	0.282
Education Years	16.7 ± 2.70	15.8 ± 2.7	16.4 ± 2.67	15.5 ± 2.35	15.7 ± 3.35	0.086	0.299
DOI (y)	-	8.01 ± 5.10	3.6 ± 0.53	5.7 ± 1.3	14.4 ± 4.29	-	***<0.0001***
LEDD (mg)	-	856 ± 605	674 ± 283	697 ± 456	1304 ± 738	-	***0.002***
UPDRS-III	-	25.7 ± 17.3	12.9 ± 8.0	23.9 ± 14.1	37.5 ± 20.3	-	***0.006***
HY Stage	-	1.7 ± 0.63	1.8 ± 0.46	1.9 ± 0.40	2.1 ± 0.90	-	0.393

Table 3.1 (continued).

	Validation Study Subjects						
	Control	**PD Overall**	**PD_E**	**PD_M**	**PD_L**	**P-values[1]**	**P-values[2]**
N Subjects (F,M)	66 (33, 33)	87 (41, 46)	18 (7, 11)	36 (19, 17)	33 (15, 18)	0.746	0.784
Age (y)	64.6 ± 8.77	66.4 ± 9.00	64.7 ± 9.30	66.8 ± 10.14	66.9 ± 7.58	0.209	0.497
Education Years	17.2 ± 2.9	16.5 ± 2.7	16.4 ± 2.7	17.0 ± 2.7	16.1 ± 2.7	0.160	0.265
MMSE	29.1 ± 1.18	28.5 ± 1.41	28.8 ± 1.35	28.6 ± 1.46	28.2 ± 1.39	***0.055***	0.189
HAM	2.7 ± 3.3	5.5 ± 4.8	4.7 ± 4.0	3.8 ± 3.0	7.8 ± 5.8	***<0.0001***	***<0.0001***
DOI (y)	-	5.56 ± 5.16	0.48 ± 0.26	3.13 ± 1.24	10.98 ± 4.27	-	***<0.0001***
LEDD (mg)	-	732 ± 671	346 ± 229	634 ± 842	1022 ± 468	-	***0.002***
UPDRS-III	-	27.8 ± 17.0	20.2 ± 9.2	25.3 ± 14.0	34.6 ± 20.7	-	***<0.0001***
HY Stage	-	1.9 ± 0.77	1.5 ± 0.62	1.7 ± 0.70	2.2 ± 0.81	-	***<0.0001***

[1]P-values for comparisons between all PD and Control subjects using two-sample t-tests.
[2]P-values of ANOVA across PD subgroups (and Controls as appropriate).
[*]P-values obtained using Fisher's exact test.
Abbreviations –DOI: duration of illness; HAM: Hamilton depression scale; HY: Hoehn-Yahr; LEDD: levodopa daily equivalent dosage; MMSE = Mini Mental State Examination; PDE: PD-Early; PDM: PD-Middle; PDL: PD-Late; UPDRS: Unified Parkinson's Disease Rating Scale;

Table 3.2. *Comparisons of regional local gyrification index among PD subgroups and Controls.*

Longitudinal Cohort						
	PD_E		**PD_M**		**PD_L**	
LGI in PD Subgroups vs. Controls						
	β_{PDE}	P-Value	β_{PDM}	P-Value	β_{PDL}	P-Value
Overall LGI	-0.008	0.766	-0.012	0.581	-0.074	***0.001***
Inf. Parietal	-0.048	0.151	-0.012	0.661	-0.104	***0.001***
Postcentral	0.033	0.383	-0.028	0.364	-0.105	***0.002***
Precentral	0.000	0.993	-0.023	0.446	-0.123	***<0.001***
Sup. Frontal	-0.013	0.536	-0.012	0.507	-0.076	***<0.001***
Supramarginal	0.019	0.638	0.000	1.000	-0.088	***0.015***
Annual Δ LGI in PD Subgroups vs. Controls						
	β_{PDE}	P-Value	β_{PDM}	P-Value	β_{PDL}	P-Value
Overall LGI	-0.005	0.056	-0.007	***0.000***	-0.002	0.257
Inf. Parietal	-0.007	0.184	-0.008	0.055	-0.002	0.642
Postcentral	-0.005	0.289	-0.008	***0.026***	-0.002	0.614
Precentral	-0.008	0.096	-0.008	***0.016***	0.000	0.905
Sup. Frontal	-0.002	0.513	-0.006	***0.020***	0.001	0.812
Supramarginal	-0.006	0.275	-0.011	***0.016***	-0.006	0.187
Validation Study Sample						
	PD_E		**PD_M**		**PD_L**	
	β_{PDE}	P-Value	β_{PDM}	P-Value	β_{PDL}	P-Value
Overall LGI	0.017	0.511	-0.022	0.261	-0.070	***0.002***
Inf. Parietal	0.012	0.693	-0.045	0.061	-0.065	***0.020***
Postcentral	0.037	0.333	-0.020	0.511	-0.070	***0.040***
Precentral	0.003	0.938	-0.024	0.448	-0.126	***<0.001***
Sup. Frontal	0.040	0.166	-0.027	0.226	-0.058	***0.023***
Supramarginal	0.076	***0.043***	-0.015	0.595	-0.064	0.054

Abbreviations – DOI: duration of illness; PD_E: PD-Early; PD_M: PD-Middle; PD_L: PD-Late;

Figures

Figure 3.1. *PD_L subjects (duration of illness > 5 years) demonstrated significantly reduced gyrification bilaterally in the inferior parietal, pre- and post-central, and superior frontal areas, compared to Controls at baseline visit (left). PD subgroup v. Control color maps represent adjusted p-values using an expected false discovery rate of 0.05. PD_L subjects demonstrated significantly reduced gyrification in several neocortical areas, compared to PD_E and PD_M subjects at baseline (right). Post-hoc PD subgroup comparison color maps represent significant beta values using an FDR-adjusted p-value threshold of 0.05.*

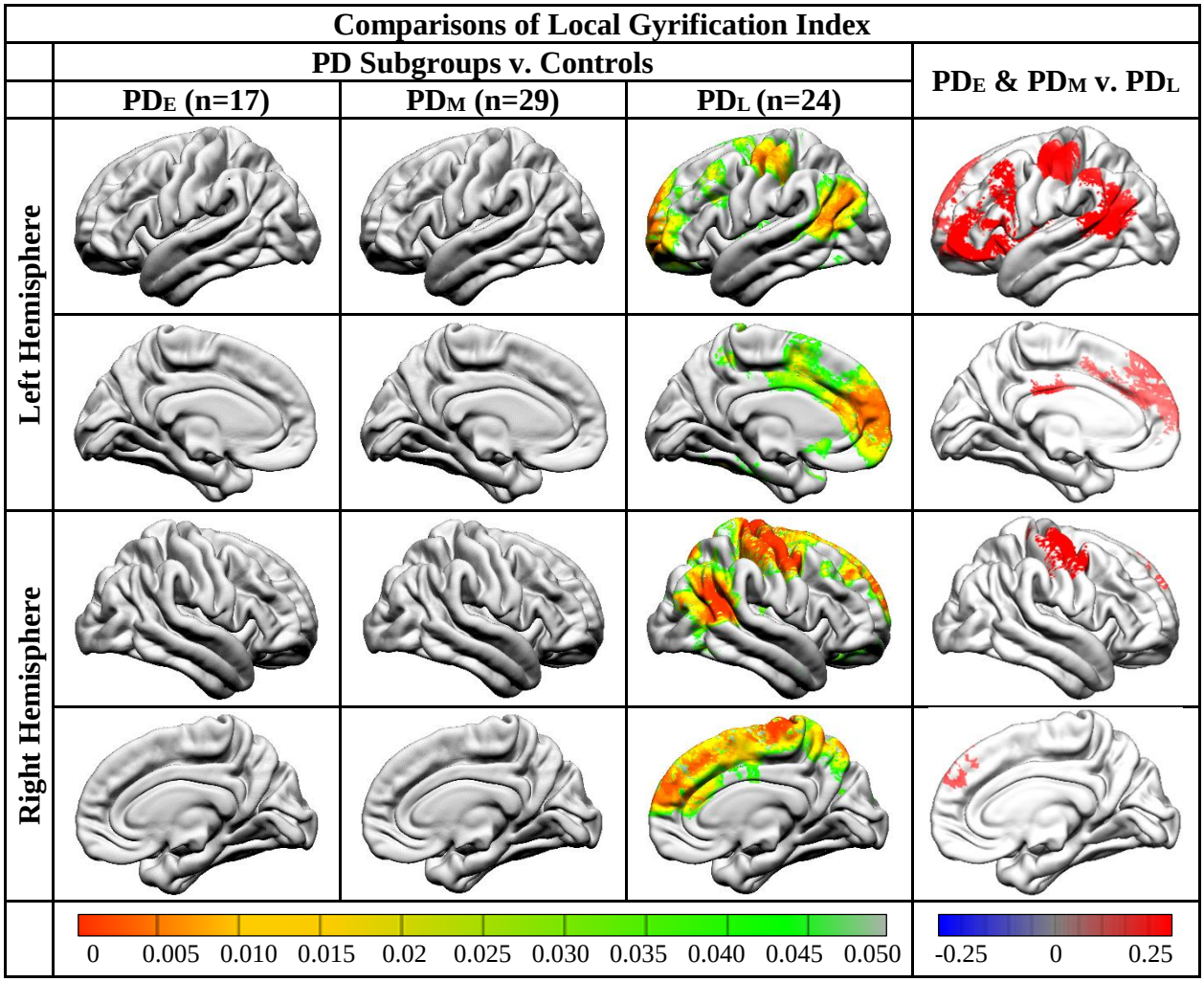

Comparisons of Local Gyrification Index
PD Subgroups v. Controls
PDE & PDM v. PDL
PDE (n=17)
PDM (n=29)
PDL (n=24)
Left Hemisphere
Right Hemisphere
0 0.005 0.010 0.015 0.02 0.025 0.030 0.035 0.040 0.045 0.050
-0.25 0 0.25

Figure 3.2. *Descriptive plots illustrate the relationship between regional local gyrification and clinical measurements among PD subjects in the longitudinal cohort. Center lines (black) and gray areas represent the moving average and 95% bootstrapped confidence region for local gyrification index as a function of clinical measurements. Beta and p-values were obtained via multiple linear regression for each clinical variable. Two data points were not shown in the LEDD plots because they had LEDD > 1800mg/day.*

(see below)

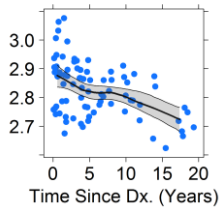
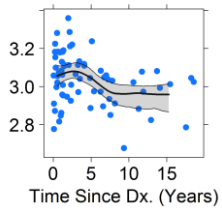
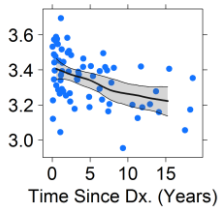
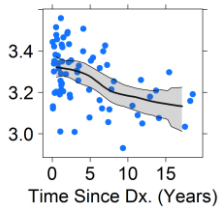
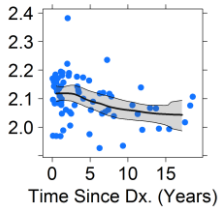
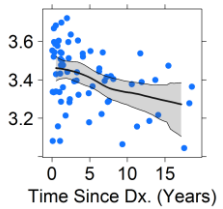
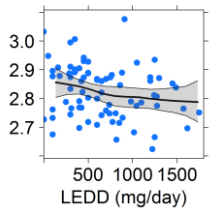
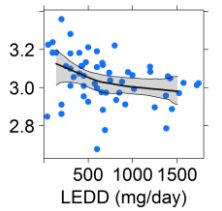
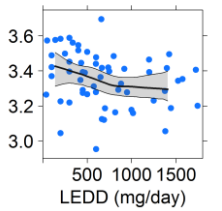
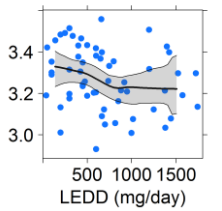
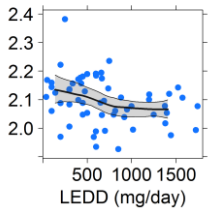
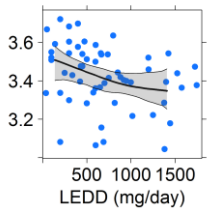
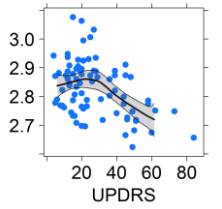
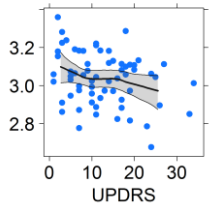
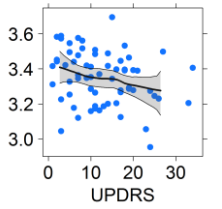
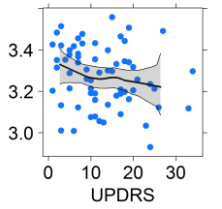
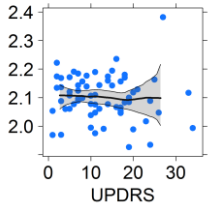
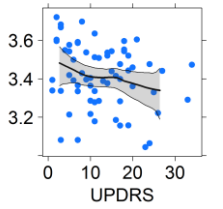
Longitudinal Cohort (Baseline Visit)					
Clinical correlations of Local Gyrification Index (Vertical Axis) by Cortical Region in PD Subjects					
Overall LGI	**Inferior Parietal**	**Postcentral**	**Precentral**	**Superior Frontal**	**Supramarginal**
Disease Duration (Years)					
β: -0.007 P: 0.001	β: -0.006 P: 0.060	***β: -0.011 P: 0.001***	***β: -0.012 P: 0.000***	***β: -0.006 P: 0.002***	***β: -0.009 P: 0.012***
					
LEDD (mg/day)					
β:-2.2E-05 P: 0.002	***β:-2.8E-05 P: 0.003***	***β:-7.1E-06 P: 0.004***	***β:-3.2E-05 P: 0.009***	***B:-1.8E-05 P: 0.003***	***β:-2.6E-05 P: 0.003***
					
UPDRS-III Score					
β: -0.002 P: 0.235	***β: -0.005 P: 0.026***	β: -0.005 P: 0.057	β: -0.004 P: 0.080	β: -0.001 P: 0.488	β: -0.004 P: 0.096
					

Figure 3.3. *Descriptive plots illustrate the relationship between regional local gyrification and clinical measurements among PD subjects in the validation study sample. Center lines (black) and gray areas represent the moving average and 95% bootstrapped confidence region for local gyrification index as a function of clinical measurements. Beta and p-values were obtained via multiple linear regression for each clinical variable. Two data points were not shown in the LEDD plots because they had LEDD > 1800mg/day.*

(see below)

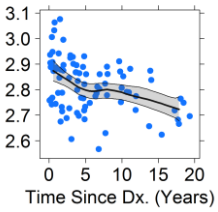
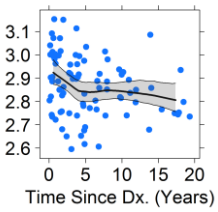
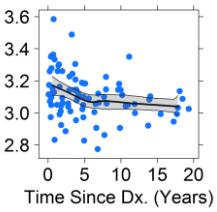
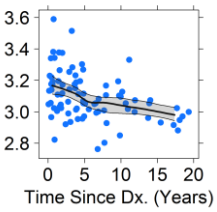
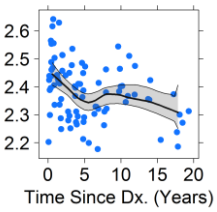
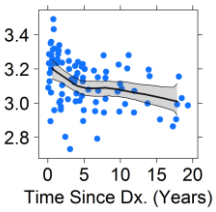
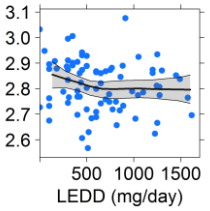
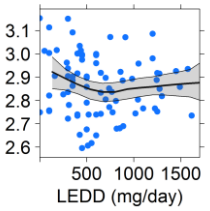
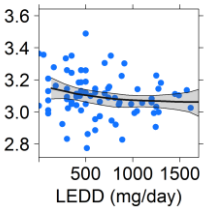
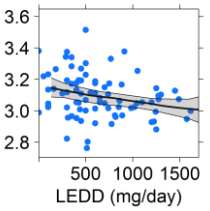
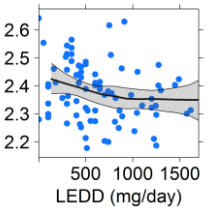
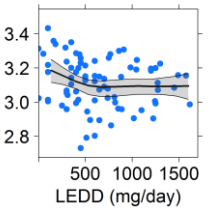
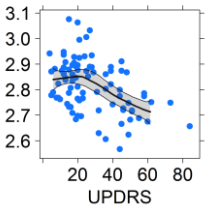
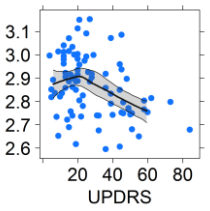
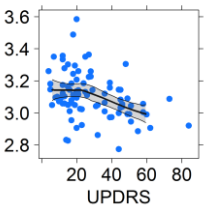
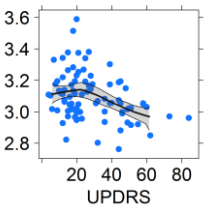
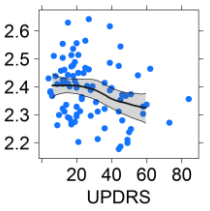
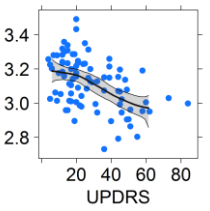
Validation Study Sample					
Clinical correlations of Local Gyrification Index (Vertical Axis) by Cortical Region in PD Subjects					
Overall LGI	**Inferior Parietal**	**Postcentral**	**Precentral**	**Superior Frontal**	**Supramarginal**
Disease Duration (Years)					
β: -0.007 P: 0.000	β: -0.005 P: 0.052	***β: -0.006 P: 0.034***	***β: -0.010 P: 0.001***	***β: -0.007 P: 0.002***	***β: -0.009 P: 0.002***
					
LEDD (mg/day)					
β: 0.000 P: 0.442	β: 0.000 P: 0.346	β: 0.000 P: 0.896	β: 0.000 P: 0.463	β: 0.000 P: 0.523	β: 0.000 P: 0.613
					
UPDRS-III Score					
β: -0.003 P: 0.000	***β: -0.002 P: 0.031***	***β: -0.003 P: 0.011***	***β: -0.003 P: 0.014***	***β: -0.002 P: 0.012***	***β: -0.003 P: 0.001***
					

Figure 3.4. *Mean local gyrification index at baseline, 18-month, and 36-month visits for regions that differed significantly in later stage PD. Points represent observations and lines represent trajectories of individual subjects. Center lines (black) and gray areas represent the moving average and bootstrapped confidence region for local gyrification index as a function of clinical measurements.*

(see below)

Longitudinal Cohort

Local Gyrification Index by Cortical Region in PD Subjects

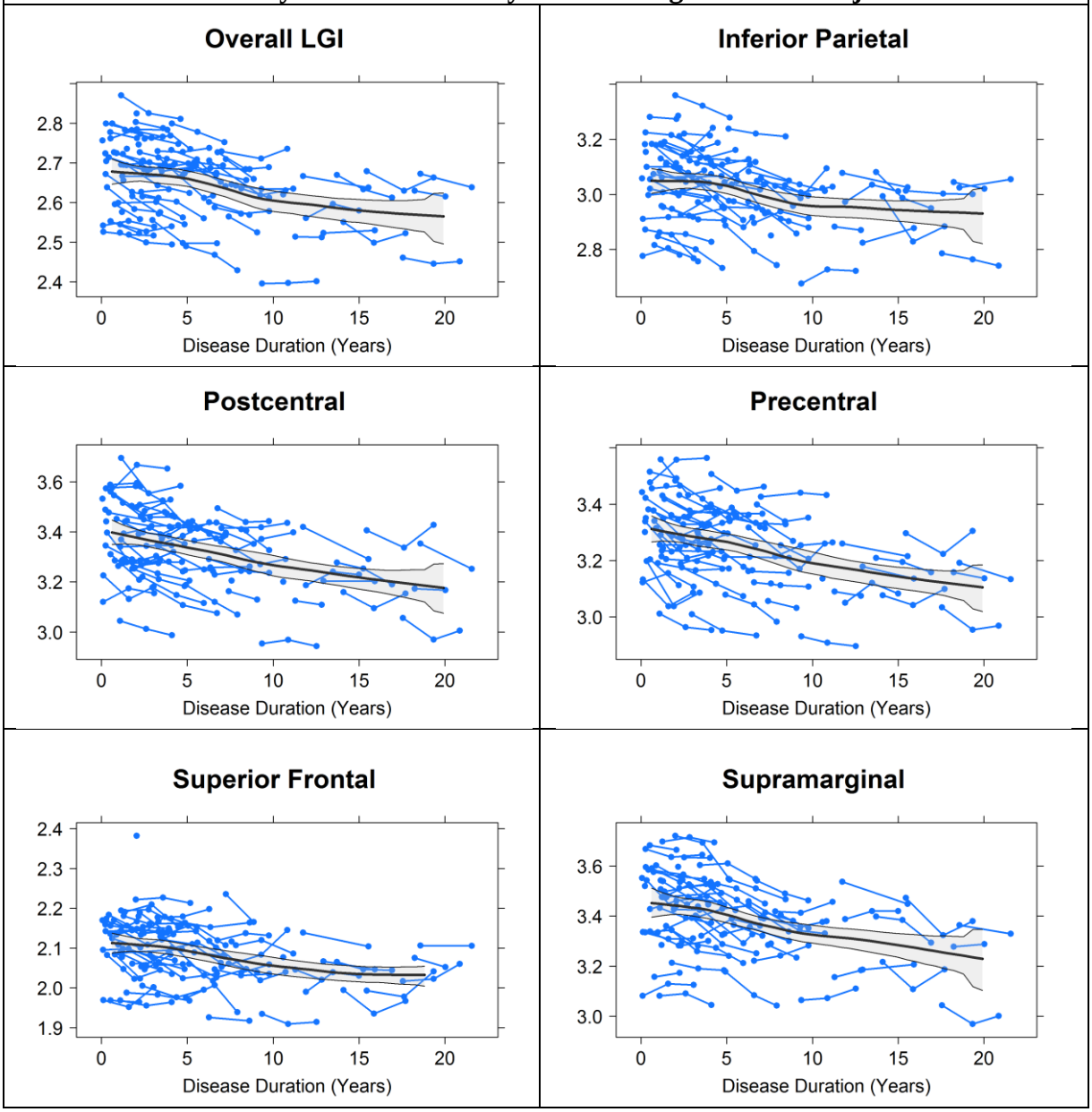
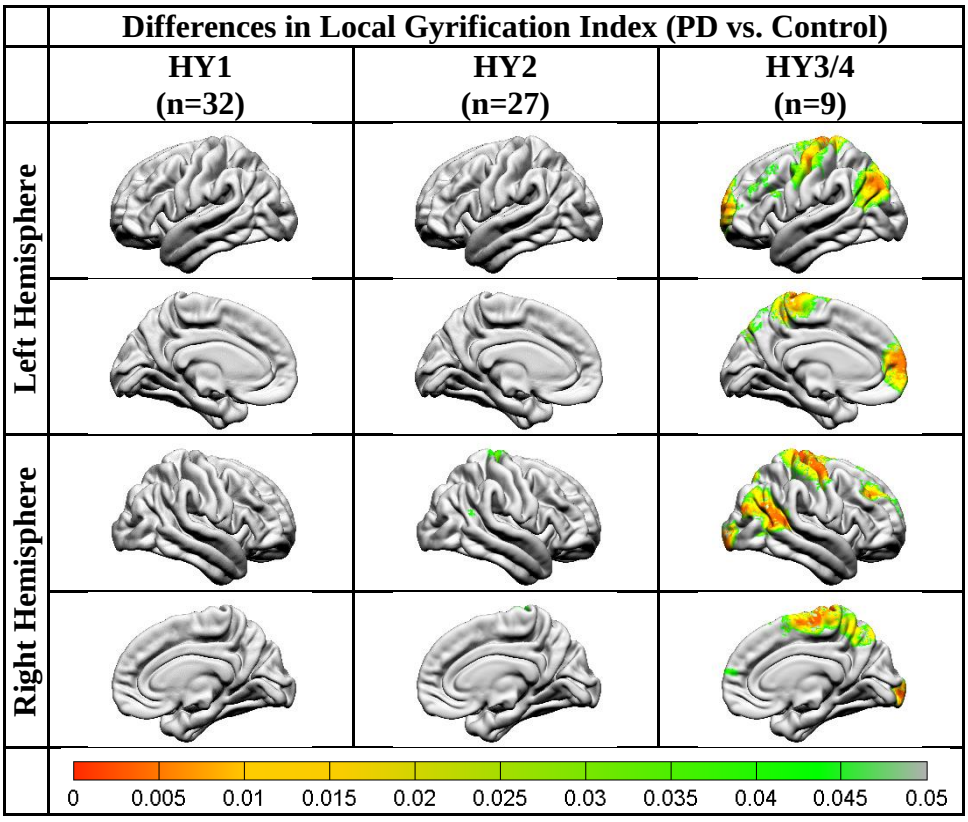


Figure 3.5. *HY 3 & 4 subjects demonstrated significantly reduced gyrification bilaterally in the inferior parietal, pre- and post-central, and paracentral areas, compared to Controls at the baseline visit. Color maps represent adjusted p-values using an expected false discovery rate of 0.05.*



Summary & future directions

This section began with a search for structural markers of PD progression by using a broad longitudinal study of several brain areas, both near and distant from the primary site of pathology in PD (substantia nigra pars compacta). I utilized a spatiotemporal approach to structural imaging in PD that was based on the notion that structural changes in different brain areas would depend upon the clinical and/or temporal stage of disease. In my initial exploration, I found that cortical volume, in particular, was correlated with disease duration and cognitive and motor function in PD. Cortical volume loss tended to persist throughout multiple disease stages, in contrast to striatal (putamen and caudate) volume losses, which tended to plateau after a few years (similar to niugrostriatal radioligand studies) (29). These findings were consistent with the notion that nigrostriatal dopamine losses which have been associated with atrophy of the dentrites on medium spiny neurons (48, 49, 51), occur very early in PD (4). In contrast, the cortex is involved at a later time (2) and may be more useful to track progression. Since cortical volume loss is a general measure of cortical atrophy (as the product of thickness and volume), I subsequently investigate more specific measures of cortical structure, such as gyrification. Indeed, cortical gyrification showed particularly robust correlations with multiple measures of disease progression. Thus, cortical volume and gyrification were utilized in the subsequent sections to investigate factors that might influence the progression of PD.

SECTION III: THE ROLE OF WHITE MATTER IN PARKINSON'S DISEASE PROGRESSION

Preface

While gray matter atrophy in PD is well documented (33-41), the extent of white matter involvement has been controversial (167). Past imaging studies have considered gray and white matter changes separately in PD. However, cortical gray and subcortical white matter are related intimately. Subcortical white matter tracts contain the myelinated axons of cortico-cortical and cortico-subcortical projections from neuron cell bodies residing in cortical gray matter (168). Thus, degenerative processes affecting one substrate (i.e. cortical gray or subcortical white matter) may manifest as changes in the other.

While there are known neurodegenerative changes that occur in cortical gray matter in PD such as Lewy pathology, apoptotic signaling, and loss of interneurons and neurotransmitters (2, 5-7), the mechanisms of reported white matter degeneration are unknown. One possibility is that PD-related injury to cell bodies might result in anterograde degeneration of axons in white matter tracks. Conversely, it has been suggested that the properties of axons in white matter tracks might modulate the vulnerability of neurons to PD-related pathology (163). Thus, to guide future research on imaging biomarkers and understanding PD pathology, it will be important to understand white matter changes and their relationships with cortical gray matter changes throughout the PD process.

The research of this *Section* investigated the relationships between cortical gray matter atrophy and subcortical white matter diffusion characteristics, with three main goals. The first goal was to investigate the potential white matter changes in PD. The second goal was to explore the relationship between axonal myelination and overlying cortical gray matter. Axonal myelination has been suggested to be protective against PD-related pathology, based on observations that Lewy

pathology tends to affect neurons that are long, thin, and poorly myelinated (146, 163). The third goal of this section was to understand the relationship between cortical gyrification and diffusion characteristics of underlying subcortical white matter in PD. In normal development, cortical folding in development is thought to results from tangential forces of expanding gray matter and constrains of subcortical white matter (34, 164). The mechanisms of cortical "unfolding" in neurodegenerative disorders, to our knowledge, are unknown. The latter two goals were addressed by studying the associations between cortical gray matter structural measurements and diffusion characteristics of the underlying subcortical white matter.

[*Chapter 4* is in the final stage of preparation for submission. I am the lead author.]

Chapter 4: Cortical gray & subcortical white matter associations in Parkinson's: hints of lost neuroplasticity?

Abstract

Background: Cortical gray matter loss in Parkinson's disease is well established, and recent literature suggests that subcortical white matter degeneration also may occur. The associations between cortical gray and subcortical white matter, however, remain unknown. The current study investigated the relationships between cortical gray and subcortical white matter in Parkinson's disease.

Methods: T1- and diffusion-weighted magnetic resonance images were obtained from 76 Parkinson's and 70 control subjects at baseline, 18-, and 36-months. Cortical volumes and subcortical white matter axial and radial diffusivities were compared between groups. Correlations between cortical volume (total and regional) and subcortical white matter diffusivity (overall and underlying regional cortex areas) were analyzed.

Results: At baseline, Parkinson's subjects had lower total cortical volumes and higher subcortical white matter axial and radial diffusivity than control subjects. Total cortical volume was correlated inversely with overall subcortical white matter axial ($p=0.010$) and radial diffusivity ($p<0.001$) in Parkinson's, but not in control subjects (group interaction $p<0.05$). The relationship tended to be stronger in later-stage vs. earlier-stage Parkinson's subjects (group interaction $p=0.052$ for axial diffusivity, $p=0.098$ for radial diffusivity). Among 21 regional volumes that were significantly different between groups, nine were correlated significantly with underlying subcortical white matter axial diffusivity and/or radial diffusivity in Parkinson's ($p<0.05$) but not control subjects. Longitudinally, Parkinson's subjects had accelerated rates of cortical atrophy. The rate of cortical atrophy was associated with the rate of axial diffusivity and/or radial diffusivity

increases in a number of regions in Parkinson's, and opposite associations were found in control subjects (interaction $p<0.05$). Subgroup analysis revealed that there were no correlations between annual changes in total cortex volume and white matter axial diffusivity ($p=0.358$) in earlier-stage Parkinson's subjects. In later-stage Parkinson's subjects, however, annual loss of cortex volume had a trend association with increasing subcortical white matter axial diffusivity that did not reach significance ($p=0.096$).

Conclusions: Cortical atrophy in Parkinson's disease is associated with poorer subcortical white matter microstructure. Thus, cortical atrophy and white matter degeneration may not be independent processes in Parkinson's. The opposing associations found in Parkinson's vs. control subjects suggest that the gray-white matter link might reflect impaired capacity to compensate for neuronal losses during the Parkinson's process.

Introduction

In Parkinson's disease, atrophic and degenerative changes have been well documented in a wide range of brain areas, including the cerebral cortex (5-7). Postmortem analysis of Lewy pathology has supported the notion that the progression of Parkinson's disease might follow a characteristic pattern, involving the brain stem early and cortical regions in more advanced stages (2). The recent discovery that Lewy pathology can spread across neurons has fueled the notion that neuronal disease may spread in a prion-like process (169). In addition, it has been suggested that long, thin, and poorly myelinated neurons (such as cortical projection neurons) are preferentially vulnerable to Lewy pathology (163). Thus, it seems likely that subcortical white matter tracts, which contain axonal projections from cortical neurons, could be an integral part of the Parkinson's disease process.

Ontogenetically, cortical neurons are born closely to each other in the ventricular zone and migrate along a common pathway to form cortical columns (170). The final number of ontogenetic cortical columns and neurons in each column determines the cortical surface area and thickness, respectively (171). Cortical thickness and surface area are thought to be independent genetically and may reflect separate processes (172). Cortical volume, however, may be a useful measure to detect overall structural changes during aging or disease processes, since it is the combined property of thickness and surface area. Indeed, loss of cortical volume has been documented both in healthy aging (117, 173) and Parkinson's disease (41), although the underlying mechanisms might be different. In Parkinson's disease, cortical cell losses (5, 174) may contribute to the loss of cortical volume. In healthy aging, dendrite losses and/or shrinkage of larger neurons (175) may drive the

changes in cortical volume, since the number of cortical neurons is thought to remain relatively constant (176-179).

The relationships between cortical gray matter and underlying subcortical white matter might be determined developmentally, but can be plastic and may change dynamically throughout the lifespan due to relevant experience, aging, and neurodegeneration. Although many studies have investigated the roles of cortical gray matter and subcortical white matter separately in Parkinson's disease, few studies have investigated the inter-relationship between them. I hypothesized that the neurodegenerative process occurring during Parkinson's disease might alter the relationships between cortical gray matter and subcortical white matter. The current study utilized cortical gray matter volume and subcortical white matter tract properties (axial and radial diffusivity) to investigate this hypothesis. I also explored the influence of disease duration on the gray-white matter association with the hope of shedding light on potential changes in neuroplasticity during Parkinson's progression.

Methods

Study subjects

Parkinson's disease (n=76) and control (n=70) subjects having a Mini Mental State Examination score ≥26 (148, 149) were selected from a longitudinal cohort study (Table 4a.1). Parkinson's disease patients were recruited from a tertiary movement disorders clinic, and control subjects were recruited from spousal populations and the local community. Parkinson's disease diagnosis was confirmed using published criteria (150). All subjects were free of major and acute medical issues or neurological disorders except Parkinson's disease. All brain images were inspected and deemed free of any major structural abnormalities or motion artifacts. In accordance with the

Declaration of Helsinki, written informed consent was obtained for all subjects. The study protocol was approved by the Penn State Hershey Institutional Review Board.

Clinical information and evaluation

Disease duration was defined as years since initial Parkinson's disease diagnosis. Hamilton Depression Rating Scale scores were obtained and recorded at each visit (128). Unified Parkinson's disease Rating Scale motor scores and Hoehn-Yahr stages were assessed for Parkinson's disease subjects in the "on-medication" state at each visit (26, 129). Levodopa-equivalent daily dose was calculated according to published criteria (153).

MRI data acquisition

All subjects were scanned using a 3.0 Tesla magnetic resonance scanner (Trio, Siemens Magnetom, Erlangen, Germany, with an 8-channel phased array head coil) at baseline, 18 months, and 36 months. A magnetization-prepared rapid acquisition gradient echo sequence was used to obtain T1-weighted images with TR/TE = 1540/2.34, FOV = 256 mm x 256 mm, matrix = 256 x 256, slice thickness = 1 mm (with no gap), slice number = 176. For diffusion tensor imaging, acquisition parameters were as follows: TR/TE=8300/82 ms, b value=1000 s/mm^2, diffusion gradient directions=42 and 7 b=0 scans, FOV=256 mm × 256 mm, matrix=128 × 128, slice thickness=2 mm (with no gap), and slice number=65.

Structural image processing

T1-weighted brain images were processed automatically using FreeSurfer's longitudinal pipeline (version 5.1.0) (154). Briefly, this pipeline creates unbiased within-subject templates that then were used to initialize image processing (skull stripping, Talairach transformations, atlas registration, spherical surface maps) for scans at each visit (155, 156). Cortical volumes were

computed by multiplying cortical thickness and surface area at each cortical surface vertex. Gray matter volumes in each cortical region were computed using regional means extracted from cortical parcellations (Freesurfer's Desikan atlas) (180).

Diffusion tensor image processing

Diffusion image quality control and tensor reconstruction was performed using DTIPrep (Neuro Image Research & Analysis Laboratory, University of North Carolina, Chapel Hill, NC USA). This software first checks diffusion weighted images for quality by calculating the inter-slice and inter-image intra-class correlation, and then corrects for the distortions induced by eddy currents and head motion (181). Diffusion tensor images subsequently were estimated via weighted least squares (182). Additional quality control was performed by visually inspecting the images for artifacts and directionality. Diffusion tensor images were skull-stripped using brain masks generated from the T1 image segmentation step.

Atlas building was performed using a two-stage process in order to ensure that the overall final atlas was not biased by subject dropout. The first stage involved creation of within-subject atlases using images from each subject's baseline and follow-up images. The second stage involved creation of an overall atlas using the within-subject atlases of all subjects. For creation of both the within-subject and the overall atlases, we utilized the DTIAtlasBuilder program (Neuro Image Research & Analysis Laboratory, University of North Carolina, Chapel Hill, NC USA). This software employs a state-of-the-art image registration pipeline. To generate atlases, affine registration first is applied using the BRAINSFit module within Slicer (183). Second, unbiased diffeomorphic deformations fields are computed using the GreedyAtlas module within AtlasWerks (184). Third, a refinement step is applied via symmetric diffeomorphic registration with the Slicer DTI-Reg module using Advanced Normalization Tools (185, 186). The final step of

DTIAtlasBuilder concatenates the transforms of the previous steps to compute the overall transformation of the original diffusion tensor images into the final average diffusion tenor imaging atlas. This allows for mapping between the atlas and the individual diffusion tensor images recorded at each visit without the need for resampling.

For the current study, we chose to use axial diffusivity, thought to be more specific to axonal degeneration, and radial diffusivity, thought to correlate inversely with axonal myelination (187-189). We defined several specific regions of interest on the fractional anisotropy (FA) map of the average DTI atlas. These regions of interest were chosen specifically for their relation to overlying cortical gray matter. "Overall subcortical white matter" was defined as a region of interest that was generated by first thresholding the overall final atlas image at fractional anisotropy > 0.2. The thresholded mask then was edited manually to exclude white matter in the brainstem, cerebellum, thalamus, and other gray matter where fractional anisotropy fell beyond the 0.2 threshold level. This final region of interest was used to extract diffusion scalar values of "overall subcortical white matter." We also examined subcortical white matter diffusion scalars in specific subcortical regions that were named according to the overlying gray matter. This was performed by co-registering a standardized white matter parcellation atlas (JHU-MNI-SS-TypeI) with the overall final atlas, and then mapping the parcellated white matter regions back to the original diffusion tensor images (190).

Statistical analysis

Group Comparisons

Age and years of education were compared between Parkinson's disease and control subjects using two-sample *t*-tests, whereas gender frequencies were compared using Fisher's Exact Test. MMSE scores were compared between groups using Wilcoxon Rank-Sum Test. Baseline total and regional cortical gray matter volumes and white matter diffusion measurements were compared

using one-way analysis of covariance with adjustment for age, gender, education, and depression scores, with intracranial volume added as a covariate for the gray matter analysis. To limit the probability of false positive findings in our analysis of gray matter-subcortical white matter relationships (see below), we restricted our analyses to cortical areas that were significantly lower ($p<0.05$) in Parkinson's disease subjects compared to control subjects (total and 21 regions). Rates of annual change in gray matter volumes and white matter diffusion were compared between groups using a linear mixed effects model with random slopes and intercepts and the same covariates as above, with the additional term of years elapsed (since baseline) and the interaction term years elapsed × group.

Gray matter-subcortical white matter associations

The baseline relationships between gray matter volume and subcortical white matter diffusion were explored using two models. The main model (Model 1) included gray matter volume as the dependent variable and the following independent variables: subcortical white matter diffusion scalar, intracranial volume, age, gender, education, Hamilton depression score, and disease duration (as appropriate). To test whether the correlation between gray matter and subcortical white matter depended upon group status (Parkinson's disease vs. control), we performed interaction analyses by adding the additional independent variable of Parkinson's disease (yes/no) × subcortical white matter diffusion scalar. To account for the possibility that associations between gray matter and subcortical white matter were driven primarily by intrinsic characteristics of the subjects, we performed a supplemental analysis (Model 2) using a secondary model that utilized residualized values of subcortical white matter diffusion scalars based on age, gender, education, and Hamilton depression scores and restricted this analysis to voxels where the overall atlas FA was > 0.2.

Longitudinal data were analyzed by linear regression where the slope of white matter diffusion across all visits (annual rate per subject) was the dependent variable and the independent variable was the slope of gray matter volume across all visits (annual rate per subject). Group differences were assessed using the additional term group × annual rate of gray matter volume change.

To lessen the likelihood that extreme values and/or non-normally distributed distributions might drive the study results, all cross-sectional comparisons and correlations using imaging measurements were analyzed using multiple linear regression and p-values generated via permutation testing of the model residuals (10,000 iterations) (191, 192). Raw p-values are reported due to the step-wise nature of the analysis, however, values that survived correction for multiple comparisons (using expected false discovery rate of 0.05) are noted (160). We define statistical significance as $p < 0.05$, using two-tail test, and trend as $p < 0.10$, using two tail test (equivalent $p < 0.05$, using one tail test) if the direction of association is consistent with our hypothesis All analyses were completed using R version 3.1.1 (193, 194). We define statistical significance as $p < 0.05$, using two-tail test, trend as $p<0.10$, using two tail test (equivalent $p < 0.05$, using one tail test) if the direction of association is consistent with our hypothesis.

Results

Demographic and characteristics of study subjects at baseline

Compared to controls, Parkinson's disease subjects were slightly older ($p=0.011$) but did not have significant differences in education or gender frequency (Table 4a.1). Parkinson's disease subjects, however, had lower Mini Mental State Examination ($p=0.024$) and Montreal Cognitive Assessment ($p=0.007$) scores and higher depression scores ($p<0.0001$) than control subjects. Clinical characteristics of Parkinson's disease subjects also are listed in Table 1.

Cortical gray matter volume in Parkinson's disease and control subjects

Parkinson's disease subjects had lower total cortex volume compared to control subjects at baseline ($p=0.006$) and in 21 out of 102 parcellated regions ($ps<0.05$) that were used subsequently for correlation analyses between cortical regional gray matter volume and underlying subcortical white matter diffusion. Both Parkinson's disease and control subjects had loss of total cortex volume over time ($ps<0.05$). In Parkinson's disease there was accelerated atrophy in the cortex overall and in 16 of the parcellated cortical regions that were examined, with 15 regions surviving correction for multiple comparisons ($p<0.05$) (Table 4a.2).

Subcortical white matter diffusional properties in Parkinson's disease and controls

There were no differences in the diffusion characteristics of overall subcortical white matter between control and Parkinson's disease subjects at baseline. Parkinson's disease subjects, however, had higher axial or radial diffusivity in six of the 21 regions examined ($ps<0.05$, Table 3). Longitudinally, both Parkinson's and control subjects had increasing axial and radial diffusivities over time in all of the subcortical white matter areas analyzed ($ps<0.05$), except control subjects did not have increasing axial diffusivity in the left inferior temporal area over time ($p=0.369$). The rate of radial diffusivity increase was higher in Parkinson's disease compared to that of control subjects for the left postcentral area ($p=0.015$), although this result did not survive correction for multiple comparisons (Table 4a.6).

Cross-sectional correlations of cortical volume and white matter diffusion

Using the main analysis model (Model 1), total cortex volume was correlated inversely with overall subcortical white matter axial diffusivity ($p=0.010$) and radial diffusivity ($p<0.001$) in Parkinson's disease subjects but not in control subjects ($p=0.702$ and $p=0.618$, respectively; Table 4). There was a significant interaction for group status (Parkinson's disease or control subjects;

p=0.015 for axial diffusivity and p=0.006 for radial diffusivity relationships). This indicated that the correlation between subcortical white matter diffusion measurements and cortex volume was dependent upon the disease status (as shown in Figure 4a.1). These correlations persisted in a stricter analysis (ps<0.05) using thresholded regions of interest and residualized diffusion values (p=0.009 for axial diffusivity and p=0.001 for radial diffusivity). Both axial and radial diffusivities of the underlying subcortical white matter also were correlated with a number of respective gray matter regional volumes in Parkinson's disease subjects (ps<0.05). Similar results were obtained when applying stricter criteria (Model 2) that utilized thresholded regions of interest and residualized diffusion values (Table 4a.4).

To explore how the association between gray matter volume and subcortical white matter diffusion at baseline relates to disease progression, we correlated these measurements in PD-Earlier and PD-Later subjects using disease duration in Parkinson's disease using the median of 4.26 years as the separation point. Total cortex volume was correlated inversely with subcortical white matter axial diffusivity in PD-Earlier (β=-2241, p=0.032) and PD-Later (β=-5103, p=0.025), and the trend-level interaction term of Parkinson's disease subgroup × axial diffusivity suggested that PD-Later subjects had a stronger gray-white matter relationship compared to PD-Earlier subjects (p=0.065). Total cortex volume also was correlated inversely with radial diffusivity in PD-Earlier (β=-1387, p=0.009) and PD-Later (β=-3032, p=0.015), although the interaction term of Parkinson's disease subgroup × radial diffusivity did not reach significance, even at the trend-level (p=0.160). Similar results were obtained using residualized and threshold-based diffusion values (Figure 4a.2).

Longitudinal correlations of cortical volume and subcortical white matter diffusion changes

In Parkinson's disease, annual loss of cortex volume was correlated significantly with the annual increase in subcortical white matter axial and radial diffusivities in several areas ($p<0.05$), and there may have been a trend-level correlation for total gray matter and overall subcortical white matter axial diffusivity ($p=0.084$). Correlations of the opposite direction were found in several areas in controls ($p<0.05$) (Table 4a.5).

We also explored whether the longitudinal associations were modulated by disease duration. PD-Earlier subjects had no correlations between annual change in total cortex volume and annual subcortical white matter axial diffusivity change ($p=0.358$), although there might have been a trend-level association after removal of one outlier ($p=0.090$). In PD-Later subjects, annual loss of cortex volume had a trend-level association with annually increasing subcortical white matter axial diffusivity ($p=0.096$; Figure 4a.3). No correlations were found for radial diffusivity measurements in the two disease stage groups.

Discussion

To our knowledge, the current study is the first to investigate the relationship between gray matter atrophy and white matter diffusion properties in Parkinson's disease. The results suggest that in contrast with healthy aging subjects, cortical gray matter atrophy in Parkinson's disease is related to poorer microstructural integrity of underlying subcortical white matter, particularly in later-stages. This suggests that unlike healthy aging, the Parkinson's process may have impaired the neuroplastic capacity of the brain to compensate for neural losses that progressively get worse as the disease advances. Future studies with a longer duration of follow-up are needed to replicate these findings.

Furthermore, exploration of the responsible mechanisms is warranted since they may have important implications for understanding Parkinson's disease pathology and its progression.

Gray matter and white matter changes in Parkinson's disease

Gray matter atrophy in Parkinson's disease is well documented (33-41), and the current study confirmed these findings in a longitudinal cohort. In contrast, past research has yielded inconsistent results regarding the extent and nature of white matter involvement in Parkinson's disease. Some studies have suggested that Parkinson's disease subjects have worse subcortical white matter diffusion properties (195-201) particularly in advanced-stage, cognitively impaired, and depressed patients (167). Conversely, other studies have found no or minimal evidence of altered white matter changes in Parkinson's disease (202-205). The current study supports those reports of worse white matter diffusion properties in several brain regions of non-demented Parkinson's disease. No subcortical white matter diffusion differences, however, survived correction for multiple comparisons. Similarly, although the rate of cortical gray matter volume loss in most cortical regions examined were accelerated significantly in Parkinson's disease compared to control subjects, we found no differences in the rates of white matter diffusion change. This may indicate that the effects of Parkinson's disease on white matter microstructural integrity are relatively weaker than those leading to gray matter atrophy. It also may be that white matter microstructural changes are more difficult to capture via diffusion tensor imaging. Larger sample sizes and more sensitive methods for capturing white matter properties on imaging might be useful to confirm these findings in future studies.

The gray-white matter associations in Parkinson's disease

Our findings of a gray matter-white matter association in Parkinson's disease are in agreement with those of Ham et al. (206), who reported that cortical atrophy was associated with the

distribution of white matter hyperintensities in Parkinson's disease. The current study, however, utilized distinct diffusion measures (axial and radial diffusivity) that provide quantitative measurements of the microstructural properties of subcortical white matter, as opposed to categorical classification of white matter hyperintensities (206). In addition, we utilized a spatial approach to pair cortical gray matter areas with underlying subcortical white matter, and included a control cohort for comparison of correlation strengths. It is possible that the observed gray matter-white matter relationships represent separate parallel processes throughout Parkinson's disease progression. This is less likely, however, because we included disease duration as a covariate in the analyses to account for progression effects. Taken together, these findings suggest that degenerative changes in cortical gray matter and subcortical white matter are not necessarily independent phenomena in Parkinson's disease.

Possible biological mechanisms of the gray-white matter associations in Parkinson's disease

There are several possible explanations for the observed gray matter-white matter correlations in Parkinson's disease. *First,* the gray matter-white matter associations might be attributable to the death of the overlying cortical cells consequent to anterograde degeneration of axons in the subcortical white matter (207). This would be consistent with previous reports suggesting that while Parkinson's disease subjects undergo cell losses (2, 5, 6), the number of cortical neurons in normal aging remains relatively constant (175, 176) *Second,* microstructural damage to subcortical white matter axons might cause cellular changes in the overlying cortical gray matter substrate. This notion is supported by documentation of cortical atrophy in association with subcortical white matter hyperintensities in both Parkinson's (206) and non-Parkinson's (208) populations. *Third,* the extent of subcortical white matter myelination may influence cortical neuronal survival in Parkinson's disease. In support of this hypothesis, cardiac nerve biopsies show

that alpha-synuclein aggregates accumulate much more abundantly in unmyelinated axons in Parkinson's subjects (146), and the pattern of Lewy deposition has been suggested to be related inversely to the myelination pattern of the developing human brain (163). Consistent with this protective myelin hypothesis, the current study found that radial diffusivity (which is thought to vary inversely with axonal myelination) showed significant associations with total cortical gray matter volume in a number of parcelated cortical regions. Change in radial diffusivity, however, was not correlated with change in cortical volume longitudinally. Furthermore, subcortical white matter axial diffusivity was correlated with cortical gray matter volume in both cross-sectional and longitudinal analyses, suggesting that axonal integrity also is related with overlying cortical volume (187-189).

It is important to note that cortical gray matter volume is known to decrease throughout the lifespan (117, 173), and subcortical white matter axial and radial diffusivities increase sharply after the age of 60 years (209, 210). In normal aging, there is a loss of dendrites in the cortex (211) and subcortical white matter fibers (212). Consistent with this, the current study found cortex atrophy and worsening subcortical white matter diffusion over time in both Parkinson's disease and control subjects. The finding of differential cortical gray and subcortical white matter associations between Parkinson's disease and healthy aging subjects, however, was both unexpected and intriguing. Some evidence suggests that whereas healthy brain tissue can compensate for neuronal loss by proliferating new dendrites, patients with neurodegenerative diseases may have reduced plastic capacity for cortical dendritic remodeling (213-217). Consistent with this hypothesis, our study found a hint of increasing cortical volume in association with poorer subcortical white matter diffusivity in a number of parcelated brain regions in control subjects (Tables 4.4 and 4.5). In contrast, cortical volume losses in Parkinson's disease probably are due to neuronal loss and associated axonal degeneration combined with a limited capacity to compensate for damage. It also

is notable that gray-white matter associations appeared stronger in later-stage patients, perhaps because of further reduced compensatory capacity. Future research is needed to replicate our findings and elucidate the potential link between diminishing neuroplasticity and the gray-white matter associations in Parkinson's disease.

Limitations & summary

Although our longitudinal design is a strength, the sample size is still relatively small. In designing the study, we tried to balance carefully the potential of both false positive and false negative results. To limit the probability of false positive gray-white matter associations, we only analyzed the associations in areas that were found to have lower gray matter volumes in Parkinson's disease subjects at baseline. To lower the likelihood of false negatives or excluding regions of potential interest, we utilized a criteria of raw $p<0.05$ for areas to be included subsequently in the gray-white matter association analyses. Despite these efforts, it still is possible that we excluded some biologically meaningful regions of interests and/or introduced false positive regions of interests into the association analyses. It is important to point out, however, that 1) several of the reported gray-white matter associations at baseline survived correction for multiple comparisons and 2) the results of longitudinal analyses were consistent with baseline analysis. Together, these findings support the credibility of our overall conclusion that cortical gray and subcortical white matter are related in Parkinson's disease. Nevertheless, independent replication is needed because such data may yield important information regarding neuroplasticity in normal aging and progressive neurodegenerative disease.

The current study suggested that poorer subcortical white matter microstructural characteristics and cortical atrophy are related in Parkinson's disease. These results suggest that cortical atrophy and altered white matter diffusion properties in Parkinson's disease described by

previous studies should not be assumed to be independent. The differential cortical gray–subcortical white matter associations found in Parkinson's and control subjects suggests that Parkinson's patients, in contrast to healthy aging subjects, have impaired capacity to compensate for cortical gray matter losses. Further studies are warranted to confirm these findings and elucidate underlying mechanisms of the cortical gray-subcortical white matter link in Parkinson's disease.

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Table 4a.1. *Demographic and clinical characteristics of study subjects*

	PD	**Control**	**P-Value**
n (Female : Male)	76 (29 : 47)	70 (36 : 34)	0.134
Age (years)	63.3 ± 8.4	59.9 ± 7.7	0.011
Education (years)	15.9 ± 2.7	16.6 ± 2.8	0.135
MMSE	29.1 ± 1.1	29.5 ± 0.9	0.024
MoCA	24.6 ± 3.6	26.1 ± 2.5	0.007
HAM	7.7 ± 4.5	3.8 ± 2.5	<0.0001
UPDRS-III	22.5 ± 14.3	-	-
LEDD	557 ± 457	-	-
Duration of disease (years)	4.9 ± 5.5	-	-
HY Stage	1.8 ± 0.7	-	-

Abbreviations – HAM: Hamilton depression rating scale; HY: Hoehn-Yahr; LEDD: Levodopa daily equivalent dosage; MMSE: Mini mental state exam; MoCA: Montreal Cognitive Assessment; UPDRS: Unified Parkinson's disease rating scale;

Table 4a.2. *Areas of cortical gray matter volumes that were significantly different between Parkinson's disease and control subjects at baseline (least squares adjusted means) and corresponding rates of change (model estimates).*

	Comparisons of GM Volume			**Comparisons of ΔGM Volume/y**		
	PD	**Control**	**P-Value**	**PD**	**Control**	**P-Value**
Cortex	446477 ± 33247	458554 ± 34885	***0.006***	-3371 ± 4828	-1552 ± 2314	***0.001****
Bilateral Averages						
BSTS	2235 ± 409	2380 ± 430	***0.007***	-15.3 ± 31.4	-7.2 ± 16.1	***0.042***
Fus	9355 ± 1476	9759 ± 1549	***0.042***	-76.7 ± 95.6	-39.2 ± 73.1	***0.009****
IP	12711 ± 2017	13273 ± 2116	***0.040***	-73.5 ± 212.7	-5.6 ± 82.5	***0.003****
IT	10037 ± 1450	10772 ± 1522	***<0.001****	-66.1 ± 129.6	-33.7 ± 56.2	***0.024****
LOC	11071 ± 1559	11577 ± 1636	***0.015***	-81.9 ± 151.0	-25.7 ± 67.6	***0.002****
LOF	7064 ± 743	7309 ± 780	***0.013***	-34.2 ± 70.1	-20.5 ± 49.8	0.127
MT	10472 ± 1519	11186 ± 1594	***0.001****	-81.9 ± 125.0	-31.5 ± 64.5	***0.001****
PoC	9320 ± 1267	9854 ± 1330	***0.001****	-87.5 ± 128.9	-50.7 ± 79.8	***0.036****
SP	12720 ± 1713	13245 ± 1797	***0.022***	-121.0 ± 205.8	-62.8 ± 107.3	***0.020****
Left Hemisphere						
L. IT	10167 ± 1650	10691 ± 1731	***0.017***	-65.0 ± 159.3	-33.2 ± 70.7	0.055
L. LOC	11157 ± 1894	11838 ± 1987	***0.006***	-73.5 ± 158.9	-14.0 ± 63.6	***0.002****
L. MT	9916 ± 1725	10640 ± 1810	***0.002****	-74.8 ± 146.7	-21.1 ± 73.2	***0.001****
L. PoC	9509 ± 1355	10137 ± 1421	***<0.001****	-97.6 ± 126.9	-56.9 ± 80.6	***0.025****
Right Hemisphere						
R. BSTS	2144 ± 491	2308 ± 515	***0.013***	-18.8 ± 36.3	-13.6 ± 17.0	0.202
R. Fus	9232 ± 1650	9759 ± 1732	***0.018***	-79.6 ± 109.7	-40.3 ± 85.1	***0.012****
R. IP	13676 ± 2437	14561 ± 2557	***0.008***	-90.0 ± 226.3	-18.7 ± 107.1	***0.008****
R. IT	9907 ± 1780	10854 ± 1868	***<0.001****	-67.9 ± 132.4	-34.6 ± 66.4	***0.029****
R. LOF	6943 ± 821	7222 ± 862	***0.011***	-48.8 ± 82.6	-23.5 ± 66.3	***0.021****
R. MT	11027 ± 1743	11732 ± 1829	***0.002****	-86.1 ± 125.5	-39.5 ± 71.1	***0.004****
R. PoC	9130 ± 1540	9570 ± 1616	***0.032***	-78.0 ± 149.3	-44.2 ± 92.2	0.090
R. SP	12576 ± 1905	13247 ± 1999	***0.009***	-128.0 ± 221.6	-76.3 ± 135.1	0.069

* Significant after false discovery rate adjustment using 102 cortical regions

Abbreviations – AD: Axial diffusivity; BST: Banks of superior temporal sulcus; Fus: Fusiform; GM: gray matter; IP: Inferior parietal; IT: Inferior temporal; L: Left; LOC: Lateral occipital; LOF: Lateral orbitofrontal; MT: Middle temporal; PoC: Postcentral; RD: Radial diffusivity; R: Right; SP: Superior parietal;

Table 4a.3. *Comparisons of subcortical white matter regions between Parkinson's disease and control subjects at baseline.*

	Group Comparisons					
	AD (×10^{-5} mm^2/sec)			**RD (×10^{-5} mm^2/sec)**		
	PD*	**Control***	**P-Value**	**PD***	**Control***	**P-Value**
Overall SCWM	116.8 ± 4.2	116.1 ± 4.4	0.262	60.1 ± 3.9	59.6 ± 4.0	0.304
Bilateral Averages						
BSTS	106.5 ± 5.2	104.7 ± 5.4	***0.007***	62.6 ± 4.4	61.4 ± 4.6	***0.032***
Fus	96.0 ± 4.9	94.6 ± 5.1	***0.033***	68.4 ± 4.2	67.2 ± 4.4	***0.029***
IP	103.9 ± 6.2	103.4 ± 6.5	0.534	63.4 ± 6.1	62.2 ± 6.4	0.134
IT	114.5 ± 6.4	113.0 ± 6.7	0.071	66.3 ± 5.1	65.0 ± 5.4	0.064
LOC	95.6 ± 5.9	94.3 ± 6.2	0.103	62.3 ± 4.9	61.0 ± 5.1	0.058
LOF	99.1 ± 6.2	98.9 ± 6.5	0.811	65.9 ± 5.2	65.5 ± 5.5	0.494
MT	109.1 ± 5.9	107.6 ± 6.2	*0.053*	63.6 ± 4.8	61.9 ± 5.1	***0.010***
PoC	102.0 ± 5.6	100.9 ± 5.9	0.134	60.4 ± 4.9	59.7 ± 5.1	0.283
SP	109.7 ± 7.8	108.5 ± 8.2	0.260	61.7 ± 6.8	60.4 ± 7.2	0.153
Left Hemisphere						
L. IT	112.9 ± 6.3	111.4 ± 6.6	0.088	63.5 ± 5.2	62.6 ± 5.4	0.154
L. LOC	93.7 ± 7.0	92.3 ± 7.4	0.138	63.9 ± 5.8	62.9 ± 6.1	0.177
L. MT	107.0 ± 5.6	105.6 ± 5.9	*0.052*	61.5 ± 4.9	60.0 ± 5.1	***0.026***
L. PoC	103.5 ± 6.4	102.2 ± 6.7	0.116	61.9 ± 5.6	61.1 ± 5.8	0.317
Right Hemisphere						
R. BSTS	109.7 ± 6.5	107.5 ± 6.8	***0.011***	64.7 ± 5.7	63.0 ± 6.0	***0.023***
R. Fus	97.2 ± 6.6	95.7 ± 7.0	0.081	69.3 ± 5.4	68.0 ± 5.6	0.066
R. IP	103.5 ± 7.6	102.6 ± 8.0	0.383	64.6 ± 8.1	63.3 ± 8.5	0.219
R. IT	116.1 ± 8.4	114.5 ± 8.8	0.147	69.1 ± 6.3	67.5 ± 6.6	0.057
R. LOF	98.5 ± 5.6	98.8 ± 5.9	0.665	66.1 ± 5.2	66.1 ± 5.5	0.950
R. MT	111.1 ± 7.4	109.5 ± 7.8	0.116	65.7 ± 5.6	63.8 ± 5.9	***0.011***
R. PoC	103.5 ± 6.4	102.2 ± 6.7	0.112	61.9 ± 5.6	61.1 ± 5.8	0.313
R. SP	110.7 ± 9.6	109.5 ± 10.1	0.359	62.5 ± 8.8	60.8 ± 9.3	0.158

*value reported as least squares adjusted means

Abbreviations – AD: Axial diffusivity; BST: Banks of superior temporal sulcus; Fus: Fusiform; GM: gray matter; Hemi: hemisphere; IP: Inferior parietal; IT: Inferior temporal; L: Left; LOC: Lateral occipital; LOF: Lateral orbitofrontal; MT: Middle temporal; PoC: Postcentral; RD: Radial diffusivity; R: Right; SP: Superior parietal;

Table 4a.4. *Associations between cortical gray matter volumes and diffusion measurements in underlying white matter at baseline.*

	PD Subjects				**Control Subjects**				**Group Interaction**			
	AD		**RD**		**AD**		**RD**		**AD**		**RD**	
	β	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**
Cortex Total	-2566	0.010	-1776	0.001*†	-369	0.698	-248	0.623	-2149	0.029	-1347	**0.013**
Bilateral Averages												
BSTS	-16.8	0.079	-3.6	0.504	-6.6	0.500	-4.6	0.482	-9.9	0.383	-0.6	0.938
Fus	-65.3	0.034	-38.6	0.024	-67.4	0.138	-53.3	0.076	-26.7	0.520	-7.3	0.769
IP	-75.7	0.016	-47.3	0.004*†	-3.0	0.946	-20.1	0.365	-92.4	0.050	-38.5	0.078
IT	9.9	0.718	-13.7	0.357	-9.3	0.774	-26.3	0.203	-4.3	0.889	-6.5	0.731
LOC	-90.1	0.005†	-52.3	0.004*†	16.2	0.604	-9.9	0.591	-100.3	0.009	-39.6	0.061
LOF	3.5	0.824	-12.1	0.172	2.0	0.885	-7.2	0.366	-14.2	0.438	-12.3	0.220
MT	-54.0	0.090	-41.1	0.028	45.1	0.156	-0.1	0.996	-81.4	0.039	-33.7	0.130
PoC	-39.3	0.118	-39.3	0.005*	10.2	0.737	-1.9	0.911	-60.6	0.084	-34.1	0.084
SP	-21.3	0.331	-10.6	0.399	6.0	0.857	2.8	0.873	-21.0	0.534	-6.6	0.731
Left Hemisphere												
L. IT	-6.2	0.848	-21.2	0.224	-6.5	0.854	-31.2	0.160	-10.8	0.782	0.4	0.985
L. LOC	-84.0	0.015	-44.1	0.018†	29.5	0.346	4.1	0.840	-113.4	0.004	-50.9	0.024
L. MT	-13.9	0.693	-38.4	0.042	13.0	0.767	-23.6	0.352	-32.0	0.505	-17.7	0.493
L. PoC	-17.9	0.470	-24.2	0.091	8.0	0.780	3.9	0.798	-39.7	0.234	-25.6	0.176
Right Hemisphere												
R. BSTS	-1.4	0.887	0.1	0.977	1.2	0.888	4.1	0.513	-4.7	0.676	-4.2	0.553
R. Fus	-17.5	0.467	-14.4	0.329	-37.1	0.415	-31.0	0.306	-8.3	0.838	-0.2	0.994
R. IP	-71.5	0.041	-40.3	0.031	-41.7	0.316	-29.0	0.089	-54.2	0.252	-19.4	0.368
R. IT	4.5	0.853	-12.9	0.390	6.4	0.822	1.8	0.930	-18.8	0.546	-25.2	0.196
R. LOF	11.9	0.529	-5.9	0.553	18.3	0.257	-2.4	0.779	-24.7	0.269	-13.2	0.234
R. MT	-58.4	0.067	-33.0	0.098	61.3	0.025	21.6	0.228	-96.7	0.009	-41.0	0.069
R. PoC	-59.5	0.024	-50.9	0.001*†	-2.7	0.933	-8.7	0.618	-64.9	0.083	-36.9	0.079
R. SP	-3.4	0.856	-1.6	0.874	-2.2	0.944	2.2	0.891	-2.9	0.934	-1.9	0.915

Estimates and p-values in are calculated using a linear model with control & Parkinson's disease subjects. Bold denotes unadjusted p<0.05. Underlined p values denote significance ($p \leq 0.05$) when employing thresholded regions of interest (where FA > 0.2) and residualized values of diffusion scalars (age, gender, education years, Hamilton depression score).

* Significant after false discovery rate adjustment using 22 cortical regions

† Significant after false discovery rate adjustment using 22 cortical regions with thresholded regions of interest and residualized values of diffusion scalars.

Abbreviations – AD: Axial diffusivity; BST: Banks of superior temporal sulcus; Fus: Fusiform; GM: gray matter; Hemi: hemisphere; IP: Inferior parietal; IT: Inferior temporal; L: Left; LOC: Lateral occipital; LOF: Lateral orbitofrontal; MT: Middle temporal; PoC: Postcentral; RD: Radial diffusivity; R: Right; SP: Superior parietal

Table 4a.5. *Associations between annual change in cortical gray matter volume and annual change in diffusion of underlying white matter.*

	PD Subjects				Control Subjects				Group Difference			
	AD		RD		AD		RD		AD		RD	
	β	P-Val	β	P-Val	β	P-Val	β	P-Val	β	P-Val	β	P-Val
Cortex	-0.162	0.084	-0.005	0.969	0.119	0.488	0.209	0.420	-0.101	0.184	-0.011	0.922
Bilateral Averages												
BSTS	-0.040	0.654	0.118	0.260	0.149	0.410	0.117	0.587	-0.001	0.988	0.109	0.228
Fus	-0.281	0.049	-0.213	0.149	-0.161	0.339	-0.322	0.110	-0.245	0.016	-0.271	0.017
IP	-0.177	0.061	-0.063	0.595	0.427	0.018	0.819	0.002*†	-0.082	0.279	0.038	0.714
IT	-0.114	0.153	-0.064	0.598	0.174	0.329	0.389	0.195	-0.056	0.433	-0.008	0.944
LOC	-0.325	0.002*	-0.334	0.003	-0.020	0.921	-0.062	0.795	-0.222	0.013	-0.268	0.007
LOF	0.213	0.420	0.139	0.664	0.178	0.488	0.081	0.730	0.177	0.333	0.077	0.705
MT	-0.074	0.445	0.051	0.694	0.049	0.813	0.065	0.829	-0.039	0.642	0.026	0.822
PoC	-0.104	0.248	0.125	0.274	0.177	0.258	0.135	0.519	-0.041	0.571	0.106	0.258
SP	-0.228	0.032	-0.150	0.295	0.398	0.014	0.646	0.005*†	-0.097	0.246	-0.012	0.920
Left Hemisphere												
L. IT	-0.110	0.241	-0.164	0.246	0.360	0.033	0.499	0.074	-0.016	0.843	-0.049	0.694
L. LOC	-0.483	<0.001*	-0.597	<0.001	-0.290	0.206	-0.366	0.142	-0.383	<0.001	-0.514	<0.001*
L. MT	-0.129	0.070	-0.094	0.464	-0.071	0.646	0.064	0.801	-0.119	0.063	-0.094	0.378
L. PoC	-0.101	0.315	0.177	0.165	0.155	0.326	0.114	0.593	-0.036	0.638	0.155	0.135
Right Hemisphere												
R.	0.047	0.686	0.172	0.297	0.257	0.196	0.171	0.462	0.108	0.266	0.182	0.153
R. Fus	-0.090	0.600	-0.037	0.828	-0.034	0.844	-0.149	0.460	-0.063	0.591	-0.077	0.541
R. IP	-0.103	0.364	-0.024	0.874	0.318	0.042	0.644	0.006*†	-0.007	0.928	0.109	0.345
R. IT	-0.038	0.776	0.093	0.555	0.054	0.841	0.232	0.503	-0.011	0.927	0.098	0.499
R. LOF	0.407	0.046	0.337	0.202	-0.006	0.972	-0.003	0.987	0.197	0.134	0.124	0.441
R. MT	-0.015	0.922	0.196	0.258	0.192	0.463	0.095	0.780	0.052	0.663	0.163	0.280
R. PoC	-0.003	0.973	0.160	0.179	0.138	0.282	0.190	0.270	0.040	0.572	0.160	0.088
R. SP	-0.263	0.014	-0.190	0.206	0.255	0.059	0.438	0.022	-0.124	0.122	-0.038	0.737

* Significant after false discovery rate adjustment using 22 cortical regions

† Significant after false discovery rate adjustment using 22 cortical regions with thresholded regions of interest.

Table 4a.6. *Comparisons of subcortical white matter annual change between Parkinson's disease and control subjects.*

	Group Comparisons					
	Δ AD (×10^{-5} mm^2/sec)/y			**Δ RD (×10^{-5} mm^2/sec)/y**		
	PD	**Control**	**P-Value**	**PD**	**Control**	**P-Value**
Overall SCWM	0.954 ± 0.639	0.976 ± 0.412	0.868	0.789 ± 0.603	0.599 ± 0.329	0.059
Bilateral Averages						
BSTS	0.974 ± 0.810	1.036 ± 0.687	0.704	0.676 ± 0.640	0.616 ± 0.540	0.636
Fus	0.846 ± 0.852	0.864 ± 0.542	0.905	0.755 ± 0.698	0.567 ± 0.445	0.095
IP	1.314 ± 0.689	1.334 ± 0.622	0.901	1.017 ± 0.705	0.900 ± 0.604	0.394
IT	0.547 ± 0.746	0.573 ± 0.646	0.856	0.719 ± 0.622	0.585 ± 0.492	0.299
LOC	0.838 ± 0.818	0.994 ± 0.566	0.316	0.656 ± 0.704	0.540 ± 0.492	0.314
LOF	1.265 ± 1.387	1.355 ± 0.967	0.706	1.084 ± 1.134	1.019 ± 0.585	0.721
MT	0.651 ± 0.812	0.730 ± 0.657	0.598	0.751 ± 0.691	0.658 ± 0.478	0.452
PoC	1.277 ± 0.905	1.246 ± 0.512	0.834	0.891 ± 0.864	0.686 ± 0.611	0.103
SP	1.462 ± 0.912	1.541 ± 0.851	0.696	1.258 ± 0.831	1.169 ± 0.655	0.588
Left Hemisphere						
L. IT	0.288 ± 0.738	0.129 ± 0.850	0.314	0.483 ± 0.829	0.312 ± 0.676	0.279
L. LOC	1.062 ± 0.985	1.287 ± 0.780	0.235	0.870 ± 0.847	0.777 ± 0.655	0.535
L. MT	0.549 ± 0.814	0.505 ± 0.767	0.763	0.652 ± 0.833	0.542 ± 0.556	0.424
L. PoC	1.251 ± 0.900	1.130 ± 0.488	0.403	0.923 ± 0.872	0.617 ± 0.579	***0.015***
Right Hemisphere						
R. BSTS	1.056 ± 1.065	1.230 ± 1.001	0.443	0.699 ± 0.994	0.803 ± 0.913	0.596
R. Fus	0.538 ± 0.953	0.770 ± 0.710	0.297	0.486 ± 0.791	0.493 ± 0.563	0.966
R. IP	1.342 ± 0.798	1.400 ± 0.835	0.761	0.944 ± 0.779	0.928 ± 0.806	0.925
R. IT	0.812 ± 1.095	1.063 ± 0.832	0.262	0.925 ± 0.723	0.860 ± 0.677	0.679
R. LOF	1.182 ± 1.717	1.124 ± 0.838	0.781	1.079 ± 1.540	0.841 ± 0.535	0.148
R. MT	0.721 ± 1.080	0.922 ± 0.840	0.323	0.835 ± 0.958	0.758 ± 0.751	0.599
R. PoC	1.295 ± 1.025	1.349 ± 0.581	0.742	0.861 ± 0.934	0.760 ± 0.687	0.476
R. SP	1.623 ± 1.025	1.709 ± 0.987	0.700	1.343 ± 0.918	1.298 ± 0.789	0.808

Abbreviations – AD: Axial diffusivity; BST: Banks of superior temporal sulcus; Fus: Fusiform; GM: gray matter; Hemi: hemisphere; IP: Inferior parietal; IT: Inferior temporal; L: Left; LOC: Lateral occipital; LOF: Lateral orbitofrontal; MT: Middle temporal; PoC: Postcentral; RD: Radial diffusivity; R: Right; SP: Superior parietal;

Figures

Figure 4a.1. *Relationships between cortical gray matter volume and underlying subcortical white matter diffusion in Parkinson's disease vs. Controls. Parkinson's disease subjects had stronger interactions between cortical gray matter volume and subcortical white matter diffusion properties. Cortex gray matter volume are shown as a percent of intracranial volume for illustration purposes.*

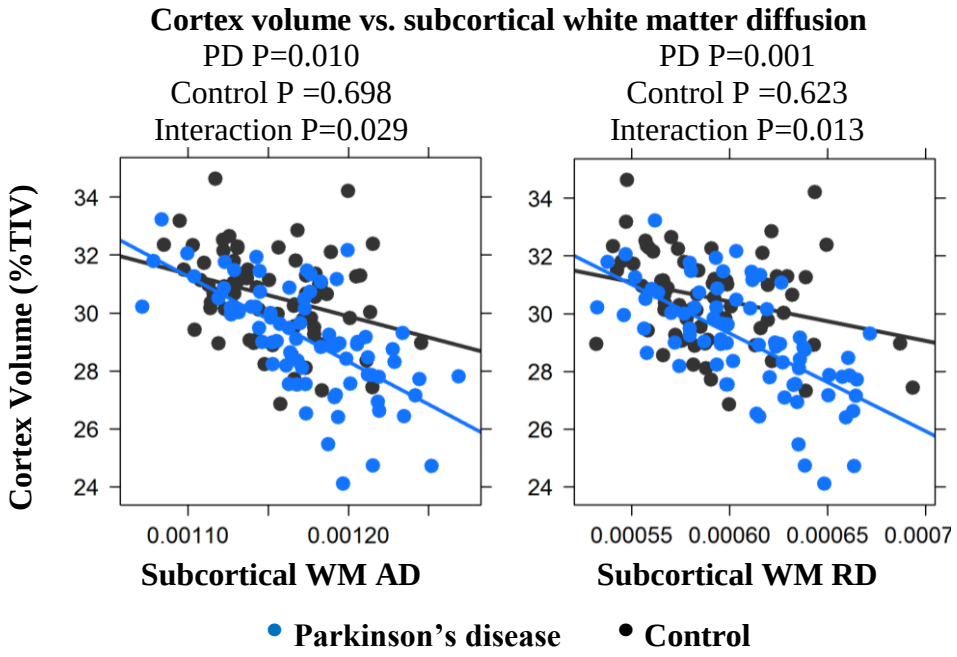


Figure 4a.2. *Relationships between cortical volume and underlying subcortical white matter diffusion in earlier- vs. later-stage Parkinson's disease subjects. Cortex volume in Parkinson's disease subjects is shown as the percent of intracranial volume for illustration purposes.*

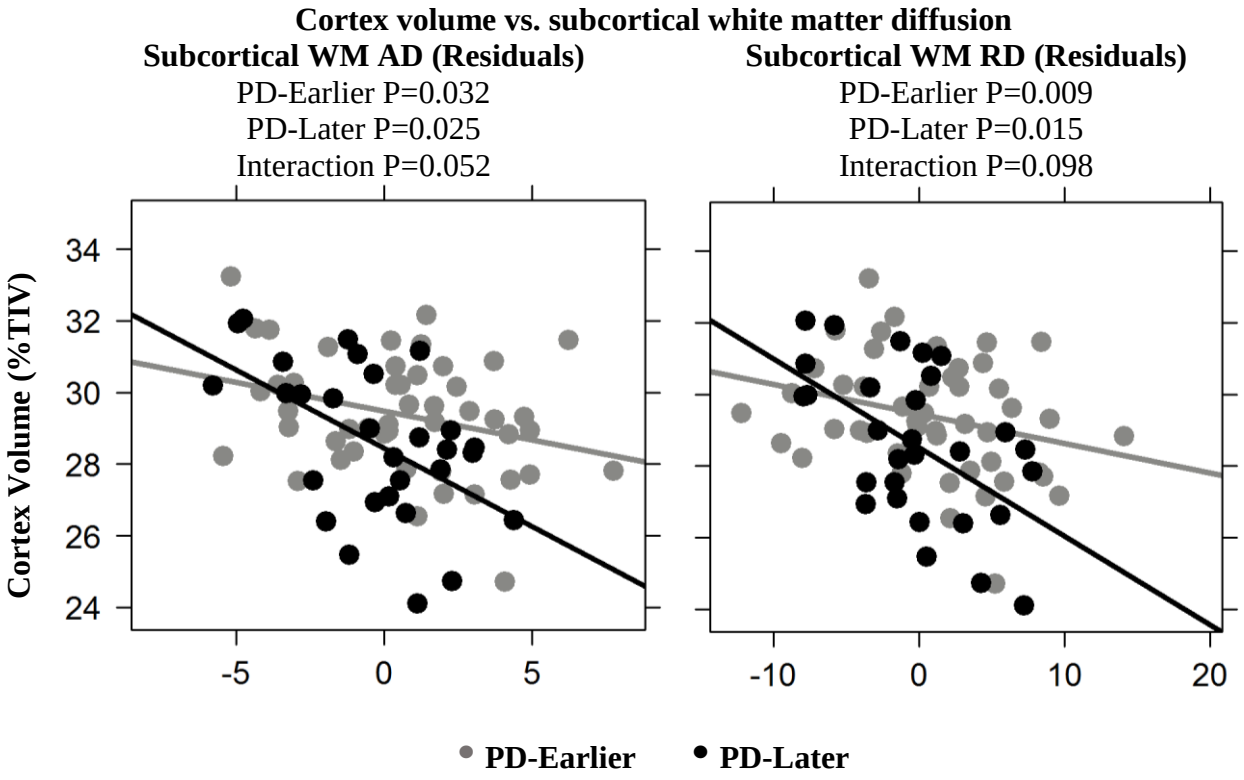
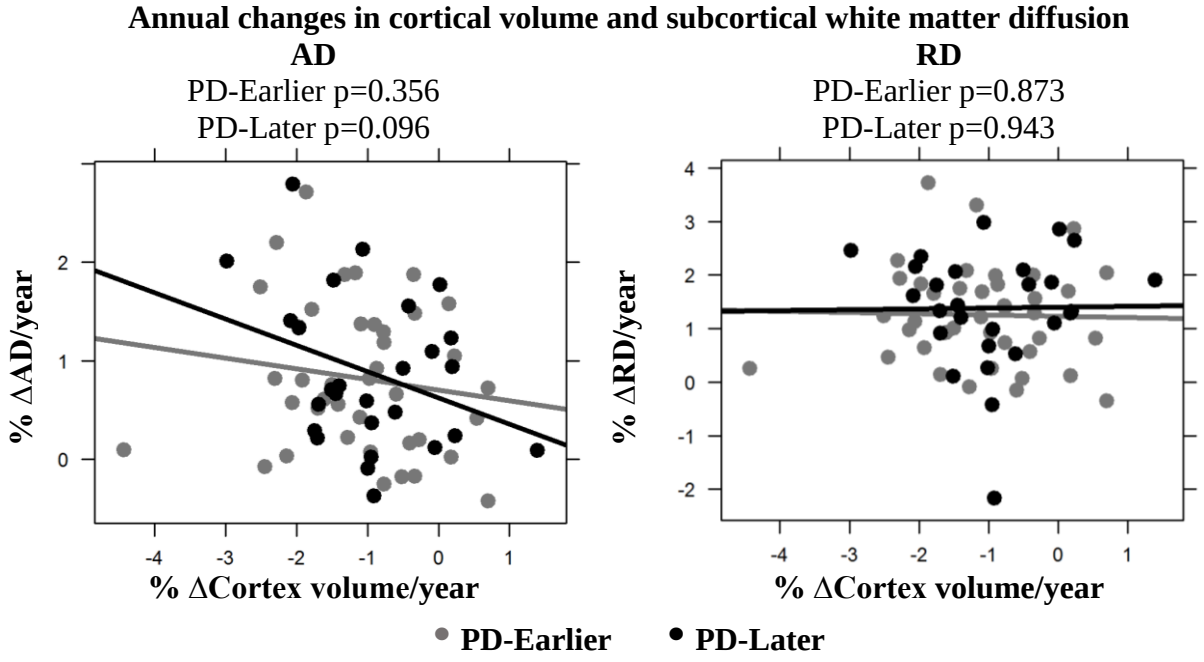


Figure 4a.3. *Relationships between annual change in cortical volume and annual change in underlying subcortical white matter diffusion in earlier- vs. later-stage PD subjects.*



Summary & future directions

The role of white matter in PD is controversial. Several past studies have reported altered subcortical white matter diffusion characteristics in PD, whereas others have reported no differences in comparison to control subjects (167). Since cortical gray and subcortical white matter contain cell bodies and myelinated axons of cortical projection neurons, respectively, it seems reasonable to suspect that the properties of one substrate might influence the other. This section addressed the relationships between cortical gray matter changes and subcortical white matter diffusion characteristics in PD, with three particular areas of interests as follows.

First, I assessed differences in white matter diffusion characteristics between PD and control subjects. This study confirmed that PD subjects have poorer white matter diffusion characteristics compared to control subjects. However, these differences are not as robust as the structural differences of gray matter areas between PD and control subjects.

Second, I explored the possible role of axonal myelination in PD, since previous studies have suggested that it may play a protective role against PD-related pathology (2, 146). To investigate the differential associations of cortical structure and axonal properties in PD, I utilized axial and radial diffusivity as gauges of general axonal integrity and axonal myelination, respectively. The results showed that axial (a surrogate for general axonal integrity) and radial (representing the extent of myelination) diffusivities were both correlated with cortical volume, but axonal diffusivity had stronger temporal associations. These findings do not strongly support the notion that axonal myelination plays a neuroprotective role in PD. However, these findings should be interpreted with a degree of caution because methods used in this research were based on diffusion characteristics and might not have been sensitive and specific enough to fully capture myelination properties of white matter. Future study with newer techniques (i.e. diffusion kurtosis imaging) may be needed to

explore white matter microstructure more deeply in PD (218). Furthermore, the time course was over 3 years, with data collection time points spaced by 18 months, and this study might not have had the temporal resolution necessary to detect associations between axonal myelination and cortical volume. Thus, further research with longer follow-up may be necessary to determine whether axonal myelination modifies the trajectory of PD-related pathology, since pharmacologic therapies that may induce myelination are also under investigation (219).

Interestingly, while I found longitudinally that worsening subcortical white matter axonal integrity was associated with cortical atrophy over time in PD, these associations was reversed in control subjects. These findings could suggest that cortical gray and subcortical white matter degeneration go hand-in-hand over time in PD, while healthy aging control subjects may still be able to compensate for age-related subcortical white matter deterioration (212) (209, 210), possibly through known processes such as increased dendrite proliferation (213-217).

This study in *Chapter 4* put forward some of the first evidence that cortical gray and subcortical white matter degeneration in PD may be related. There are some potentially worthwhile avenues for future research. In this study, I used cortical volume as a surrogate for PD-related pathology, but prior research has specifically focused on the relationship between alpha-synuclein pathology and white matter myelination (146, 163). There are specific imaging modalities under development that are thought to reflect alpha synuclein (220). In contrast to cortical volume, which may represent general pathological changes in the cortex, molecular imaging of alpha synuclein may be more specific to investigate any potentially neuroprotective effect of axonal myelination. On a similar note, more sophisticated imaging modalities or body fluid biomarkers might be employed to specifically measure axonal myelination in subcortical white matter (218). Another potential avenue for investigation of the role of white matter would be to explore the role of agents that have been

shown recently to potentially induce remyelination (219). While this could theoretically be undertaken in human subjects using a retrospective design and white matter imaging data, a more direct but clinically relevant approach would be to explore the effects of miconazole on the susceptibility of neurons to alpha-synucleinopathies (219, 221).

The third goal of this section was to investigate the nature of the relationships between cortical gyrification and underlying white matter properties. In normal development, cortical folding is thought to be caused by from tangential forces of rapidly expanding cortical gray matter and physical constraints imposed by subcortical white matter (34, 164). The mechanisms of cortical "unfolding" in neurodegeneration have not been explored extensively. The data suggest that there are some trend cross-sectional and longitudinal relationships between gyrification and subcortical white matter diffusion properties in PD, and none in healthy aging subjects (Tables 4b.1-4, Figures 4b.1-2, see below). The cortical gyrification-white matter associations in PD, if present, are much weaker compared to cortical volume-white matter associations. None of the potential gyrification-white matter associations survived the adjustment of multiple comparisons for significances. The findings that cortical gyrification loss does not correlate strongly with underlying white matter diffusion are not expected, but extremely interesting. This suggest that cortical gyrification loss may reflect degenerative processes occurring de nova in the gray matter as part of intrinsic PD progression, rather than processes of subcortical white matter changes (may or may not directly linked to intrinsic PD process, such as subcortical vascular changes) that indirectly affect cortical structure. Furthermore, these findings suggest that cortical gyrification (see *Chapter 3*), and not necessarily cortical volume might provide a more specific gauge to track gray matter changes in PD. This hypothesis, however, needs to be tested in subsequent studies.

Table 4b.1. *Associations between cortical gray matter gyrification index and diffusion measurements of underlying white matter at baseline*

	PD Subjects				**Control Subjects**				**Group Interaction**			
	AD		**RD**		**AD**		**RD**		**AD**		**RD**	
	β	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**
Overall	-285	0.441	-499	0.214	-255	0.583	-82	0.875	-495	0.274	-620	0.217
Supramarginal	-499	0.177	-773	0.077	196	0.663	-30	0.957	-1039	**0.030**	-1023	0.085
Inf. Parietal	-464	0.099	-606	**0.037**	-66	0.863	-21	0.953	-864	**0.032**	-926	**0.017**
Sup. Parietal	-420	**0.028**	-465	**0.034**	-388	0.212	-161	0.644	-277	0.368	-512	0.126
Sup. Frontal	205	0.385	131	0.613	-19	0.947	-133	0.668	130	0.708	178	0.628
Postcentral	375	0.305	320	0.446	190	0.689	349	0.497	-347	0.521	-239	0.694
Precentral	895	0.070	552	0.311	113	0.842	-229	0.710	36	0.962	319	0.672

Abbreviations – AD: Axial diffusivity; RD: Radial diffusivity;

Table 4b.2. *Associations between cortical gyrification index and diffusion measurements of underlying white matter at baseline among PD subjects*

	PD-Earlier				**PD-Later**			
	AD		**RD**		**AD**		**RD**	
	β	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**
Overall	-335	0.507	-449	0.370	-994	0.268	-1082	0.230
Supramarginal	-336	0.494	-552	0.340	-1792	**0.025**	-2024	**0.021**
Inf. Parietal	-807	**0.033**	-830	**0.036**	-370	0.556	-1046	0.079
Sup. Parietal	-451	0.064	-477	0.083	-987	**0.048**	-900	0.135
Sup. Frontal	188	0.546	186	0.628	123	0.760	-92	0.824
Postcentral	524	0.327	557	0.349	-122	0.839	-123	0.861
Precentral	1327	0.055	1058	0.160	311	0.701	-166	0.859

Abbreviations – AD: Axial diffusivity; RD: Radial diffusivity;

Table 4b.3. *Associations between annual change in cortical gyrification index and annual change in diffusion measurements of underlying white matter*

	PD Subjects				**Control Subjects**				**Group Interaction**			
	AD		**RD**		**AD**		**RD**		**AD**		**RD**	
	β	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**
Overall	0.034	0.910	-0.265	0.465	0.340	0.369	0.634	0.291	0.041	0.850	-0.179	0.540
Supramarginal	-0.045	0.871	-0.372	0.125	-0.053	0.822	0.133	0.553	-0.062	0.731	-0.214	0.192
Inf. Parietal	-0.168	0.449	-0.195	0.470	-0.180	0.346	-0.170	0.558	-0.187	0.203	-0.222	0.260
Sup. Parietal	-0.006	0.982	0.311	0.400	-0.414	0.155	-0.391	0.377	-0.127	0.513	0.097	0.719
Sup. Frontal	-0.404	**0.001**	-0.302	0.195	0.000	0.998	0.073	0.863	-0.310	**0.008**	-0.231	0.273
Postcentral	0.148	0.492	0.322	0.196	0.281	0.235	0.396	0.271	0.164	0.297	0.313	0.116
Precentral	-0.104	0.612	-0.098	0.664	0.106	0.650	0.066	0.840	-0.065	0.663	-0.086	0.645

Abbreviations – AD: Axial diffusivity; RD: Radial diffusivity;

Table 4b.4. *Associations between annual change in cortical gyrification index and annual change in diffusion measurements of underlying white matter among PD subjects*

	PD-Earlier				**PD-Later**			
	AD		**RD**		**AD**		**RD**	
	β	**P-Val**	**β**	**P-Val**	**β**	**P-Val**	**β**	**P-Val**
Overall	0.510	0.175	-0.170	0.698	-0.641	0.165	-0.417	0.527
Supramarginal	0.341	0.373	-0.052	0.862	-0.588	0.169	-0.830	**0.050**
Inf. Parietal	-0.264	0.333	-0.298	0.352	0.002	0.995	-0.029	0.951
Sup. Parietal	0.075	0.802	0.258	0.517	-0.109	0.837	0.392	0.587
Sup. Frontal	-0.442	**0.011**	-0.313	0.290	-0.361	0.060	-0.309	0.443
Postcentral	0.292	0.239	0.376	0.179	-0.132	0.752	0.217	0.682
Precentral	0.171	0.474	0.214	0.384	-0.848	**0.024**	-0.952	0.052

Abbreviations – AD: Axial diffusivity; RD: Radial diffusivity;

Figure 4b.1. *Relationships between cortical gyrification and underlying subcortical white matter diffusion measurements (excluding brainstem and cerebellar structures). Overall local gyrification index shown as residuals based on intracranial volume for illustration purposes.*

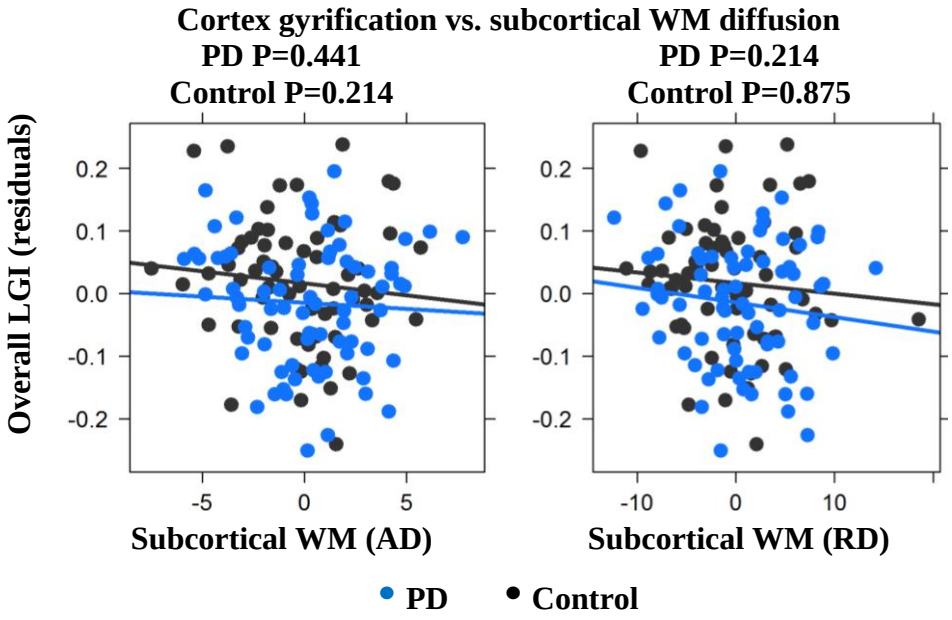
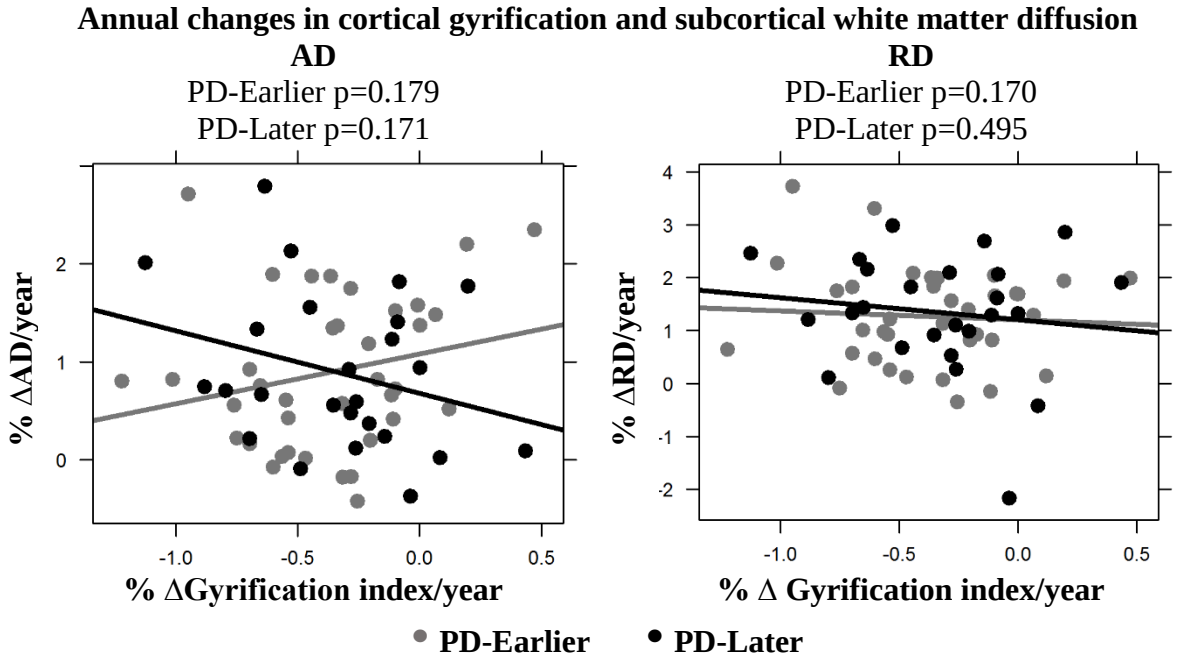


Figure 4b.2. *Relationships between annual change in cortical gyrification and annual change in underlying subcortical white matter diffusion in earlier- vs. later-stage PD subjects.*



SECTION IV: THE ROLE CHOLESTEROL IN PARKINSON'S DISEASE PROGRESSION

Chapter 5: Review: Cardiovascular disease, lipids, and Parkinson's disease in a dynamic era of statin use

Preface

Recent studies have suggested that higher plasma total and LDL-cholesterol may be associated with lower risk (10, 16-18) and delayed onset of PD (20). There is, however, a lack of studies investigating the associations between cholesterol and the progression of PD (21, 222) and the nature of the link between cholesterol and neurodegenerative diseases is poorly understood. In investigating the role of plasma cholesterol in PD, it is important to consider the many factors that could mediate and/or modify the relationship between the disorder and plasma cholesterol levels. These factors might affect both PD risk and cholesterol metabolism individually or in combination. Furthermore, investigating the link between cholesterol and PD has become complex because of the widespread use of cholesterol modifying drugs to treat hypercholesterolemia, which can make it difficult to decipher the roles of statin and hypercholesterolemia in PD. This section begins with a review article that attempts to synthesize and summarize literature regarding the current state of knowledge about PD, cholesterol, and potential mediators and modifiers of PD-cholesterol link such as cardiovascular and cerebrovascular disease, plasma cholesterol and brain cholesterol physiology, genetic associations, and cholesterol-modifying drugs. The review finishes with a meta-analysis and critical evaluation of the literature regarding PD risk with respect to statins, a widely-prescribed class of cholesterol-modifying drugs (223). In the chapters following the review, I investigate the relationships between plasma cholesterol in PD and both clinical and functional measurements of disease progression.

[*Chapter 5* is in the final preparation stages for publication. I am the leading author.]

Abstract

There is a strong and consistent association of Parkinson's disease (PD) and environmental factors (e.g., smoking, caffeine, high plasma urate associated with lower risk; various environmental factors with higher risk), although mechanisms are unclear. The newest controversy has been a suggested link between sporadic Parkinson's disease (PD) and factors related to cardiovascular health [i.e. cerebrovascular disease, plasma low density lipoprotein (LDL) cholesterol, apolipoprotein alleles, LDL receptor, and statin usage]. There is a relatively consistent body of literature that suggests that individuals with higher plasma LDL and higher statin use have lower risk of PD. The common assumption has been that these data reflect a disease-modifying effect of statins, yet significant controversy exists on how to interpret these data. Because statins are important tools for fighting cardiovascular disease and have a relatively high therapeutic index, it even has been suggested they should be put into our drinking water. As many as a quarter of adults over 40 years of age use statins for cardiovascular indications, and this is predicted to increase. We review the human and laboratory literature relating to cholesterol and statins in the context of the genetic and environmental that affect factors their role in PD. We hypothesize that rather than being protective against PD, unnecessary lowering of cholesterol may be an important risk factor. We highlight important areas of needed research, and also the need to personalize therapy in individuals who are at higher risk for sporadic PD.

Parkinson's disease, cholesterol, and statins

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder that is characterized pathologically by death of dopamine neurons in the substantia nigra pars compacta. Of the age-related neurodegenerative diseases, PD is second only to Alzheimer's disease (AD) in prevalence, and is estimated to affect more than four million people worldwide (224). Over the next 15 years, PD is expected to double in prevalence based only on population aging (225), imposing a substantial public health burden (3), Although the exact cause of PD onset is unknown in most cases, its etiology is thought to involve a complex interplay between genetic and environmental factors. Indeed, PD has offered some interesting paradoxes. For example, several environmental or physiological parameters usually thought to be detrimental to good health [e.g., smoking (11), coffee consumption (12, 226), high plasma urate (13)] have been shown to be associated with lower risk of PD. Indeed, there are even current clinical trials trying to raise plasma urate pharmacologically as a neuroprotective therapy. Unfortunately, the mechanisms behind these associations remains speculative, and in some case there is controversy about causation (11, 227, 228). Thus, it is critical to identify and understand factors that may influence the pathogenesis or progression of this disease, particularly the factors that are modifiable.

This review focuses on the current controversy about how risk and/or progression of PD risk is related to circulating LDL-cholesterol levels and the drugs that modify lipid status. The epidemiologic implications of issue are significant. Recent data from the U.S. Centers for Disease Control and Prevention show that 1-in-3 individuals in the United States aged 40 or older utilize a cholesterol-lowering drug, and 1-in-4 were prescribed a statin in 2012 (229). Although there is overwhelming evidence for the use of such medication in individuals with specific cardiovascular risk factors, there has been recent impetus to use the drugs more widely, and as we will review, in

some cases even as neuroprotective agents (230-233). Our goal is to examine critically the literature regarding the connection between PD and cholesterol to provide context on the potential non-cardiovascular implications for using cholesterol-lowering therapies.

Cardiovascular/cerebrovascular disease and Parkinson's diseases

Clinical studies

During the last few decades, the relationship between cardiovascular/cerebrovascular (CV) disease and PD has been examined, with somewhat consistent results. Two consecutive studies, one community-based (n, PD=228, control=228) and one hospital-based (n, PD=468; control=468) reported lower prevalence of heart disease among parkinsonian patients, although arteriosclerosis was paradoxically reported to be increased (234, 235). Struck et al. in 1990 (n, PD=200, control=200) reported lower cumulative incidences of stroke and myocardial infarction (MI) among PD patients, although the study did not utilize a matched design (236). Natauaj and Rajput (237) also reported lower incidence of stroke in PD in 2005 (n, PD=500, essential tremor=270, control=490), but this finding may have been attributable to lower rates of smoking in the PD group (smoking is consistently associated with lower PD risk (11). Another hospital-based study by Scigliano et al. in 2006 (n, PD=178, control=533) reported that several CV risk factors were less common among PD patients (238). This latter finding was, however, complicated by the fact that the controls were a relatively sicker sample of hospitalized patients, one-third of whom had osteoarthritis of the spine or intervertebral disk prolapse and potentially limited mobility.

Conversely, other reports have suggested that stroke and MI are more common in PD after diagnosis. A large prospective study of residents in Taiwan in 2015 (n, PD=3,211, control=3,211) reported that PD subjects, matched with controls for several relevant CV risk factors, had an increased future risk of MI (239). Despite this robust evidence that risk of MI is increased after PD

diagnosis, antiparkinson medication may have been a factor, as the PD group used ergoline dopamine agonists, many of which are known to cause cardiac valvulopathy and pulmonary fibrosis (240-242). Gorell et al., in 1994, utilized death certificate information in Michigan (U.S.A.) to show that PD patients over the age of 40 are more likely to die from cerebrovascular disease, yet cautioned that this might have attributable to detection bias (i.e., PD subjects are more likely to see a neurologist) (243). Another explanation for increased cerebrovascular disease in PD is that patients can have relatively limited mobility and loss of physical independence in later-stages. Ben-Shlomo and Marmot (1995) showed high risk of death from ischemic heart disease and cerebrovascular disease among parkinsonian subjects (n, PD=220, control=421) recruited from 40 general practice locations in England and Whales (244). Unfortunately, PD diagnoses were not confirmed, and may be confounded by atypical parkinsonism (244) .

A UK-based study of general practice records (n, PD=3,637, control=3,327) reported increased occurrence of cerebrovascular events among PD subjects, even after adjusting statistically for several chronic diseases, psychiatric conditions, body mass index (BMI), and smoking (245). A prospective cohort study by Lee et al., in 2013, utilized information from the National Insurance Research Database (n=43,810) in Taiwan and suggested that PD incidence is increased in association with ischemic heart disease, heart failure, and peripheral vascular disease. Additionally, several medications related to cardiovascular health were associated with increased PD incidence prospectively, including selective cyclooxygenase-2 inhibitors, antiplatelet agents, β-adenoreceptor antagonists, calcium channel blockers, nitrates, and anti-arrhythmics (246). This study suggested that poor cardiovascular health precedes PD diagnosis, whereas previous studies had only been able to show poor cardiovascular health in association with or after PD diagnosis. Interestingly, a large (n= 94,308) population-based prospective study suggested that future risk of PD is associated with

history of cerebrovascular accident, but not ischemic heart disease (247). There may be questions, however, regarding the validity of this study, since smoking did not correlate with lower PD risk, something almost universally found. The authors utilized information from the Clalit Health Services clinical database in Israel, and some information (i.e., smoking) might not have been recorded consistently in the medical records. It is also possible, as with other studies, that neuronal damage resulting from cerebrovascular accident or vascular parkinsonism might be misdiagnosed as sporadic PD.

When considering these data, it is important to note the evolving public health emphasis on cholesterol-lowering therapies and treatments for PD. Specifically, once statins became widely available, use increased dramatically until by 1998 more than 10% of U.S. adults had taken these drugs. Individuals in more recent studies are likely to have had prolonged exposure to lipid-lowering therapies. Moreover, there are several potential confounders in studying the relationship including smoking, blood pressure dysregulation in PD, and the potential cardiotoxic effects of some antiparkinson medications. In addition, the vascular clinical subtype of PD might confound studies of CV disease and PD, although vascular parkinsonism accounts for only a few percent of parkinsonisms (224, 248). Thus, we have chosen not to address vascular parkinsonism, as it has been reviewed comprehensively recently (249).

Imaging-based studies

Several radiographic studies have reported evidence of increased cerebrovascular disease among PD subjects. Piccini (1995) (n, PD=102, control=68) and Gattellaro (2009) (n, PD=10, control=10) for example, demonstrated increase white matter lesions (WMLs) in PD that are associated with dysfunction of cognition, posture, and/or gait (167, 195, 250). The presence of WMLs has, however, been associated with blood pressure dysregulation in PD (orthostatic

hypotension and supine hypertension) that might be either intrinsic to the disease process, or result from treatment (251, 252). Overall, evidence from clinical and radiographic studies seems to suggest that CV disease is more common after PD diagnosis. The best evidence from a carefully controlled study (239), supports the notion that treated PD subjects have a higher prospective risk of MI (239), yet it remains unclear whether CV disease modifies the risk of developing PD.

Pathology-based studies

Recent pathological autopsy data have provided important perspective. Although two post-mortem pathological studies have shown inverse relationships between the severity of Lewy pathology and cerebrovascular/small vessel disease in PD and control patients, the issue of cause and effect is not resolved by these findings alone (253, 254). Ghebremedhin et al., in 2010, reported that the extent of Lewy pathology in patients having Lewy body diseases (n=102 PD, n=13 Lewy body dementia [LBD], control=53) was inversely related to the severity of atherosclerosis, infarcts, and small-vessel disease (253). This raises the possibility that the observed increase in cerebrovascular disease after PD diagnosis in many studies might be a result of the disease or treatment process, rather than a factor that contributes to Lewy-body based neurodegeneration. An alternative explanation is that individuals with more severe cerebrovascular disease might be more likely to have smoked in their lifetime (255), which might be protective against Lewy pathology (256). These findings should be validated in future studies that can account for relevant behavioral factors as it suggests that it may be important to examine how CV risk factors influence PD risk and progression.

Plasma cholesterol and Parkinson's disease

Case-control studies of cholesterol and PD prevalence

The first hint that plasma cholesterol might be linked to PD was reported by Lamperti et al. in abstract form only in 1981. Their data (n=100 PD) showed that hyperlipidemia was less common among PD subjects. This issue lay dormant until the case control report from our group that reported that increased plasma cholesterol was associated with reduced PD risk (238, 257, 258). A case-control study by Scigliano et al. in 2006 (n, PD=178, control=533) also reported lower frequency of high blood cholesterol and triglycerides among PD subjects compared to controls discharged from the hospital for other neurologic diseases (238). As mentioned previously, the control subjects in this latter study may have suffered from limited physical activity due to orthopedic diagnoses, yet a third of the patients were diagnosed with depression or anxiety, which also have been associated with plasma cholesterol (259, 260). This bias might be expected to minimize the difference between PD and control cholesterol, thus strengthening the study. One limitation is that cholesterol-lowering drugs and smoking history were not taken into account. Huang et al. also reported that higher cholesterol is associated with a lower occurrence of PD, even after adjusting for smoking and the use of cholesterol-lowering drugs (257). In a case-control study, Miyake et al., in 2010, reported an association between hypercholesterolemia and decreased risk of PD [n, PD=249 (within six years of onset) control=368] after employing a similar statistical control for relevant covariates (258). This study was, however, hospital-based and might have been subject to ascertainment bias.

Prospective studies of cholesterol and PD incidence

Several prospective studies have addressed whether low cholesterol is a cause or effect of PD (10, 16-18, 261). de Lau et al., in 2006, (n=6,465 age ≥ 55 years) reported a dose-related lower prospective risk of PD after controlling for several behavioral and genetic factors (17). An analysis

of a large community-based study (Nurses' Health Study, n=121,046 women and Health Professionals Follow-up Study, n=50,833 men) by Simon et al., in 2007, showed that after adjusting for multiple behavioral factors, the association between PD and lower total cholesterol was present, but marginal, and limited to women (18). Another prospective study by Huang et al., in 2008, reported decreased risk of PD in association with higher cholesterol in 3,233 Japanese men, although the authors noted that the association might be driven by cholesterol-lowering drugs (16). The most recently published prospective cohort study by Huang et al., in 2015, showed that higher total and LDL cholesterol were associated with lower future risk of PD, even after accounting for statin usage (10).

There is one report that offered a contradictory finding. Hu et al., in 2008, reported that higher total cholesterol may increase PD risk over a study period of 18.1 years in a Finnish population of 24,773 men and 26,153 women (261). It is interesting, however, that the association was limited to subjects ages 25-54 years, since individuals younger than age 50 years account only for roughly 4% of new cases, and incidence increases rapidly after age 60 (262). The findings of Hu et al. might represent a distinct early-onset subtype of parkinsonism (261).

Cholesterol and PD progression

Although most previous studies of PD and cholesterol have been focused on the risk of PD, the role of cholesterol in the progression of PD "pre-PD" also has been studied. Postuma et al., in 2015, examined this issue in a study of 279 idiopathic rapid eye movement sleep behavior disorder (RBD) patients, a group known to have a high risk of converting to PD. RBD subjects having hypercholesterolemia were less likely to convert to dementia with Lewy bodies or PD (21). Interestingly, no association was found between conversion and the use of lipid-lowering drugs. In a prospective study of 774 subjects having early PD (~2 years since diagnosis) enrolled between

1987 and 1988, Huang et al., in 2011, showed a trend of slower motor symptom progression over 2 years in association with higher plasma cholesterol. Notably, very few of the patients were taking statins at baseline (222). Finally, a recent study of 300 PD subjects by Mahlknecht et al., in 2015, reported that higher LDL-cholesterol levels, but not total cholesterol levels, were associated with later age of disease onset, even after adjusting statistically for statin usage, age, gender, and body mass index (20). Thus, the weight of the literature by several independent groups, and across several geographic areas supports an association between higher risk, faster progression, and earlier onset of PD and lower cholesterol.

Plasma cholesterol in non-PD neurobehavioral disorders

Although the PD-cholesterol link may seem counter-intuitive when viewed from the cardiovascular perspective, several studies have suggested similar associations in non-PD populations. Lower levels of total cholesterol, for example, have been related to multiple system atrophy, depression, and suicide (259, 260, 263). In otherwise normal individuals, for example, higher cholesterol in late-life (age ~70-80 years) has been associated with better cognitive function and lower risk of dementia (264-266). Conversely, higher plasma cholesterol in mid-life (age ~50 years) is thought to be associated with higher risk of dementia and mild cognitive impairment many years later (267, 268). Thus, the timing and duration of cholesterol levels throughout an individual's lifespan may play a role in later cognitive function. The case of Alzheimer's disease (AD) is unique as will be discussed in the following section.

Parkinson's disease and cholesterol-related genes

The link between plasma cholesterol and neurodegenerative disorders (i.e. PD and AD) seems quite paradoxical. A link between PD and plasma cholesterol is particularly difficult to reconcile with the standard notion that CNS and plasma compartments generally do not to

communicate. There is some evidence to suggest uptake of LDL particles and other apolipoproteins across the blood-brain barrier (269-273), possibly via low-density lipoprotein receptor, low-density lipoprotein receptor related proteins 1 and 2 (274), scavenger receptor class B type 1 (275), and/or ABCA1 (275), but the extent of transport across the blood-brain barrier is unclear.

One explanation is that the link between PD and cholesterol is behavioral (i.e., based on nutritional habits). Another explanation is that the association between PD and plasma cholesterol might be driven by parallel processes in the plasma and brain (i.e. via genetic pleiotropy). Apolipoproteins E (ApoE) seem to provide one plausible candidate for such a mechanism, since they are secreted from astrocytes and subsequently endocytosed into the cytoplasm of neurons (23, 276, 277), and are involved in numerous process including oxidative stress (278), Indeed, ApoE4 has been consistently associated with high LDL cholesterol and also increased lifetime risk for AD (279). Thus, it is logical to ask how this class of proteins may play a role.

ApoE-ε2 and Parkinson's disease

ApoE2 is known to associate with a lower risk of AD (280), but because it also associates with lower LDL-cholesterol (281), it raises the question of how it relates to PD. A meta-analysis of twenty-two studies published by Huang et al. in 2004 showed that ApoE2 was associated with a small, but significantly increased risk of PD (282), and a later updated meta-analysis published in 2009 showed similar results (283). Three recent reports published since 2009 also reported similar or trend-level associations between ApoE2 and PD (284-286). ApoE2 allele frequency has also been shown to be greater among patients having tardive dyskinesia after antipsychotic (dopamine antagonist) therapy (287).

Because the effect size of the ApoE2 allele on PD risk is modest (OR 1.2), and because of the low frequency of this allele (283), it is not surprising that several studies have found no

significant effect (288-291). Two recent studies of PD in Mexican patients, for example, showed no statistically significant associations, probably because the number of subjects carrying ApoE2 was very small (n=6 and n=10 PD patients carrying ApoE2) (292, 293). A trend-level association, however, was present in one of these studies and suggested increased ApoE2 frequency in PD (292). Similarly, there was a very small number of ApoE2 carriers in a study by Ghebremedhin et al., who reported a trend of increasing ApoE2 frequency with advancing PD pathological stage (n carrying ApoE2, control=15, Stage 3=2, Stage 4=8, Stage 5/6=12) (294). More recently, a large study of 2,412 cases and 1053 controls by Federoff et al., in 2012, concluded that there was no association between ApoE2 occurrence of PD (295). However, there may have been a trend-level association between ApoE2 carrier status/ApoE2 dosage and earlier age of PD onset in females. A limitation of this study was a lack of matching or statistical adjustment for lipid-lowering therapies, which mare likely to be more common in ApoE alleles that correlate with higher LDL cholesterol (281). Thus, future studies will need to capture information regarding lifetime exposure to lipid-lowering therapies and make appropriate design or statistical adjustments.

ApoE-ε4 and Parkinson's disease

The ApoE4 allele is clearly associated with significantly increased risk of AD and of higher plasma LDL cholesterol (279, 296). Thus, it is not surprising that the ApoE4 allele has been consistently associated with increased prevalence of dementia in PD (283, 297). Conversely, prior to 2004, no study reported a significant effect of ApoE4 on the risk of PD itself (282). It is interesting that some post-statin era studies have found associations between ApoE4 and PD. In 2006, for example, Ghebremedhin et al. reported increasing frequency of ApoE4 with worsening PD pathologic stage (n, PD=108, control=108) (294). Several recent case-control studies reported significantly or trend-level increased occurrence of PD in association with ApoE4 allele frequency,

although prolonged use of lipid-lowering drugs and the tendency for higher baseline cholesterol in ApoE4/- patients might have confounded this relationship (285, 289, 290, 292, 293, 296). Other recent case-control studies, however, have not demonstrated a strong relationship between PD risk and ApoE4 allele frequency (283, 288, 298).

Combined ApoE- ε2/ε4 genotype and Parkinson's disease

A meta-analysis by Huang et al., in 2004, showed that the ApoE2/4 genotype is associated with higher occurrence of PD (282). Kiyohara et al., in 2011, indicated that PD occurrence may be increased in the combined ε2/ε4 genotype (n, PD=238 control=296) (285). Although more recent studies since 2004 have indicated no effect of the combined ε2/ε4genotype on PD risk, this question has been difficult to address due to the relative infrequency of ApoE2 and ApoE4 alleles in the general population (283, 284, 286, 291-294). There is reason to speculate that the combination of two "defective" alleles may be worse for PD risk than possessing only one. Large-scale studies that can account for lifetime exposure of lipid-lowering therapies are needed to address this question adequately.

Weighing the possible roles of ApoE and cholesterol

There is evidence that both plasma cholesterol levels and different ApoE alleles have associations with PD risk and progression. The relative contributions and causal pathways in these associations, however, remain unknown. This question is particularly relevant, since ApoE2 and ApoE4 alleles are known to be associated with lower and higher plasma LDL cholesterol, respectively. Interestingly, Singh et al., in 2014, (n, PD=70, control=100) showed lower LDL cholesterol among control subjects despite the more frequent occurrence of the ApoE4 allele (290). This suggests that cholesterol and ApoE alleles might associate somewhat independently with PD. Furthermore, two recent studies found no association between ApoE2 and PD, but showed higher

LDL cholesterol among controls compared to PD (290, 298). These findings, although pending validation by future studies, suggest that plasma cholesterol might play a role in PD that is not solely attributable to differences in ApoE allelic frequency.

There may be reason to suspect an association between ApoE2 and PD, since this allele has been associated with lower LDL cholesterol (281). However, as with studies of CV disease and cholesterol, it is important to consider the confounding effect of prescriber practices of lipid-lowering drugs. Davies et al., for example, showed in 2011 that ε3/ε4 individuals had 50% greater likelihood of statin prescription than ε3/ε3. Further, ε3/ε2 individuals were 50% as likely as ε3/ε3 individuals to be prescribed a statin (281). Thus, prescriber practice may represent a critical confounder that may reduce our ability to discern effects of different ApoE alleles, especially in more recent cohorts.

Possible mechanisms – Brain cholesterol, apolipoproteins, and iron

Although the association between PD and apolipoproteins status and PD has a modest effect size, the involved mechanisms are still of interest. As noted earlier, the association between plasma cholesterol and PD risk might be merely correlative, reflecting ongoing parallel processes. In this case, individuals having ApoE-ε2 would be expected to have lower plasma cholesterol, and accordingly, higher PD risk. Another explanation draws upon the role of iron in the central nervous system. Nigral iron accumulation is thought to be an important feature of PD, although the mechanisms are not completely clear (299). Du et al., in 2012, reported that nigral and basal ganglia R2* signals (thought to reflect brain iron accumulation) is inversely correlated with plasma total and LDL cholesterol among PD subjects (133). Bush et al., in 2015, showed that higher levels of ferritin in the cerebrospinal fluid (CSF) correlate with higher concentrations of CSF APOE-ε4 and reduced cognitive function in human subjects (300).

It is also possible that the link between PD and plasma cholesterol might be based upon parallels in brain cholesterol handling. In mature brains, cholesterol primarily is synthesized by astrocytes and is transported to neurons via endocytosis by interacting with the LDL receptor (LDLR) and apolipoprotein E (ApoE) (22). Interestingly, there are reports of increased cholesterol in PD cortices (24) and increased cholesterol breakdown products in the cerebrospinal fluid of PD subjects (301, 302). There is also evidence suggesting that levels of the LDL receptor related protein (LRP) is increased in the CSF of PD patients. These lines of evidence seem paradoxical at first glance. However, one explanation is that an increase in brain cholesterol and CSF levels of LRP might be compensatory responses to neural stress. Indeed, cholesterol is necessary for synaptogenesis (22) and blocking the LDL receptor has been shown to reduce this process *in vitro* (23). The synthesis of cholesterol, its oxidation products, and ApoE, are increased in response to neural cell injury, possibly to increase turnover in the repair process (303, 304). Furthermore, ApoE, and its receptors (LDLR and LRP) have been shown to be increased in striatal and hippocampal tissue after lesioning with MPTP (305). Thus, it seems reasonable that brain and/or cholesterol might play a protective role in PD, and neurons may be more vulnerable when there is decrease availability or synthesis of cholesterol, or when ApoE delivery of lipid content is defective.

There is also evidence to suggest that brain cholesterol metabolism is influenced by iron regulatory genes and may be altered in PD subjects, although the role of specific variants is still a topic of considerable controversy (306-312). Ali-Rahmani et al., in 2014, showed that the H63D variant of the hemochromatosis (HFE) gene is associated with a 50% reduction in cholesterol content in neuroblastoma cells, which was accompanied by a decrease in HMG-CoA reductase and increase in cholesterol 24-hydroxylase. In a mouse model of human H63D (H67D-HFE), there was a greater age-dependent decline in brain cholesterol, decrease in synapse proteins, increase in caspase-3

expression, and impaired memory compared to wild type (313). Another variant of the HFE locus, C282Y, has been shown to associate with reduced LDL cholesterol in the general population (313).

Statins and Parkinson's disease

Statins were first introduced to the market in 1989 and have since become among the most widely-used drugs worldwide, attributable to their success in preventing cardiovascular disease. Recent guidelines in the United States have broadened the current usage of these drugs to 1 in 4 adults over the age of 40 years for the prevention of heart disease and related conditions (229). Newer guidelines published in 2013 will expand the use of these drugs, although it has been noted that not all individuals tolerate statin treatment well (223, 314). Thus, it is important to consider the possible effects of these drugs on the risks and/or presentations of other disease (i.e. neurodegenerative diseases) (315).

Multiple effects of statins of potential relevance to PD

Statins inhibit hydroxyl-methyl-glutaryl (HMG) CoA reductase, the enzyme which catalyzes the rate-limiting step in cholesterol synthesis. The biochemical product of this rate-limiting step is mevalonate, the substrate for the biosynthesis of cholesterol. Based on the previously described relationship between serum cholesterol and PD, one might predict statins to have a deleterious effect on PD, yet the situation has numerous complexities, including the fact that individual statins differ in their ability to cross the blood-brain barrier and in their effects on several different cellular pathways (316).

Co-enzyme Q10

In addition to lowering cholesterol synthesis, by reducing mevalonate synthesis, statins also reduce the production of Co-enzyme Q10 (CoQ10) (317-319). CoQ10 deficiency occurs frequently in PD, and there is evidence that there are lower levels CoQ10 in PD brain tissue (320, 321).

Interestingly, clinical trials suggest that CoQ10 may have some beneficial effect on the rate of disease progression in PD (322, 323), although the response to treatment may depend upon baseline levels of CoQ10 and chemical properties of the drug formulation (324-326). This notion is supported by the finding that orally administered nanomicellar CoQ10 may attenuate neurodegeneration in paraquat and MPTP mouse models of PD (327, 328). Unfortunately, numerous other clinical trials have failed to show significant benefit of oral CoQ10 administration on motor or pharmacologic progression in PD (329-333).

Anti-inflammatory and anti-apoptotic effects

Statins have been shown to have anti-inflammatory and anti-apoptotic effects and inhibit oxidative stress in laboratory studies (233). In 6-hydroxydopamine (6-OHDA) lesioned mice, simvastatin and atorvastatin caused significant attenuation of dopaminergic neurotoxicity and of indices of inflammation independent of plasma or brain cholesterol concentrations (334, 335). The mechanisms for these effects are unknown, and while scientifically interesting, such findings usually fail to translate into clinical efficacy, often because PD is a multifactorial progressive disorder and the available models differ significantly.

Alpha synuclein aggregation

Third, statins have been shown to also have been shown to attenuate alpha synuclein aggregation in two transgenic mouse models of PD (336). The limitations, however, of translating an animal model directly to understanding of human diseases must be acknowledged. For example, Tison et al. provided evidence to suggest that simvastatin may decrease levodopa-induced dyskinesia in a macaque model of PD treated at high doses, although these effects were not seen in humans treated at lower doses (231).

Cerebral blood flow

Fourth, long-term use of statins is associated with markedly improved cerebral blood flow, perhaps via the nitric oxide synthase pathway (337). Indeed, reduced cerebral blood flow has been associated with cognitive decline in PD (338). Thus, statins may have a theoretic application to improve the cognitive aspects of PD.

Symptomatic modification

Simvastatin has been reported to reverse the downregulation of D1 and D2 dopamine receptors in 6-OHDA lesioned rats when administered over 4 weeks (339). Statins have also been shown to modify the expression of NMDA receptors in parkinsonian animal models. Thus, it seems possible that statins could have a masking effect of PD symptoms, delaying the clinical diagnosis.

Low-density lipoprotein receptor-related protein 1 (LRP-1) expression

Statins influence plasma cholesterol via several mechanisms. In addition to lowering intracellular cholesterol synthesis via inhibition of HMG-CoA reductase, statins also increase LDLR and LRP-1 expression, resulting in increased hepatic LDL cholesterol particle uptake (340). Few studies have examined the effect of statins on LDLR or LRP-1 expression in neurons. Sato et al., in 2012, however, showed that statins enhanced Aβ clearance via upregulation of LRP-1 in mice (341). Furthermore, LDLR activity has been shown to be increased in astrocytes after administration of lovastatin *in vitro*, perhaps in efforts of synaptogenesis, although neurotoxicity was observed with long-term exposure (342).

Inhibition of remyelination

Although PD is typically considered to be a disease of the gray matter, recent evidence suggests that white matter lesions and alterations in white matter diffusion properties may be more common in PD compared to control subjects (167, 195, 196). Although this proposal is still quite controversial, it is relevant to the PD-statin question because a cholesterol is needed in high

concentrations for myelin formation (343). Indeed, statin therapy has been shown to impair remyelination in the brain in disorders such as multiple sclerosis by interfering with cell signaling in oligodendrocytes (344, 345). Braak et al. reported preferential Lewy body deposition mainly in long, poorly myelinated neurons (163). Recent research has provided some, albeit correlative, evidence that myelin may serve a protective role against Lewy pathology in neurons (146).

Summary of statin mechanisms

In summary, although statins might be expected to have mixed effects on PD-related pathways. Namely, they might be expected to worsen PD-related pathology through lowering of serum cholesterol and suppression of CoQ10 and myelin synthesis, whereas they may have beneficial effects through several other independent pathways (i.e. anti-inflammation, anti-apoptotic, reduction of oxidative stress, nitric oxide synthase activity). To make things more complex, the effects of statins on PD risk may depend upon the time when they are used. Indeed, PD is a progressive neurodegenerative disorder and there may be a window of opportunity to the influence the disease process.

Clinical evidence

The relationship between statins and PD in clinical studies has been controversial. Several early reports statin usage to concerning behavioral or neurological conditions (346-348). In 1995, well before the link between PD and cholesterol gained considerable attention, Müller et al. published a description of two patients in whom PD was "unmasked" acutely by the use of lovastatin (349). In both cases, withdraw of lovastatin was associated with remission of PD-like symptoms. Of course, it is important to consider the possibility that these cases represented chance finding or specific drug-genetic interactions. Nonetheless, the case series report and emerging evidence of a PD-cholesterol link sparked interest in the potential relationship between PD and statin usage. A

recent meta-analysis of eight observational studies suggested that statin use may be associated with a decreased occurrence of PD. Interestingly, this analysis suggested significant publication bias toward an apparent protective effect of statins (350). At the present time of writing, no randomized trials have examined the effect of statins on PD conversion, although such trials may be warranted in high-risk patients (i.e. rapid eye movement sleep behavioral disorder [RBD]). Accordingly, the observational studies that have been conducted to date have only been able to control for a subset of potential confounders as available data allow (i.e. smoking, caffeine use, ibuprofen use, coronary heart disease, diabetes, hypertension, history of hypercholesterolemia) (351).

Case-control studies

In 2007, Huang et al. reported a decreased occurrence of individuals who had ever used cholesterol-lowering drugs or statins in PD. These results persisted in a relatively small sample (n, PD=124, control=122) even after adjusting for LDL cholesterol levels and smoking (257). Most subsequent studies (except two prospective studies) have not adjusted for LDL-cholesterol levels, although this factor may be important, since subjects having high LDL cholesterol might be more likely to use cholesterol-lowering drugs.

Three case-control studies published in 2008 examined the issue of PD and statin usage. In a UK-based study of general practice records, Becker et al. examined the association between current (last prescription <90 days) or past use (last prescription ≥90 days) and PD occurrence in 3,327 controls and 3,637 PD cases, adjusting statistically for several chronic diseases, psychiatric conditions, body mass index (BMI), and smoking. Neither current nor past statin use was associated significantly with PD occurrence. There was, however, a weak association between use of low- or medium-dose pravastatin (a more hydrophilic statin) and higher PD occurrence. Interestingly, there may have also been a trend toward higher PD risk in individuals who had ever received ≥30 statin

prescriptions (OR 5.02, 95% CI 0.53 to 46.38, p=0.16), although there were only eight subjects (7 PD and 1 control) in this subgroup (352).

In another community-based study in British Columbia (n, PD=4756, control=19,024), Samii et al., in 2008, reported no association between PD occurrence and statin use one year prior to the index date (OR 0.94, 95% CI 0.82–1.09). However, cases and controls were matched on age only and the statistical analysis adjusted for total number of prescriptions prior to the index date, which might not accurately measure type of severity of other chronic diseases that would influence statin use. Another limitation of this study was that no case confirmation process was employed (353).

In a population-based study of 312 recent diagnosed PD cases (<3 years) and 342 controls in rural California, Wahner et al., in 2008, reported an inverse association between having ever used statins and PD occurrence. The association persisted after 5-year lag analysis, was stronger among individuals who had never smoked, and was present in participants both older and younger than 60 years. Increasing duration of statin usage was associated with decreasing PD occurrence, and all statins except pravastatin (a hydrophilic statin) were found to be protective (354).

Finally, a recent study by Ritz and colleagues (2010) in Denmark (n, PD=1,931, control=9,651) showed an inverse association between PD occurrence and statin use (two or more prescriptions) two year prior to the index date. These associations were significant for short-term users (OR 0.57, 95% CI 0.36–0.89) and trend-level for high-intensity (OR 0.69, 95% CI 0.45–1.04). Interestingly, the risk estimates were not different between hydrophilic and lipophilic statins and did not depend upon age. Also interestingly was the lack of association between PD and medium (OR 1.0, 95% CI 0.72–1.46) or long-term statin use (OR 1.08, 95% CI 0.76–1.52), even though the sample size was comparable to the short-term use subgroup. Of note, the use of a composite score

(Charlson index) to co-vary for chronic diseases might have mask particular confounding factors. However, the risk estimates did not change significantly after adjusting for cardiovascular drugs, dementia, cerebrovascular disease (355).

There are several considerations to mention when considering these case-control studies. First, PD is known to have a long "pre-clinical" phase that begins years before diagnosis and the onset of classic motor symptoms (356). Thus, statin usage might have a more important role in determining PD risk when used during this prodromal phase time period, in which cell losses are occurring at a sub-clinical level (357). Indeed, several studies employed lag analyses to investigate this question. Second, one might expect that PD subjects have an increased likelihood of being prescribed with statins simply because they are more likely to visit their physicians. However, control subjects tended to have higher rates of statin use. Thus, the case for a protective role of statins, therefore, is actually strengthened by this notion. Third, is it is possible that PD subjects had lower baseline cholesterol levels, thus reducing the likelihood of statin use.

Prospective cohort studies

Several prospective cohort studies have examined the potential relationship between statins and PD risk. In 2007, de Lau et al. studied PD incidence in Rotterdam according to statin usage over a period of 9 years. PD risk tended to be lower (95% CI 0.08-1.35) among the 1,008 patients who had received any statin prescriptions during the 9-year study period, although statistical significance was not reached, perhaps due to the sparsity of PD subjects in this subgroup. This finding, however, might have been expected, since PD subjects tend to have lower LDL-cholesterol, and it is unclear whether prescription of statins was made before or after PD diagnosis (358).

A large study of roughly 1 million subjects based on the US Veterans Affairs database by Wolozin et al., in 2007, reported lower incidence of PD among patients who had taken simvastatin

for at least 7 months, and trend-level lower incidence of PD was reported for atorvastatin. Interestingly, simvastatin is thought to cross the blood-brain barrier more readily than atorvastatin, which might explain these results at least partially. The control sample of this study was composed of individuals over the age of 65 years who were taking any cardiovascular drug, except statins. Although the study was generally well-designed, it did not take into account LDL cholesterol levels before statin usage (359).

Gao et al., in 2012, incorporated information about cholesterol levels indirectly by utilizing "duration of hypercholesterolemia" as a surrogate, as well as several other relevant factors. Using 12-year follow-up data from two community-based studies (Nurses' Health Study and Health Professional Follow-up Study) (n, total=129,006 PD=644), the authors reported an association between cholesterol-lowering drugs and lower risk of PD. These associations were particularly prominent for long-term use (≥ 6 years), but not for short-term use. Interestingly, the association between cholesterol-lowering drugs use and decreased PD risk was also dependent upon age, and was present in those who were younger than 60 years at the beginning of follow-up. However, the younger patients might have been more likely to receive statins, and this may have driven the age-dependency of the association between cholesterol-lowering drugs and decreased PD risk. One limitation of this study is that duration of hypercholesterolemia might have been influenced by statin usage. Participants who were placed on statins early in their lives might have had shorter durations of hypercholesterolemia, which could lower PD risk. Furthermore, utilizing duration of hypercholesterolemia as a surrogate variable for pre-statin LDL levels might not capture the magnitude of pre-statin cholesterol levels (351).

Lee et al., in 2013, utilized data from the National Health Insurance Research Database in Taiwan to examine the effect of statin discontinuation on PD risk prospectively among 43,810 statin

initiators recruited between 2001 and 2008. The National Health Insurance Reimbursement Policy of Taiwan stipulates that statin prescriptions should be discontinued when LDL cholesterol levels reach the clinical target. Although continued use of hydrophilic statins such as rosuvastatin and pravastatin showed no associations with PD risk, the continued use of lipophilic statins were associated with decreased PD risk, especially in women. Among the lipophilic statins, simvastatin and atorvastatin showed the strongest associations, while there were only trends for lovastatin and fluvastatin and no dose-response was observed. The main limitations of this study were inability to control for pre-statin LDL cholesterol levels and smoking, and a lack of ability to discern secondary parkinsonism. The authors also acknowledged the possibility of a "sick stopper" effect (discontinuation of medication due to higher PD risk of preclinical disease). A similar explanation is that individuals having relatively low pre-statin cholesterol were more likely to discontinue statins (246).

In a large prospective study of 368 general practices in England and Wales (n, total=2,004,692, PD=1,534), Hippisley-Cox (2010) reported associations between statin use and lower PD risk in both women and men that was slightly stronger for simvastatin and atorvastatin. The more hydrophilic pravastatin and rosuvastatin showed no associations. Although the author dismissed the PD-statin associations as "not clinically significant," these data suggested approximately a 10-20% decrease in PD risk among simvastatin and atorvastatin users ($0.01 < p < 0.05$). However, there was no case confirmation in this study, which might have led to misdiagnoses of PD. In addition, although the hazard ratios were adjusted for BMI, smoking, tricyclic antidepressants, depression, and cardiovascular disease, there was no adjustment for pre-statin cholesterol levels. Furthermore, since the statistical association did not reach $p<0.01$, the authors did not investigate dose response or the effect of discontinuation (315).

In 2013, Freidman et al. utilized a historical cohort of 93,308 participants in Isreal over the age of 45 years, reporting that statin (at least 6 monthly prescriptions over 9 months) usage decreased PD risk. This effect was especially prominent in short term users (n=15,593, p=0.001), but not in intermediate-duration (n=5,152, p=0.08) or long-term users (n=2,830, p=0.50). Although it is tempting to try to interpret the lack of beneficial association in the long-term uses, it is important to note the drastic difference in sample size between duration of use subgroups. A limitation of this study was that smoking was not found to be associated with lower-risk of PD. This is a widely replicable association and raises concern about the validity of the study. Although the authors employed statistical adjustment for baseline LDL cholesterol, no association was observed with PD risk, raising further suspicion about the validity of this study (247).

Although most prospective cohort studies suggested a beneficial association between statin use and PD risk, a recent prospective study by Huang et al., in 2015, (n, total=15,291, PD=106) suggested an opposite association. Statin between 1989 and 1998 was associated with increased risk of PD diagnosis after 1998. This was the second prospective study to adjust statistically for pre-statin cholesterol, and interestingly, a significant association between statins and PD risk was not significant until this adjustment was made. A particular strength of this study was the use of a rigorous case confirmation process. This dramatically reduced the number of PD cases in the analysis and is seen as a key difference from previously published studies. The study does, however, suffer from two main limitations. First, PD patients may represent a sicker population that is in contact with the medical community more frequently, thus increasing the likelihood of statin use. Second, the analysis did not incorporate information about other cardiovascular drugs that have been associated with PD and may be concurrently used with statins (i.e. calcium channel blockers, beta receptor blockers, antiarrhythmic agents) (10).

While most clinical evidence suggests that statins, especially lipophilic ones, have a protective role against PD, it is very important to consider the possibility of publication bias because this topic may have far-reaching public health implications. We conducted a meta-analysis of the effect of statins on PD risk, distinguishing case-control studies from prospective cohort studies using random effects testing. For the case-control studies, the meta-analysis revealed no significant effect of statins on PD risk (OR 0.78, 95%CI 0.56-1.07). For the prospective studies, the meta-analysis revealed an inverse association between statins and PD risk (OR 0.88, 95% CI 0.77-1.02). The overall estimate from both case-control and prospective cohort studies suggested an inverse association between statins and PD risk (OR 0.87, 95% CI 0.76-0.98) (Figure 5.1). However, there was evidence of significant publication bias (p=0.009), which can be observed visually in Figure 5.2 Thus, there is a need for randomized controlled trials in patients at higher risk of PD in order to establish a definitive and unbiased answer to the relationship between statin usage and PD.

Summary

The weight of clinical evidence suggests that higher plasma LDL cholesterol is clearly associated with lower risk, later onset, and/or slower progression of PD. The mechanisms of this relationship, however, need to be elucidated. Given the increase in cardiovascular disease after PD diagnosis, it seems unlikely that this relationship is driven by cerebrovascular disease. ApoE genotype seems to play a role in PD risk, but its associations are modest compared to plasma cholesterol. Since ApoE has shown association with PD risk, it is possible that both brain and peripheral cholesterol metabolism are affected by this or other genetic factors (i.e. genetic pleiotropy). Furthermore, recent evidence suggests that lipoproteins cross the blood-brain barrier, and this may also be a relevant mechanism to investigate in future studies. Finally, the literature regarding the relationship between statins and PD is likely influenced by publication bias. The roles

of statins and other lifestyle factors affecting cholesterol in PD should be investigated carefully, since this information will be critical to make informed clinical decisions in patients who are at higher risk for PD.

Figures

Figure 5.1. *Funnel plot of studies investigating the relationship between statins and PD risk.*

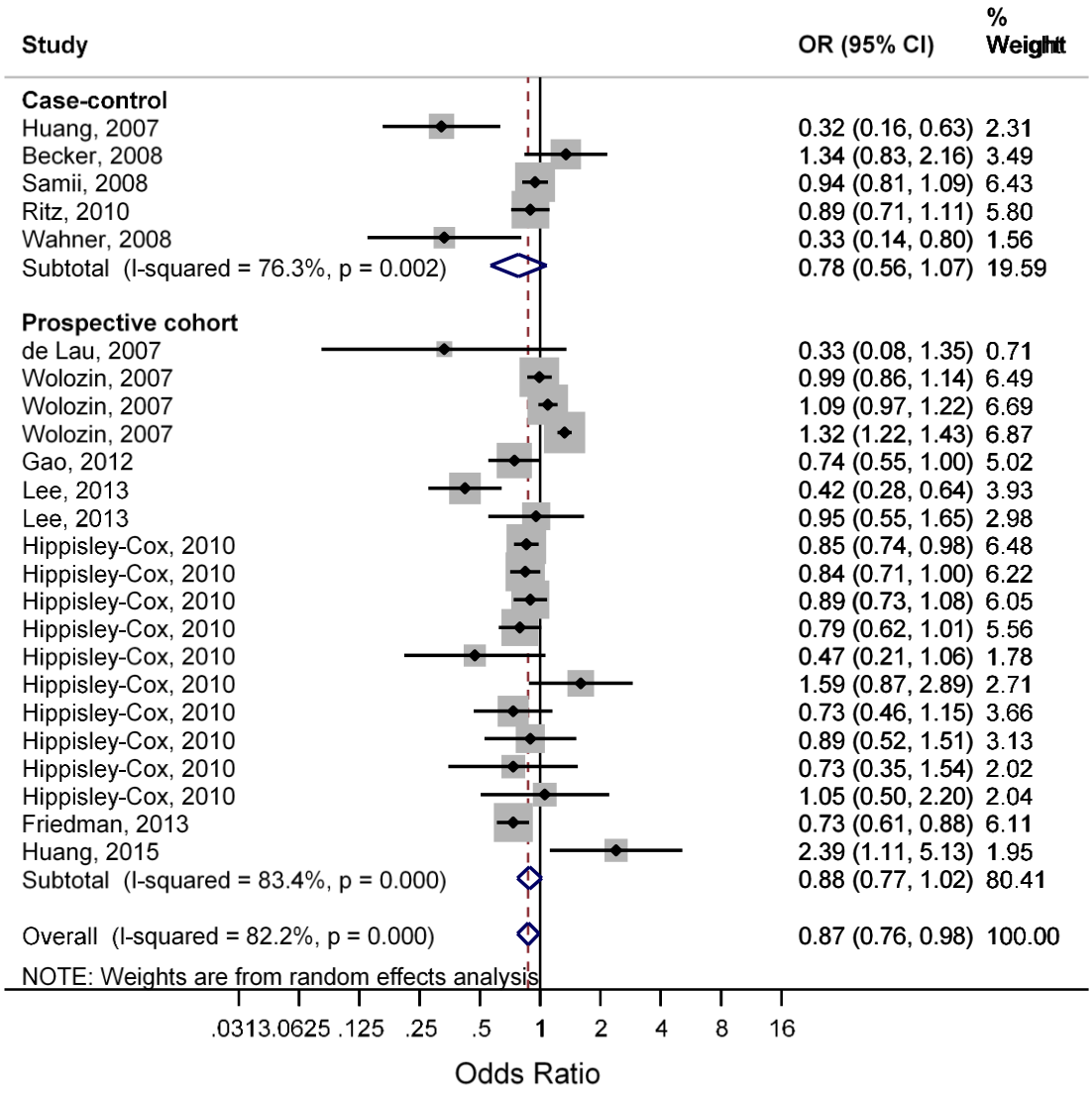
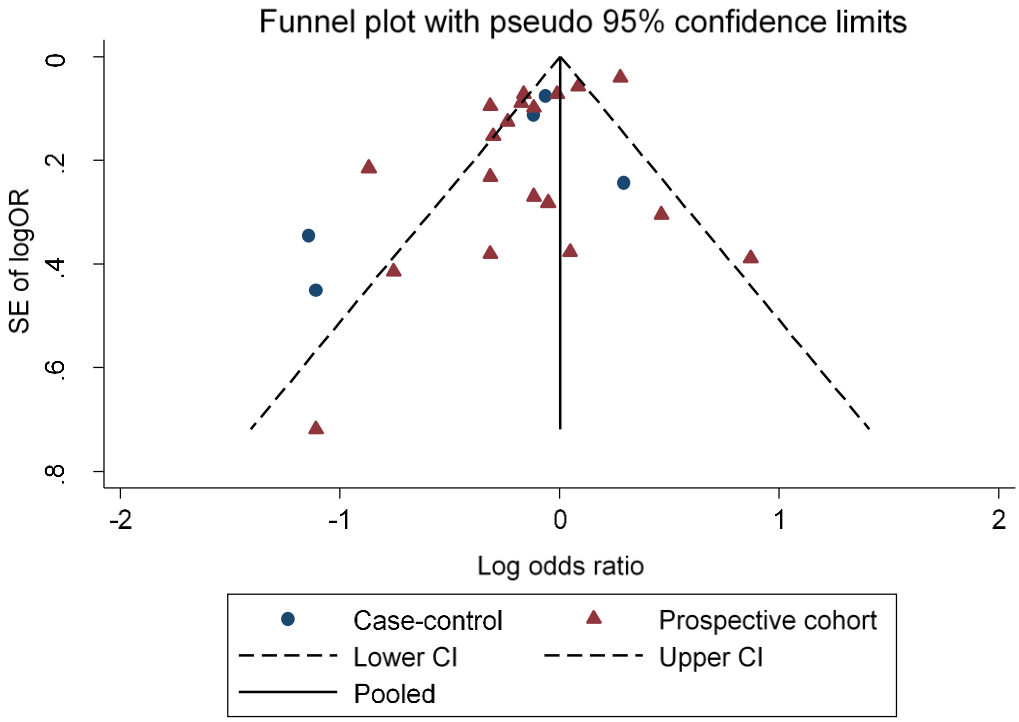


Figure 5.2. *Funnel plot of studies investigating the relationship between statins and PD risk.*



Chapter 6: Higher plasma LDL-cholesterol is associated with preserved executive and fine motor functions in Parkinson's disease

Preface

Although several case-control and prospective studies have suggested that higher plasma cholesterol levels are associated with lower PD occurrence (238, 257, 258) and risk (10, 16-18), respectively, there is a lack of studies investigating the relationship between cholesterol and the progression of PD after diagnosis. As described in the *Section I*, this is in part due to lack of objective markers for PD progression. Clinical measurements may be "contaminated" mandatory symptomatic drugs. In a drug naïve and newly diagnosed PD cohort, low density lipoprotein (LDL) cholesterol has been associated in one a trend of with slower progression to the need of levodopa (222). Cognitive decline is a particularly debilitating aspect of PD, and 80% of patients are estimated to have dementia 20 years from disease diagnosis (8). In addition, cognitive decline may be related to the progression of pathological processes outside of nigrostriatal system, since it is relative resistant to dopaminergic treatments and becomes particularly noticeable in later stages of disease These attributes make cognitive decline a particularly well-suited clinical outcome for us to investigate the potential effect of cholesterol in PD progression. The aim of this study, accordingly, was to assess the relationships between plasma cholesterol and cognitive function in PD.

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Abstract

Plasma low density lipoprotein (LDL) cholesterol has been associated both with risk of Parkinson's disease (PD) and with age-related changes in cognitive function. This prospective study examined the relationship between baseline plasma LDL-cholesterol and cognitive changes in PD and matched Controls. Fasting plasma LDL-cholesterol levels were obtained at baseline from 64 non-demented PD subjects (62.7 ± 7.9 y) and 64 Controls (61.3 ± 6.8 y). Subjects underwent comprehensive neuropsychological testing at baseline, 18-, and 36-months. Linear mixed-effects modeling was used to assess the relationships between baseline LDL-cholesterol levels and longitudinal cognitive changes. At baseline, PD patients had lower scores of fine motor ($p<0.0001$), executive set shifting ($p=0.018$), and mental processing speed ($p=0.049$) compared to Controls. Longitudinally, Controls demonstrated improved fine motor and memory test scores ($p=0.044$, and $p=0.003$), whereas PD patients demonstrated significantly accelerated loss in fine motor skill ($p=0.002$) compared to Controls. Within the PD group, however, higher LDL-cholesterol levels were associated with improved executive set shifting ($\beta=0.003$, $p<0.001$) and fine motor scores ($\beta=0.002$, $p=0.030$) over time. These associations were absent in Controls ($p>0.7$). The cholesterol – executive set shifting association differed significantly between PDs and Controls (interaction $p=0.005$), whereas the cholesterol – fine motor association difference did not reach significance (interaction, $p=0.104$). In summary, higher plasma LDL-cholesterol levels were associated with better executive function and fine motor performance over time in PD, both of which may reflect an effect on nigrostriatal mediation. Confirmation of these results and elucidation of involved mechanisms are warranted, and might lead to feasible therapeutic strategies.

Introduction

Parkinson's disease (PD) is a common age-related neurodegenerative disorder marked pathologically by Lewy pathology and the death of dopamine neurons in the substantia nigra pars compacta. Although the cardinal manifestations of PD are related to motor symptoms, cognitive decline often is present, even in early-stage disease, and this can be severely disabling in later stages (360). Twenty years after diagnosis, dementia affects more than 80% of PD patients (8).

Previous research has suggested an age-dependent relationship between plasma cholesterol and cognition throughout the lifespan. For example, higher plasma cholesterol in mid-life (age ~50 years) has been associated with higher risk of dementia and mild cognitive impairment many years later (267, 268), whereas higher cholesterol in late-life (age ~70-80 years) has been associated with better cognitive function and lower risk of dementia (264-266). Similarly, several studies have suggested that higher total- and/or LDL-cholesterol levels may be associated with lower risk and beneficial outcomes in PD. For example, three recent case-control studies suggested that higher plasma cholesterol levels may be associated with lower occurrence of PD (238, 257, 258). In addition, four independent prospective studies indicated that higher plasma cholesterol may be associated with lower future risk of PD (10, 16-18), although one prospective study reported an opposite association (261). Higher LDL-cholesterol also has been associated with a trend of slower motor symptom progression in PD (222). Most recently, a longitudinal study demonstrated that idiopathic rapid eye movement sleep behavior disorder patients with hypercholesterolemia are less likely to convert to dementia with Lewy bodies or PD (21). These findings raise the possibility that there may be a beneficial relationship between higher plasma LDL-cholesterol and PD.

There are no known previous studies investigating cognition and cholesterol in PD, but we hypothesized that higher baseline LDL-cholesterol would be associated with slower cognitive

decline in PD participants. This was tested by examining the association between baseline plasma cholesterol levels and prospective changes in cognitive scores over 36 months in PD subjects and matched Controls.

Materials and Methods

Participants

Based on criteria outlined below, 64 PD subjects and 64 Controls who had completed blood collection and cognitive testing were selected from an ongoing longitudinal cohort study of 70 PD and 70 Control subjects using nearest neighbor propensity scoring for age, gender, and dropout rate over 36 months (361). PD diagnosis was confirmed by an experienced movement disorders specialist according to published criteria (150). Controls were recruited from spouses and from the local community. All subjects were deemed to be free of acute medical issues and major neurological disease (other than PD) by reviewing full medical histories, medication lists, and laboratory data that included chemistry panels, liver enzyme tests (aspartate transaminase and alanine transaminase), creatinine, and thyroid stimulating hormone. Subjects having Mini Mental Status Examination (MMSE) score <26 at baseline were excluded from the analysis (362). Written informed consent was obtained for all subjects and the study was conducted in accordance with the Declaration of Helsinki. The research study protocol and procedures were reviewed and approved by the Penn State Hershey Institutional Review Board.

Blood cholesterol, exposures, and potential confounding factors

Blood was collected at baseline after an 8-12 hour overnight fast. Total plasma cholesterol and triglycerides were measured by enzymatic methods as described previously using an Ortho Vitros 4600 (363). LDL-cholesterol was calculated using the Friedwald equation: $LDL_C = Chol_{total} - (Triglycerides/5 + HDL_C)$. Statin use (yes/no) and factors that might influence cognition (age,

gender) were recorded at baseline. Education level was recorded as total years of schooling. Cigarette smoking information was obtained as part of a comprehensive demographic survey. Subjects were considered to be smokers (yes/no) if they had smoked one cigarette per day for at least six months (364). Depression was considered as a continuous variable at each visit and symptoms of depression assessed using the Hamilton depression scale (HAM-D) (128).

Neuropsychological examinations

A comprehensive neuropsychological battery was administered at baseline, 18-, and 36-month visits (Supplemental Table 6.1). For PD subjects, this was done in a practically defined "off" state following overnight withdrawal from PD medications, to minimize the influence of anti-Parkinson's disease drugs (130).

As detailed below, the battery of neuropsychological tests assessed eight cognitive domains: (i) fine motor speed; (ii) memory; (iii) executive function (spontaneous flexibility); (iv) executive function (set shifting); (v) attention/working memory; (vi) processing speed; (vii) language; and (viii) spatial cognition. Cognitive domain scores were defined as the mean of the population z-scores of individual cognitive tests (Supplemental Table 6.1).

Fine motor scores were calculated as the mean time of the dominant and non-dominant hands using the Grooved Pegboard test (365). *Memory* was assessed using the Brief Visuospatial Memory Test (366) and Hopkins Verbal Learning Test (367). *Executive functions requiring spontaneous flexibility* were evaluated using the design and verbal fluency tests of the Delis–Kaplan Executive Function System. *Executive functions requiring set shifting* were assessed using the color-word interference test of the Delis–Kaplan Executive Function System (368). *Attention/working memo*ry was measured by letter number sequencing, spatial span, and digit span tests from the Wechsler Memory Scale-III (369) . The assessment of *processing speed* was based on scores from the color

naming portion of the color-word interference test of the Delis–Kaplan Executive Function System (368), and the symbol search test of the Wechsler Adult Intelligence Scale IV (370). *Language* was evaluated using the word reading portion of the color-word subtest of the Delis–Kaplan Executive Function System (368) and the Boston Naming Test (371). *Spatial cognition* was assessed using the Judgment of Line Orientation test (372).

Statistical analysis

Demographic information, clinical characteristics, and neuropsychological domain z-scores were compared between PD and Control subjects using one-way analysis of covariance (ANCOVA) with adjustment for age, gender, and education as appropriate. Gender was compared between groups using Fisher's exact tests. Cross-sectional comparisons of individual cognitive scores between PD and Control subjects were performed using ANCOVA with age, gender, and education level as covariates. At baseline, multiple linear regression analysis was used to evaluate the relationships between cholesterol level and cognition, adjusting for age, gender, education, statin usage, levodopa daily equivalent dosage (LEDD), and PD status, using the following interaction terms: 1) statin usage × cholesterol, and 2) PD status × cholesterol levels.

Longitudinal analyses of cognitive change over time were performed using linear mixed-effects modeling. The group difference was examined before and after the adjustment of other covariates of interest, respectively. The mixed-effects model to investigate the correlations between baseline cholesterol levels and cognitive change included random slopes and intercepts, with cognitive score as the dependent variable and the following independent variables: age at baseline, years elapsed (since baseline), gender, LEDD, group, education years, depression score, statin usage, and cholesterol level. The interaction terms included the following: 1) statin usage × cholesterol level, 2) group × years elapsed, 3) years elapsed × cholesterol level, 4) group × cholesterol level,

and 5) years elapsed × group × cholesterol level. The coefficient associated with the three-way interaction (years elapsed × group × cholesterol level) indicates whether the association of lipid and rate of cognitive change was different between PD and Controls. The association between cholesterol and cognition was estimated for each group (PD and Controls) and tested using F-testing of the fixed effects. Due to the stepwise nature of this analysis, all statistical results were reported as raw p-values. Results that survived adjustment for multiple comparisons using the Bonferroni method across eight tests were noted (373). All analyses were completed using R version 3.1.1.

Results

Characteristics of subjects

As shown in Table 6.1, there were no differences in age or gender frequencies between PD and Controls at baseline. PD subjects, however, had fewer years of education ($p=0.008$). There were no significant group differences in cholesterol levels, statin usage, or MMSE scores. The average disease duration for the PD group at baseline was 4.4 ± 4.4 y (mean ± SD). The average Hoehn & Yahr score was 1.7 ± 0.7.

Overall, PD subjects showed higher depression scores at each visit ($p\leq0.001$) compared to Controls. We also compared characteristics of subjects who remained in the study and those who were lost to follow-up. For both cases and Controls, there was no statistical difference in baseline LDL-cholesterol, total-cholesterol, age, gender, education years, MMSE and cognitive scores between subjects who remained in the study and those lost to follow-up.

Neuropsychological scores in PD and Controls

Cross-sectional analyses showed that PD subjects performed significantly worse than Controls in fine motor skill at all three visits ($p<0.0001$). PD subjects also demonstrated lower performance in executive set shifting and processing speed at baseline and 18 months ($p<0.05$), and

tended to have lower executive set shifting function at 36 months (p=0.059). Full details of cognitive scores at each visit are summarized in Table 6.3.

Longitudinal analysis indicated that Controls showed improved fine motor (p=0.044) and memory (p=0.003) performance over time, perhaps due to practice effects and adaptation to the testing (374-376). No other cognitive domain demonstrated changes within the Control group. PD subjects, however, had worsening performance in fine motor (p=0.009), processing speed (p=0.013), executive spontaneous flexibility (p=0.015) and attention/working memory (trend-level, p=0.054) tasks over time.

Compared to Controls, PD subjects showed accelerated loss of fine motor skill (p=0.002) and a trend of accelerated loss of memory (p=0.066) and language (p=0.080). PD and Control subjects, however, had no significant differences in rate of executive set shifting changes (p=0.937).

Correlation between cholesterol and cognition at baseline

In Controls, there were no significant associations between baseline cholesterol and cognitive scores. Within PD subjects, higher LDL cholesterol was associated with lower language scores at baseline (β=0.008, p=0.015).

Relationship between baseline LDL-cholesterol and longitudinal cognitive changes

Longitudinal analysis demonstrated that higher baseline LDL-cholesterol was significantly associated with improved executive set shifting scores over time in the PD group (β=0.003, p<0.001), whereas this association was not seen in Controls. There was a significant three-way interaction between baseline LDL-cholesterol level, group, and years elapsed related to executive set shifting (β=0.003, p=0.005). This indicated that the predictive association of LDL-cholesterol

level and rate of change in executive set shifting was significantly different between PD and Controls (Table 6.2).

Higher baseline LDL-cholesterol was significantly associated with improved fine motor skill over time in the PD group (β=0.002, p=0.030), whereas this association was absent in Controls. The three-way interaction analysis between baseline LDL-cholesterol level, group, and years elapsed (since baseline) related to fine motor skill did not reach statistical significance (β=0.002, p=0.104). This indicated that the predictive association of LDL-cholesterol level and rate of change in fine motor skill did not differ significantly between PD and Controls.

Influence of statin use on cholesterol-cognition relationship

To assess potential modifying effects of statin use in the longitudinal cholesterol-cognitive relationship in PD, we performed a sensitivity analysis by stratifying subjects according to baseline statin use. Average (mean ± SD) baseline LDL-cholesterol in PD *statin non-users* was 136 ± 33.1 mg/dL and 105 ± 20.2 mg/dL in PD *statin users*. Average (mean ± SD) baseline LDL-cholesterol in Control *statin non-users* was 134 ± 38.7 mg/dL and 100 ± 40.0 mg/dL in Control *statin users*.

Among PD *statin non-users*, higher baseline LDL-cholesterol continued to be associated significantly with improved executive set shifting (β=0.004, p<0.001) and fine motor speed (β=0.003, p=0.030) scores over time. This association was not observed in Control *non-statin users*. In addition, the significant group differences in cholesterol-cognition associations persisted for executive set shifting (interaction β=0.003, p=0.002), but not in fine motor speed (interaction β=0.002, p=0.283). This indicated that the association of LDL level on the rate of change in executive set shifting (but not fine motor speed) was significantly different between PD and Control statin non-users.

Among *statin users*, there was no statistically significant difference between PD and Controls regarding LDL-cholesterol-cognition associations for executive set shifting (interaction β=0.004, p=0.195) or fine motor scores (interaction β=0.004, p=0.269). In PD statin users, baseline LDL-cholesterol had no association with fine motor (β=0.003, p=0.277) or executive set shifting over time (β=0.003, p=0.206). Interestingly, there was a positive association between higher baseline LDL cholesterol and improvement in language performance over time in PD-statin users (β=0.003, p=0.036). No associations between baseline LDL-cholesterol and cognition over time were observed in Control statin users.

Discussion

The current study provided the first evidence that in non-demented PD subjects, higher plasma LDL-cholesterol may be associated prospectively with better executive set shifting and fine motor function longitudinally. These cholesterol-cognitive relationships were specific to PD and not found in the matched Controls. These findings are consistent with previous studies suggesting beneficial outcomes in PD (222) and lower risk (10, 16-18) with plasma higher LDL-cholesterol. Future studies are warranted to investigate the mechanisms underlying these relationships.

In the current study, baseline LDL-cholesterol was associated prospectively with better performance in cognitive domains that are more specifically affected in PD (e.g., executive set shifting and fine visuomotor speed). Each mg/dL increase in LDL-cholesterol was associated with a z-score increase of 0.003 per year in the executive set shifting task. According to these results, a baseline LDL-cholesterol difference of 20 mg/dL could be extrapolated to predict an increase or decrease in the executive set shifting z-score of 0.3 over five years (20 mg/dL x 0.003/year x 5 years). Recent studies, for example, have suggested that performance reduction in z-scores by 1 or 1.5 in more than 2 subtests or domains could be meaningful clinically to define MCI or predict future

conversion to dementia (377) Thus, the cholesterol-cognitive association in PD may be meaningful clinically over the span of several years in terms of development of dementia.

Although the LDL-cholesterol relationship may be etiological (*vide supra*), it is possible that this relationship could be driven by behavioral factors associated with the disease (i.e. nutritional and/or lifestyle). Even if the cholesterol-cognition association is causal, careful risk-benefit analyses would be needed to establish the appropriate cholesterol target and therapeutic choices given the well-known adverse effects of LDL-cholesterol on cardiovascular disease.

It is interesting to note that cognitive domains associated with cholesterol in PD have been correlated with striatal functioning. For example, lower performance on the Grooved Pegboard test is associated closely with nigrostriatal denervation (378). Similarly, frontostriatal dysfunction in PD results in poor performance on set-shifting tasks (379). This raises the possibility that the interaction between cholesterol-cognition might occur at the level of nigrostriatal function. Although speculative, there are several supportive data. For example, higher plasma LDL-cholesterol levels are associated with markers of lower iron accumulation in the substantia nigra (133). It also has been reported that higher cholesterol in late-life (age ~70-80 years) is associated with better cognition in healthy populations (264-266), and this may be relevant because impairment of nigrostriatal integrity is known to occur in the normal aging process, although at a much slower rate compared to PD subjects (380). Together, these results raise the possibility that the association between cholesterol and cognition may be nigrostriatal-based.

Although the exact biological basis of the cholesterol-cognition relationship is unclear, we speculate that one possibility is that cholesterol could facilitate compensatory repair of injured neuronal pathways in PD. Indeed, cholesterol is necessary for synaptogenesis, and antagonizing the LDL receptor disrupts this process (22, 23). In normal brain, however, cholesterol is synthesized

primarily by astrocytes and then is transported to neurons via endocytosis and interaction with the LDL receptor and apolipoprotein E (22). There is limited ability for plasma cholesterol to transverse the blood brain barrier (381). Thus, brain cholesterol levels are made mainly *de novo*, and might not be linked directly to plasma cholesterol level (382). In PD, some reports have suggested an increased cholesterol content in cortices (24), and increased levels of cholesterol breakdown products in the cerebrospinal fluid of patients (301, 302). Together, it may suggest that brain cholesterol turnover is increased in PD. It will be very interesting to determine if the ability of plasma cholesterol to traverse the blood-brain barrier is changed in the disease condition. On the other hand, if the cholesterol in the CNS and periphery are highly compartmentalized, it may be that the effects of cholesterol are indirect (e.g., reflecting toxicant metabolism or transport).

In our sensitivity analysis, we examined the effect of baseline statin use on the cholesterol-cognitive correlations in PD. Consistent with the results of the main analysis, baseline LDL-cholesterol was correlated with better executive set shifting and fine motor performance over time in *statin non-users*. It is interesting to note that these correlations were not extended to *statin users*. This later finding may be related to a lower amount of variance in baseline plasma levels and a smaller sample size in the PD *statin users* subgroup than in the PD *statin non-users* subgroup. There have been suggestions that statins are neuroprotective in PD, although this is controversial (336, 383). Future studies with bigger sample sizes and detailed and stratified analyses of statin effects on PD progression are warranted.

Strengths and limitations

The strengths of the study included: 1) prospective data analysis with both 18- and 36-month follow-up; 2) comprehensive cognitive batteries to evaluate domain-specific cholesterol-cognitive associations; and 3) inclusion of controls of similar age ranges that enabled us to find the differential

cholesterol-cognitive relations between PD and Controls. Major limitations include: 1) the study design could not tease out if the cholesterol-cognitive relationship is causative, reverse causative, or a parallel process; 2) the sample size was relatively small as this is the first explorative study of the relationship between cholesterol and cognition. Nevertheless, the association of cholesterol with executive set shifting in PD was strong enough to survive correction for multiple comparisons; and 3) the study could not tease out the effects of statin subgroups on PD progression.

Summary

To our knowledge, this is the first study investigating the relationship between LDL-cholesterol and cognitive function in PD subjects. The results indicated that higher LDL-cholesterol in PD is associated with better performance in functional domains that depend on proper nigrostriatal functioning (i.e. fine motor skill and executive function). Although the LDL-cholesterol relationship may be etiological (*vide supra*), it also is possible that this relationship could be driven by behavioral factors associated with the disease (i.e., nutritional, medical, and/or lifestyle). Similarly, reduced plasma cholesterol may serve simply as a warning sign and may not be connected directly to brain cholesterol metabolism. Even if the cholesterol-cognition association is causal, careful risk-benefit analyses would be needed to establish the appropriate cholesterol target and therapeutic choices given the well-known adverse effects of LDL-cholesterol on cardiovascular disease. Future studies are warranted to confirm these findings and explore potential mechanisms.

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Table 6.1. *Demographics of study subjects at baseline (averages presented as mean ± SD)*

	PD, n=64	**Control, n=64**	**P-values**
n, Female : n, Male	26 : 38	32 : 32	0.374
Statin Use (No : Yes)	46 : 18	49 : 15	0.686
Smoker (No : Yes)	48 : 16	49 : 15	1.000
Age (years)	62.7 ± 7.9	61.3 ± 6.8	0.268
Education (years)	15.6 ± 2.8	16.9 ± 2.6	***0.008***
LDL-cholesterol (mg/dL)	127.3 ± 33.0	126.5 ± 41.3	0.616
MMSE	29.3 ± 1.0	29.5 ± 0.9	0.698
Hamilton Depression Scale	7.7 ± 4.8	4.0 ± 2.6	***<0.0001***
LEDD (mg)	709 ± 481	NA	-
Disease duration (years)	4.4 ± 4.4	NA	-
Hoehn & Yahr Score	1.7 ± 0.7	NA	

Table 6.2. *Association of baseline LDL-cholesterol levels with change in cognitive function over time (using baseline, 18-month, and 36-month scores)*

	PD		**Controls**		**Group Difference**	
	Estimate	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**
Fine motor speed	0.002	**0.030**	0.000	0.801	0.002	0.104
Memory	0.001	0.289	0.001	0.394	0.001	0.726
Executive function: SF	0.000	0.741	0.000	0.482	0.000	0.900
Executive function: SS	0.003	**<0.001***	0.000	0.905	0.003	**0.005***
Attention	0.001	0.500	0.001	**0.035**	-0.001	0.526
Processing speed	0.000	0.742	0.000	0.811	0.000	0.889
Language	0.001	0.240	0.000	0.967	0.001	0.348
Spatial cognition	0.001	0.563	0.000	0.875	0.001	0.705

** Significant after correction for multiple comparisons.*

Table 6.3. *Neuropsychological assessment of PD and Control subjects. Performance in cognitive domains presented using average z-scores (mean ± SD).*

	PD	**Control**	**P-values**
Baseline Visit			
Fine Motor Speed	-2.03 ± 1.11	-0.46 ± 1.08	***<0.001***
Memory	-0.59 ± 0.91	-0.45 ± 0.92	0.403
Executive Function: SF	0.11 ± 0.78	0.43 ± 0.70	0.090
Executive Function: SS	-0.01 ± 0.94	0.44 ± 0.47	***0.018***
Attention	0.29 ± 0.661	0.37 ± 0.53	0.803
Processing Speed	0.08 ± 0.57	0.29 ± 0.32	***0.049***
Language	0.29 ± 0.80	0.51 ± 0.49	0.246
Spatial Cognition	-0.27 ± 0.93	-0.19 ± 0.77	0.787
18-Months Visit			
Fine Motor Speed	-2.03 ± 1.06	-0.31 ± 0.98	***<0.001***
Memory	-0.60 ± 0.81	-0.24 ± 0.80	0.067
Executive Function: SF	0.00 ± 0.87	0.27 ± 0.73	0.095
Executive Function: SS	0.01 ± 0.88	0.46 ± 0.41	***0.008***
Attention	0.19 ± 0.75	0.36 ± 0.55	0.456
Processing Speed	-0.02 ± 0.59	0.23 ± 0.31	***0.022***
Language	0.19 ± 0.76	0.54 ± 0.48	***0.015***
Spatial Cognition	-0.18 ± 0.91	-0.15 ± 1.00	0.610
36-Months Visit			
Fine Motor Speed	-2.09 ± 1.09	-0.19 ± 0.97	***<0.001***
Memory	-0.45 ± 0.92	-0.05 ± 0.74	0.098
Executive Function: SF	-0.08 ± 0.73	0.37 ± 0.76	***0.022***
Executive Function: SS	0.21 ± 0.74	0.52 ± 0.47	0.059
Attention	0.33 ± 0.59	0.34 ± 0.56	0.949
Processing Speed	0.08 ± 0.56	0.21 ± 0.38	0.300
Language	0.32 ± 0.86	0.64 ± 0.44	0.053
Spatial Cognition	-0.11 ± 0.59	-0.13 ± 0.83	0.952

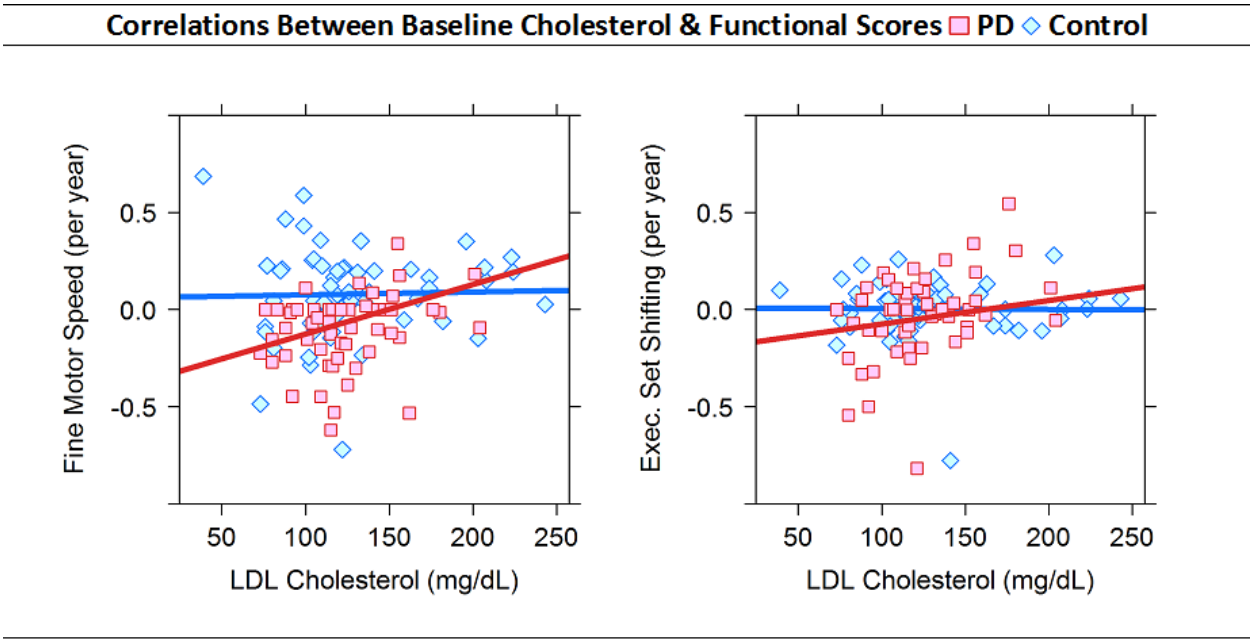
P-values reflect the comparison between PD and Control subjects after adjustment for age, gender, and years of education.

Table 6.4. *Individual cognitive tests comprising cognitive domains*

Cognitive Domain	**Individual Tests**
Fine motor speed	Grooved Pegboard Test
Memory	Brief Visuospatial Memory Test-Revised (BVMT-R)
	Hopkins Verbal Learning Test-Revised (HVLT-R)
Executive function: Spontaneous flexibility	DKEFS Design Fluency Test (DesFlu)
	Verbal Fluency Test (VerbFlu)
Executive function: Set-shifting	CWInt-Switch and CWInt-Inhibition subtests, including error scores
Attention	Digit Span
	Spatial Span
	Letter-Number Sequencing Test
Processing speed	CWInt color
	Symbol search
Language	Boston Naming Test (BNT)
	CWInt-Word subtest
Spatial cognition	Benton's Judgment of Line Orientation (JoLO)

Figures

Figure 6.1. Annual rates of changes in cognitive function over time associated with baseline plasma LDL-cholesterol levels. Annual rates of change estimated using linear regression of baseline, 18-month, and 36-month cognitive z-scores.



Chapter 7: Higher low density lipoprotein cholesterol and delayed loss of gyrification in Parkinson's disease

Preface

Past studies have suggested that higher LDL-cholesterol may be associated with lower risk (10, 16-18) and better outcomes in PD (21, 222). Although associations between LDL-cholesterol and PD risk have been undertaken in the past, there is a relative lack of studies investigating the associations of LDL-cholesterol and PD progression. As discussed in the *Section I*, this may be partially due to lack of objective markers to gauge PD progression. Equipped with newly discovered structural MRI biomarkers described in *Section II*, I hypothesized that PD patients having higher LDL-cholesterol would have fewer progression-related brain changes. Thus, the aim of this study was to understand the relationships between plasma cholesterol and brain atrophy PD patients. Since cortical folding had shown particularly strong associations with measurements of PD progression (*Chapter 3*), I first utilized cortical gyrification as a metric to investigate the effect of cholesterol on PD progression.

[This chapter is submitted to *Neurology*. I am the first author.]

Abstract

Objective: Plasma low density lipoprotein (LDL) cholesterol has been associated with Parkinson's disease (PD), but few studies have investigated its relation to PD progression. Cortical gyrification has been suggested to be a marker of PD progression, particularly prominent after five years. We examined the relationships between LDL-cholesterol and cortical gyrification in PD.

Method: Fasting plasma LDL-cholesterol levels were obtained from 71 non-demented PD subjects [63.7 ± 8.4 (SD) y] and 67 Controls (59.7 ± 7.8 y). Cortical local gyrification indices were computed using automatic segmentation of T1-weighted brain images. The relationships between baseline LDL-cholesterol and cortical local gyrification in Controls, PD, PD_{Early} (disease duration < 5 y), and PD_{Later} (disease duration ≥ 5 y) were analyzed using a linear model that accounted for potential confounders. Longitudinal correlations between baseline LDL-cholesterol and annual change in gyrification were investigated using a mixed effects model.

Results: No associations between LDL-cholesterol and cortical gyrification were found in Control, PD or PD_{Early} groups. Within the PD_{Later} group, LDL-cholesterol was associated positively with overall and regional gyrification in supramarginal, superior frontal, superior and inferior parietal, pre-, and post-central areas ($p<0.05$ after correction for multiple comparisons). Longitudinally, higher baseline LDL-cholesterol was not significantly associated with rate of gyrification changes, although there was a trend of faster loss in PD_{Later} ($p=0.063$, after correction for multiple comparisons).

Interpretation: Higher LDL-cholesterol may be associated with delayed progression of PD to later-stage cortical pathology. If confirmed, these results may provide insights into underlying mechanisms of PD progression and possible therapeutic strategies.

Introduction

Although the pathologic hallmark of Parkinson's disease (PD) is loss of the dopamine cells of the substantia nigra pars compacta and the presence of Lewy pathology (2), other degenerative cortical changes such as apoptotic signaling, reduction in neurotransmitters, and interneuron loss (5-7) are well-documented, particularly in later stages of PD. Thus, it is critical to identify factors that might modify the progression of PD-related pathology in cortical regions. To date, however, the elucidation and understanding of such factors has been hindered by the lack of objective *in vivo* biomarkers to gauge such pathology. MRI studies have been shown to capture cortical gray matter atrophy (33, 38, 40, 41, 384) that is widespread in PD, and is related to disease duration and clinical symptoms. Recent studies have demonstrated that cortical gyrification may be a particularly useful marker to gauge PD progression in the cortex, especially in the later stages (i.e., for disease durations longer than five years) (44, 79). Thus, metrics of cortical folding may facilitate an understanding of factors associated with PD progression.

The overall weight of recent literature favors higher plasma LDL-cholesterol being associated with lower PD risk and beneficial outcomes. For example, three recent case-control studies (238, 257, 258) and four prospective studies (10, 16-18) have suggested that higher cholesterol levels may be associated with lower risk of PD. In addition, higher LDL-cholesterol in PD has been associated with slowed loss of motor and executive function (222, 385). Furthermore, a recent longitudinal study suggested that patients having idiopathic rapid eye movement sleep behavior disorder have a lower likelihood of converting to dementia with Lewy bodies or PD if they have hypercholesterolemia (21). There are, however, few studies investigating the relationships between plasma cholesterol and PD-associated brain changes. Recently, Du et al., in 2012, investigated the relationship between cholesterol and R2* in 40 PD patients, (133) and found that

higher cholesterol was associated with lower nigrostriatal R2*, consistent with the hypothesized protective effect of cholesterol (133).

The aim of this study was to investigate the associations between LDL-cholesterol and cortical gyrification. We hypothesized that: 1) higher LDL-cholesterol would be associated with higher cortical gyrification in PD, specifically in select areas that have been shown to associate strongly with disease progression (overall, supramarginal, superior frontal, superior parietal, inferior parietal, postcentral, and precentral); and 2) the cholesterol-GM relationships would be strongest in PD patients with disease duration ≥ 5 years, since cortical gyrification in PD subjects has been shown to differ significantly from Controls particularly after 5 years (44).

Methods

Participants

Seventy-one PD subjects and 67 Controls were selected from a longitudinal cohort study of 76 PD subjects and 70 Controls if they had fasting blood LDL-cholesterol levels at baseline. PD diagnosis was confirmed by an experienced movement disorders specialist according to published criteria (150). Controls were recruited from the spousal population and from the local community. PD and Control subjects were free of major and acute medical issues such as liver, kidney, or thyroid abnormalities, or deficiencies of B_{12} or folate. Subjects having a Mini Mental Status Examination (MMSE) score < 26 were excluded from the study at baseline (362, 386). Depression was assessed in all subjects using the Hamilton Depression Scale (128). Written informed consent was obtained for all subjects and the study was conducted in accordance with the Declaration of Helsinki. The research study protocol and procedures were reviewed and approved by the Penn State Hershey Institutional Review Board.

Duration of illness for PD subjects was based on the date of visit minus the date of clinical PD diagnosis. PD subjects with disease duration < 5 years were defined as PD_{Early} and those with duration ≥ 5 years were defined as PD_{Later}. Unified PD Rating Scale part III-motor scores (UPDRS-III) and Hoehn & Yahr (HY) stage were obtained for all PD subjects after withholding PD medications overnight (~12 hours) as a practically defined "off-medication" state (129). Levodopa-equivalent daily dose (LEDD) was calculated according to published criteria (153) .

Blood cholesterol, exposures, and potential confounding factors

At baseline, blood was collected after an 8-12 hour overnight fast. Plasma triglycerides and total plasma cholesterol were measured enzymatically and serum albumin measured by reflectance spectrophotometry using an Ortho Vitros 4600 (363, 387). The Friedwald equation was used to calculate LDL-cholesterol (LDL_C = $Cholesterol_{Total}$ – [Triglycerides/5 + HDL_C]). Statin usage (yes/no) and cigarette smoking information were obtained as part of a comprehensive demographic survey. Subjects were considered as smokers (yes/no) if they had ever smoked one cigarette per day for six months or more (364). Education was defined as total years of formal schooling beginning with first grade. Body mass index was defined as mass (kg)/height $(m)^2$.

MRI data acquisition

All subjects were scanned using a 3.0 Tesla MR Scanner (Trio, Siemens Magnetom, Erlangen, Germany, with an 8-channel phased array head coil) at baseline, 18, and 36 months. A magnetization-prepared rapid acquisition gradient echo sequence was used to obtain T1-weighted images with TR/TE = 1540/2.34, FOV = 256 mm x 256 mm, matrix = 256 x 256, slice thickness = 1 mm (with no gap), slice number = 176.

Image processing

T1-weighted images were processed automatically using FreeSurfer (version 5.1.0) (154). Local gyrification indices (LGI) were computed as described previously (114). Historically, gyrification index was defined as the ratio of cortical surface over the perimeter of a two-dimensional brain section (113). LGI offers a method to quantify gyrification across the surface of the cortex (114). Briefly, for each vertex v_i, a circular region of interest was defined on the mesh surface having a radius of 25 mm and center at vertex v_i. Outer and pial surface areas (A_O, A_P) were computed as the sum of surface areas assigned to vertices that were within the region of interest. LGI was defined as the ratio of A_P/A_O. The final LGI at each vertex was calculated by inverse weighting based on distance. To examine the relationship between LDL-cholesterol and cortical folding, we examined specific cortical regions where LGI had been shown to have robust associations with PD progression (44). We calculated bilateral averages of LGI for each cortical region (44).

Statistical analysis

Demographic information was compared between PD and Control subjects using Student's *t*-tests, Fisher's exact tests, and one-way analysis of covariance (ANCOVA), as appropriate. Cortical gyrification was compared between PD and Controls using ANCOVA with adjustment for intracranial volume, age, gender, education years, and depression score.

The relationships between cortical gyrification and LDL-cholesterol first were analyzed within Control, PD, and PD subgroups (PD_{Early} and PD_{Later}), respectively, using a linear model that included LGI as the dependent variable and the following independent variables: intracranial volume, disease duration (as appropriate for PD or Control), age, gender, education years, history of smoking, albumin, body mass index, depression score, and LDL-cholesterol. Interaction analyses were utilized to confirm the dependency of the cholesterol-gyrification correlations on PD stage.

The aforementioned model also was extended to include the independent variables of $PD_{Earlier/Later}$ and LDL-cholesterol × $PD_{Earlier/Later}$. To account for non-normality of distributions and to increase robustness against potential outliers, correlation p-values were computed using permutation testing of the model residuals (10,000 iterations) (191, 192).We also explored the possibility that gyrification depended upon the interaction between LDL-cholesterol and age in Controls by including the interaction term LDL-cholesterol × age.

Prospective correlations between baseline LDL-cholesterol and longitudinal changes in cortical gyrification were analyzed using data from baseline, 18-month, and 36-month visits. Linear mixed effects models with random slopes and intercepts were fitted to assess the pattern of changes in cortical gyrification. The models included gyrification as the dependent variable and the following independent variables: albumin; body mass index; intracranial volume; disease duration; age at baseline; time (years elapsed since baseline); gender; years of education; smoking status; depression scores; statin usage; baseline LDL-cholesterol; time × baseline LDL-cholesterol; group (or terms for $PD_{Earlier}$ and PD_{Later} subgroups, as appropriate); group (or terms for subgroups) × time, group (or terms for subgroup) × baseline LDL-cholesterol; and group (or terms for subgroup) × baseline LDL-cholesterol × time. Due to the exploratory and step-wise nature of this analysis, we reported raw p-values. We have denoted, however, results that survived correction for multiple comparisons or correlations using an expected false discovery rate (FDR) of 0.05 (160). All comparisons were two-tailed, and all analyses were completed using R version 3.1.1 (194).

Results

Baseline characteristics of subjects

Table 7.1 describes the demographic and clinical characteristics of study subjects. PD subjects were significantly older than Controls (p=0.004), and had increased depression scores

($p<0.001$), but did not differ in gender frequencies, education, smoking history, statin usage, LDL-cholesterol, or MMSE score. Compared to $PD_{Earlier}$ subjects, PD_{Later} subjects were significantly older ($p=0.005$) and had higher motor UPDRS scores and LEDD, as expected.

PD subjects had a significantly lower superior frontal LGI ($p<0.05$) compared to Controls. $PD_{Earlier}$ showed no difference in the LGI of any region examined compared to Controls. PD_{Later} had lower overall, superior frontal, inferior parietal, and precentral LGIs compared to Controls ($p<0.05$) and trend-level lower postcentral ($p=0.066$) (Table 7.1).

Baseline correlations between LDL-cholesterol and cortical gyrification

No association was found between LDL-cholesterol and gyrification in Control, PD, or $PD_{Earlier}$ groups (Table 7.2). There also were no significant correlations between gyrification and the interaction term of age × LDL-cholesterol in either PD or Control subjects (Table 7.3). Unlike the $PD_{Earlier}$ group, within the PD_{Later} group LDL-cholesterol was associated positively with overall ($p=0.010$), supramarginal ($p=0.020$), superior frontal ($p=0.020$), superior parietal ($p=0.044$), inferior parietal ($p=0.038$), postcentral ($p=0.010$), and precentral ($p=0.015$) LGIs. These correlations survived correction for multiple comparisons (FDR adjusted $p<0.05$) (Table 7.2; Figures 1 & 2).

Disease- and stage-dependent nature of cholesterol-gyrification associations

For PD subjects overall, there was a significant association between the interaction term of $PD_{Earlier/Later}$ × LDL-cholesterol and the superior frontal LGI ($p=0.014$). Although this suggested that the association between LDL-cholesterol and superior frontal LGI was significantly stronger in PD_{Later} compared to $PD_{Earlier}$ (Figure 7.1, right column), this finding did not survive correction for multiple comparisons. Among PD_{Later} and Control subjects, there were significant correlations between supramarginal, superior frontal, superior parietal, and inferior parietal LGI, and the interaction term LDL-cholesterol × PD_{Later} ($p\leq0.05$). This indicated that the associations between

LDL-cholesterol and the LGIs of these regions were significantly stronger in PD_{Later} compared to Controls. Although these interaction correlations did not survive correction for multiple comparisons, it is compelling that in each region there was exactly the same pattern (i.e., positive slope for the PD_{Later} and lack of correlations for the $PD_{Earlier}$).

Longitudinal correlations between baseline LDL-cholesterol and annual change in cortical gyrification

There were no significant correlations between baseline LDL-cholesterol and annual change in gyrification among Controls. Among PD and PD_{Later} subjects, there was a negative association between baseline LDL-cholesterol and annual change in the postcentral LGI ($p=0.009$) that became trend-level ($p=0.063$) after correction for multiple comparisons. There also were significant interaction terms, suggesting that the effect of LDL-cholesterol on the rate of gyrification change was different in PD_{Later} and Control subjects for the superior parietal ($p=0.048$) and postcentral areas ($p=0.012$), although none of these correlations survived correction for multiple comparisons (Table 7.4). There were no longitudinal correlations when using mean LDL-cholesterol (averaged across all visits per subject) in place of baseline LDL-cholesterol ($p\geq0.078$). In addition, there were no significant correlations between annual change in gyrification and the interaction term of age × LDL-cholesterol in either PD or Control subjects (Table 7.5).

Figure 7.2 depicts the longitudinal age trajectories of gyrification in relation to disease status, subgroup ($PD_{Earlier}$/PD_{Later}), and LDL-cholesterol (lower/higher). Visual inspection suggested that overall, PD subjects in later stages of disease with lower cholesterol (< 50^{th} percentile of 115 mg/dL) had the lowest gyrifications and may have a faster course of gyrification loss, whereas PD subjects

in the later stages with higher cholesterol (≥ 50th percentile of 115 mg/dL) have higher gyrifications and may have a slower course of gyrification loss (the latter similar to that in $PD_{Earlier}$).

Discussion

The current study demonstrated that higher LDL-cholesterol is associated with higher cortical gyrification in PD at later, but not earlier, disease stages. Whereas most previous studies utilized clinical measurements as endpoints to investigate the link between PD and cholesterol, the current study utilized cortical gyrification (a newly-described and validated measurement of PD progression) as a surrogate marker for PD-associated pathology (44). These results suggest that higher LDL-cholesterol is associated with delayed loss of cortical gyrification in later stages of PD.

The role of cholesterol in PD

In recent years, several studies have provided clinical evidence that LDL-cholesterol is related to PD, but most studies have focused on the effect of LDL-cholesterol on PD risk (10, 16-18, 21, 238, 257, 258, 261). Only a few studies investigated the association between LDL-cholesterol and clinical progression of PD, partially due the lack of objective markers for PD progression when patients are treated with symptomatic medications. Huang et al., in 2011, explored data from the DATATOP study that contained only drug-naïve, newly diagnosed PD patients and found a trend that higher baseline cholesterol related to delayed need for levodopa initiation (p=0.045, one tailed) (222). More recently, we reported that higher baseline cholesterol was related prospectively with better motor and executive performance (385). Our current findings of a positive relationship between baseline LDL-cholesterol and cortical gyrification (a marker for later-stage PD cortical pathology) suggests that there may be a beneficial role of LDL-cholesterol in PD progression.

Together, they underscore the importance of considering cholesterol and its relationship with central and peripheral mechanisms in understanding and treating PD.

The importance of using stage-dependent biomarkers

Recent *in vivo* research has suggested that imaging metrics may be useful to measure PD progression in a stage-dependent manner. Specifically, striatal changes may be associated with earlier disease stage and plateau after five years, whereas cortical changes tend to continue into later stages of PD (41, 44). Consistent with this notion, cortical, but not striatal, structure has been shown to correlate better with measures of clinical progression (41, 44, 79). In addition, we found in the current study that reduced cortical gyrification was not pronounced until PD patients reached disease durations of five or more years (44). Thus, we used five years as an arbitrary cutoff to define PD subgroups for this study.

Overall, past literature has been relatively consistent in finding that LDL-cholesterol is inversely correlated with PD risk and slower progression in early disease (44, 222). No studies, however, have investigated the effects of cholesterol in later-stages of PD. In part, this may be attributable to the notion that clinical measurements in later stages may be "contaminated" by symptomatic treatments. In the current study, we utilized cortical gyrification, a metric not known to be affected by the symptomatic drugs used in PD, to reflect PD-related cortical changes in the later stages of disease. As predicted, cholesterol-gyrification associations indeed were found and are present primarily in later-stage PD subjects, but not in Controls or earlier-stage PD subjects. This study underscores the utility of investigating the associations of potential disease-modifying factors and choosing progression biomarkers in a stage-dependent manner.

The potential mechanism of the cholesterol-PD link

Despite the burgeoning evidence, the biological basis of the cholesterol-PD relationship is unknown. Because cortical gyrification loss might represent cumulative cortex pathological changes as PD advances, the positive cholesterol-gyrification relationship at later stages implies that higher LDL-cholesterol may be associated with delayed onset of gyrification loss. Although statistical analysis did not reveal how baseline cholesterol related to prospective changes in cortical gyrification, visual inspection of the age trajectory of PD subgroups relative to cholesterol levels was compelling. First, cortical gyrification is very stable in Controls over the age span of 45-75 years in this study. Second, PD subjects in an earlier stage (within five years of diagnosis) demonstrated similar levels of gyrification as Controls and were relatively stable over the similar age span; Third, PD subjects in the later stages with lower cholesterol had the lowest cortical gyrification, whereas the PD_{Later} subgroup with higher cholesterol showed higher cortical gyrification and annual changes that were similar to patients in earlier stages. This pattern is consistent with the notion that higher cholesterol may have delayed the occurrence of cortical gyrification loss.

Most intriguingly, PD subjects in earlier stages with higher cholesterol seemed to have higher gyrification, although this trend was not seen in the same PD subgroup with lower cholesterol levels. The sample size, however, was too small to test formally. If this trend is found in future studies with larger sample sizes, it suggests that higher cholesterol may be associated with either resistance to degeneration or better repair, either process resulting in compensatory "gain" of cortical gyrification. The speculation is not totally without support. For example, cholesterol is known to be necessary for synaptogenesis (23). In normal brain, however, cholesterol is synthesized primarily by astrocytes and then transported to neurons via endocytosis and interaction with the LDL receptor and

apolipoprotein E (22). Recent studies have shown uptake of LDL particles and other apolipoproteins across the blood-brain barrier (269-273), possibly via low-density lipoprotein receptor, low-density lipoprotein receptor related proteins 1 and 2 (274), scavenger receptor class B type 1 (275), and/or ABCA1 (275). Future studies are warranted to determine the extent of cholesterol and/or apolipoprotein uptake through the blood-brain barrier in both control and PD subjects. Traditionally, however, the extent of this transport has been thought to be limited (275). Even studies of acute nerve injury have shown little transport from plasma to nervous tissue (388). Yet the notion that plasma cholesterol is excluded from brain has come mainly from studies in experimental animals. Humans may be different either natively or when responding to disease. In addition, it may be that plasma cholesterol is only a marker for a cholesterol metabolite that is the actual active compound. Since some studies in PD report increased cholesterol content in cortices (24) and increased levels of cholesterol breakdown products in the cerebrospinal fluid of patients (301, 302), it seems possible that brain cholesterol turnover may be increased in PD in response to increased demands for repair and regeneration.

Strengths and limitations of the study

The current study had several strengths, including 1) utilization of objective metrics to gauge the effect of cholesterol on brain structure in PD, 2) sub-grouping of PD subjects into earlier and later stage disease, 3) the use of interaction analyses to confirm disease duration dependency of the cholesterol-gyrification associations, and 4) the use of a permutation-based method for computing p-values to ensure robustness against outliers and non-normal distributions.

There also are important limitations. First, higher cholesterol later in disease might be influenced by nutritional status, although we attempted to minimize potential confounding effects by including albumin, body mass index, and disease duration as covariates in the analysis. Second,

the sample size was relatively small and our stringent multiple comparison corrections can result in false negatives. For this reason, we reported both raw p-values and denoted the results that survived correction for multiple comparisons. Lastly, the total follow-up of each individual subject was relatively short (≤36 months). Future confirmation of the results is warranted and should include larger sample sizes and longer individual follow-up.

Summary

To our knowledge, this is the first study to provide direct neuroimaging evidence of an association between LDL-cholesterol and PD progression in later-stage disease. The results suggest that higher plasma LDL-cholesterol is associated with delayed loss of cortical gyrification, perhaps representing an increased compensatory repair mechanism. Our results should be interpreted cautiously, since the underlying nature of the PD-cholesterol link is unclear. Further investigations of the cholesterol-PD relationship, however, are warranted and may shed additional light on PD pathophysiology and potential therapeutic strategies.

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1. Research project: A. Conception, B. Organization, C. Execution;
2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique;
3. Manuscript: A. Writing of the first draft, B. Review and Critique;

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Lan Kong: 1A, 1B, 1C, 2A, 2B, 2C, 3B
Richard Mailman, 2C, 3B
Xuemei Huang: 1A, 1B, 1C, 2A, 2C, 3A, 3B

Potential Conflicts of Interest

Dr. Sterling has nothing to disclose.

Dr. Lee has nothing to disclose.

Dr. Lewis reports grants from NINDS during the conduct of the study; grants from NIEHS, grants from NINDS, grants from PSU-Internal grants, other from GE HealthCare, outside the submitted work.

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Dr. Styner has nothing to disclose.

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Dr. Mailman reports grants and personal fees from Pfizer outside the submitted work.

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Tables

Table 7.1. *Baseline demographic, clinical, and imaging characteristics of PD, PD subgroups, and Controls*

	PD All (n=71)	**$PD_{Earlier}$ (n=47)**	**PD_{Later} (n=24)**	**Control (n=67)**	**P-values PD v C**	**P-values PD_L v PD_E**	**P-values PD_L v C**
Clinical & demographic information							
Female : Male	27 : 44	20 : 27	7 : 17	34 : 33	0.170	0.312	0.113
Statin Use (N:Y)	48 : 23	33 : 14	15 : 9	55 : 12	0.077	0.595	0.094
Smoker (N:Y)	50 : 21	32 : 15	18 : 6	51 : 16	0.565	0.594	1.000
Age (years)	63.6 ± 8.4	61.7 ± 8.1	67.5 ± 7.6	59.7 ± 7.8	**0.004**	**0.005**	**<0.001**
Education (years)	15.9 ± 2.7	15.9 ± 2.3	16.1 ± 3.3	16.6 ± 2.7	0.166	0.787	0.506
LDL-c (mg/dL)	121 ± 34	124 ± 34	116 ± 33	128 ± 38	0.968	0.747	0.644
MMSE	29.1 ± 1.1	29.1 ± 1.2	29.0 ± 0.8	29.4 ± 0.8	0.282	0.839	0.915
HAM	7.8 ± 4.6	7.3 ± 3.9	8.6 ± 5.5	3.9 ± 2.4	**<0.001**	0.294	**<0.001**
UPDRS-III	22.9 ± 14.4	18.4 ± 10.6	33.1 ± 16.8	-	-	**<0.001**	-
LEDD (mg)	554 ± 454	372 ± 324	911 ± 466	-	-	**<0.001**	-
DOI (years)	4.82 ± 5.4	1.59 ± 1.4	11.14 ± 4.9	-	-	**<0.001**	-
HY Stage	1.8 ± 0.7	1.5 ± 0.6	2.3 ± 0.5	-	-	**<0.001**	-
Cortical gyrification index							
Overall	2.87 ± 0.10	2.89 ± 0.10	2.84 ± 0.10	2.89 ± 0.11	0.118	0.078	**0.020**
Supramarginal	3.41 ± 0.16	3.44 ± 0.16	3.35 ± 0.13	3.45 ± 0.13	0.718	0.057	0.111
Sup. Frontal	2.11 ± 0.08	2.12 ± 0.08	2.08 ± 0.08	2.13 ± 0.08	**0.037**	0.020	**0.002**
Sup. Parietal	2.79 ± 0.11	2.81 ± 0.11	2.75 ± 0.11	2.82 ± 0.11	0.405	0.127	0.094
Inf. Parietal	3.03 ± 0.12	3.05 ± 0.13	2.98 ± 0.10	3.08 ± 0.12	0.065	0.046	**0.007**
Postcentral	3.32 ± 0.14	3.35 ± 0.15	3.26 ± 0.13	3.34 ± 0.14	0.551	0.042	0.066
Precentral	3.27 ± 0.14	3.29 ± 0.15	3.22 ± 0.14	3.32 ± 0.15	0.113	0.125	**0.031**

Table 7.2. *Correlations of LDL-cholesterol with cortical gyrification among Controls and PD subgroups at baseline*

	Control		**$PD_{Earlier}$**		**PD_{Later}**		**PD_{Later} v. $PD_{Earlier}$**		**PD_{Later} v. Control**	
Region	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**
Overall	2.18E-04	0.593	-2.20E-04	0.677	2.55E-03	**0.010***	1.33E-03	0.073	1.38E-03	0.072
Supramarginal	-1.35E-04	0.797	-3.49E-04	0.680	3.11E-03	**0.020***	1.72E-03	0.126	2.16E-03	**0.027**
Sup. Frontal	1.29E-04	0.684	-3.72E-05	0.935	1.88E-03	**0.020***	1.55E-03	**0.014**	1.25E-03	**0.050**
Sup. Parietal	2.93E-04	0.493	-1.35E-04	0.822	2.50E-03	**0.044***	1.40E-03	0.101	1.67E-03	**0.046**
Inf. Parietal	-4.36E-04	0.326	4.53E-05	0.948	1.95E-03	**0.038***	8.96E-04	0.296	2.06E-03	**0.015**
Postcentral	7.15E-04	0.156	-2.69E-04	0.737	3.44E-03	**0.010***	1.56E-03	0.152	1.75E-03	0.074
Precentral	9.45E-04	0.081	-3.30E-04	0.681	3.39E-03	**0.015***	1.92E-03	0.088	1.70E-03	0.101

* Significant after correction for multiple correlations using an expected false discovery rate of 0.05.

Table 7.3. *Baseline correlations of gyrification and interactions of age and LDL-cholesterol among Controls and PD*

	Control Age × LDL-c		**Control $Age_{High/Low}$ × LDL-c**		**PD Age × LDL-c**		**PD $Age_{High/Low}$ × LDL-c**	
	Estimate	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**
Overall	3.67E-05	0.507	1.10E-03	0.150	1.27E-05	0.783	5.96E-04	0.454
Supramarginal	1.55E-05	0.795	5.56E-04	0.547	3.39E-05	0.627	-1.76E-05	0.989
Sup. Frontal	5.05E-05	0.165	1.08E-03	0.063	-5.80E-07	0.989	3.05E-05	0.964
Sup. Parietal	4.19E-05	0.401	4.04E-04	0.594	-4.01E-05	0.435	-1.25E-04	0.890
Inf. Parietal	8.73E-05	0.097	1.05E-03	0.191	-1.60E-05	0.759	-2.08E-04	0.822
Postcentral	4.59E-05	0.436	8.69E-04	0.343	1.02E-05	0.874	3.86E-04	0.736
Precentral	6.08E-05	0.342	1.26E-03	0.194	5.24E-05	0.442	1.06E-03	0.366

* Significant after correction for multiple correlations using an expected false discovery rate of 0.05.

Table 7.4. *Correlations of baseline LDL-cholesterol with prospective annual change in gyrification among Controls and PD subgroups*

	Control		**$PD_{Earlier}$**		**PD_{Later}**		**PD_{Later} v. $PD_{Earlier}$**		**PD_{Later} v. Control**	
	Estimate	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**
Overall	3.79E-06	0.890	-5.73E-06	0.893	-7.77E-05	0.172	7.20E-05	0.312	-8.15E-05	0.197
Supramarginal	7.79E-05	0.217	-7.56E-05	0.462	-1.70E-04	0.216	9.46E-05	0.581	-2.48E-04	0.102
Sup. Frontal	-8.31E-06	0.808	3.58E-05	0.516	-2.81E-05	0.704	6.39E-05	0.487	-1.98E-05	0.808
Sup. Parietal	5.38E-05	0.225	6.53E-05	0.363	-1.55E-04	0.106	2.20E-04	0.065	-2.09E-04	**0.048**
Inf. Parietal	3.72E-05	0.522	1.87E-05	0.843	-7.93E-05	0.531	9.81E-05	0.534	-1.17E-04	0.403
Postcentral	1.75E-05	0.729	-8.59E-05	0.288	-2.83E-04	**0.009**	1.97E-04	0.145	-3.00E-04	**0.012**
Precentral	-4.33E-05	0.378	-2.10E-05	0.790	-1.27E-04	0.229	1.06E-04	0.420	-8.36E-05	0.472

* Significant after correction for multiple correlations using an expected false discovery rate of 0.05.

Table 7.5. *Correlations of annual change in gyrification and interactions of age and mean LDL-cholesterol (per subject across all visits) among Controls and PD*

	Control Age × LDL-c		**Control $Age_{High/Low}$ × LDL-c**		**PD Age × LDL-c**		**PD $Age_{High/Low}$ × LDL-c**	
	Estimate	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**	**Estimate**	**P-Value**
Overall	1.92E-06	0.632	-6.30E-06	0.963	-2.93E-06	0.430	-4.54E-05	0.735
Supramarginal	8.06E-06	0.327	-1.46E-05	0.162	1.95E-04	0.407	-3.12E-04	0.298
Sup. Frontal	9.57E-07	0.827	-7.46E-06	0.765	-2.04E-05	0.409	-1.12E-04	0.468
Sup. Parietal	-9.83E-06	0.122	1.32E-05	0.549	-6.38E-05	0.338	2.58E-04	0.275
Inf. Parietal	-4.13E-07	0.962	7.34E-06	0.153	1.93E-04	0.663	1.96E-04	0.504
Postcentral	-5.29E-06	0.441	-1.30E-05	0.351	-1.02E-04	0.349	-2.42E-04	0.303
Precentral	-6.20E-06	0.394	-7.54E-06	0.215	-1.44E-04	0.576	-1.22E-04	0.590

* Significant after correction for multiple correlations using an expected false discovery rate of 0.05

Figures

Figure 7. 1. Correlations of cortical gyrification and LDL-cholesterol in PD and Control subjects

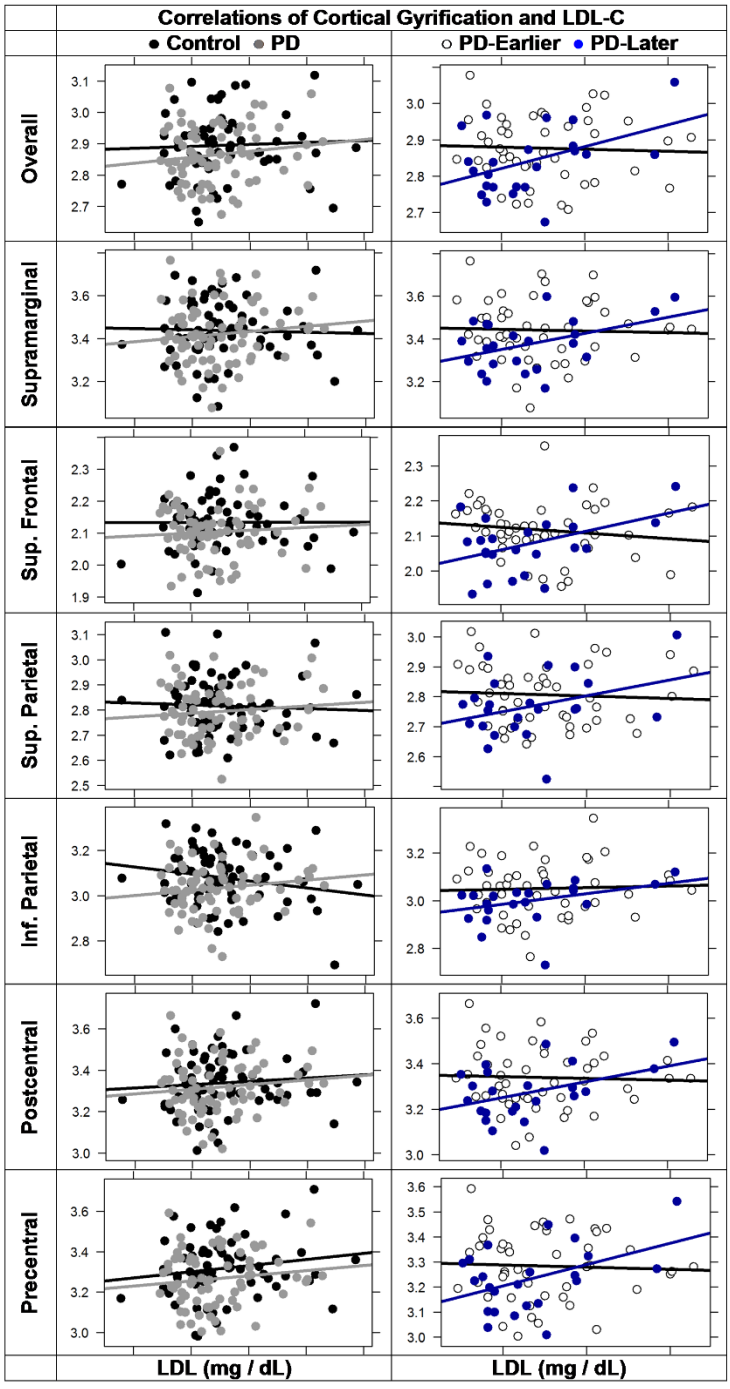
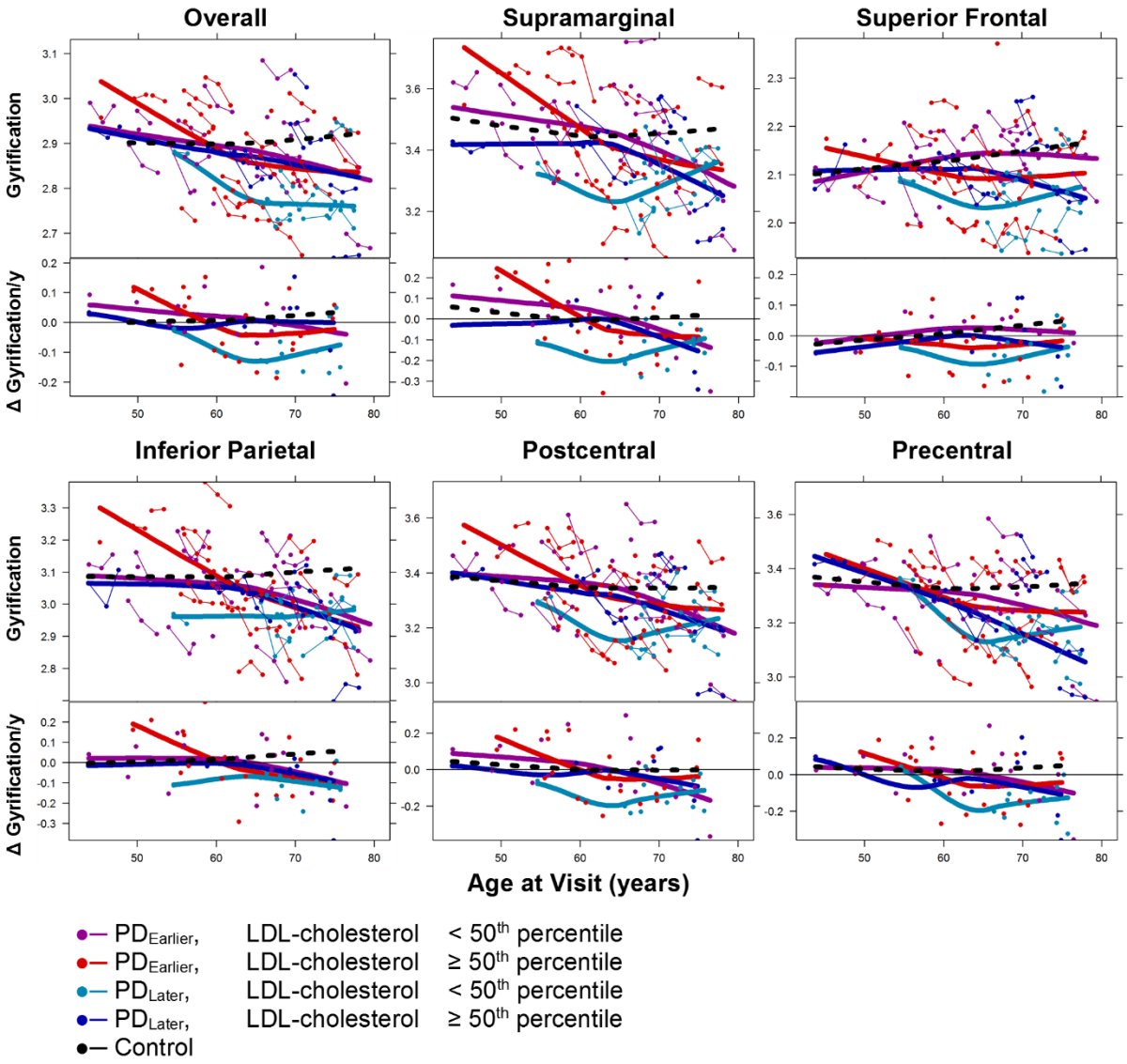


Figure 7. 2. *Age trajectories of cortical gyrification index (adjusted for total intracranial volume) by PD subgroup ($PD_{Earlier}$/PD_{Later}) and LDL-cholesterol (higher/lower)*



Summary & future directions

There is a growing body of evidence suggesting an association between plasma cholesterol and PD. Higher plasma total- or LDL-cholesterol, for example, has been associated with lower PD risk (10, 16-18), delayed age of onset (20), and beneficial outcomes (222, 385). *Chapter 5* reviewed the literature regarding the PD-cholesterol link and explored possible factors (i.e. cerebrovascular, genetic, and pharmacologic) and mechanisms that could mediate or modulate the association. In *Chapter 6*, I investigated the associations between LDL-cholesterol and cognitive cognitive decline, a particularly relevant clinical outcome. The results suggest that higher LDL-cholesterol is associated with preserved executive function and fine motor skill over time in PD, but not necessarily control subjects. In *Chapter 7*, I utilized cortical gyrification as an *in vivo* marker of PD progression to gauge the relationship between LDL-cholesterol and PD-related brain changes. The results suggested that higher LDL-cholesterol is associated with delayed loss of cortical gyrification in PD, paralleling recently reported findings of delayed onset of disease in association with higher LDL-cholesterol (20). Taken together, these results warrant future investigations of the possible mechanisms underlying the PD-cholesterol link, which may guide future treatment strategies.

Since the mechanisms underlying the PD-cholesterol link are unknown, there are several avenues that may potentially offer future insights. One possible explanation for the link between PD and cholesterol is via genetic pleiotropy (i.e. genetic influences on cholesterol metabolism in both brain and plasma compartments). Thus, future experiments should take into account the underlying genotype of individuals (ApoE, low density lipoprotein receptor, low density lipoprotein receptor related peptides 1 and 2) as well as plasma cholesterol and relevant

modifiers. On a similar note, it is possible that cholesterol metabolism requirements are different and/or altered in PD brains, in parallel with plasma cholesterol. Thus, future studies should examine the relationship between PD progression and cholesterol hydroxylated metabolites (i.e. 24- and 27-hydroxycholesterol) in the cerebrospinal fluid of PD subjects (301, 303). Although cortical gyrification was used as the biomarker for later-stage PD subjects, other imaging biomarkers may reflect changes in earlier stages of disease and may also be informative. In addition, it will be important to account carefully for nutritional and exercise-related information in future studies, since both of these can modify plasma cholesterol levels. Another unknown in the association between PD and cholesterol is the extent to which LDL-cholesterol could potentially cross the blood brain barrier. Recent studies have shown uptake of LDL particles and other apolipoproteins across the blood-brain barrier (269-273), possibly via low-density lipoprotein receptor, low-density lipoprotein receptor related proteins 1 and 2 (274), scavenger receptor class B type 1 (275), and/or ABCA1 (275). Future studies are warranted to determine the extent of cholesterol and/or apolipoprotein uptake through the blood-brain barrier in both control and PD subjects. Finally, although I have focused much attention on LDL-cholesterol in this section, it is important to note that LDL-cholesterol could be a precursor or metabolite of some other active biomolecule that is associated with beneficial outcomes in PD.

The consistency of studies reporting beneficial associations between LDL-cholesterol and PD is encouraging, but should be taken with a degree of caution. Even if there are robust associations between cholesterol and PD-related outcomes, no studies have been able to address the issue of causality. At some point in the future, it may be necessary to conduct randomized controlled trials to establish causality. To ethically justify such a trial, the potential benefits of

higher LDL-cholesterol would need to outweigh the increased risk of adverse cardiac outcomes. Since PD is a relatively rare disorder and cardiovascular disease is much more common (389), such a trial would ideally be restricted to high-risk individuals (i.e. rapid eye movement sleep behavioral disorder, male gender, no history of smoking, lower LDL-cholesterol) and/or PD subjects that have lower cardiovascular risk (i.e. lower LDL-cholesterol) (1).

SECTION V: SUMMARY OF RESEARCH, IMPLICATIONS, AND FUTURE DIRECTIONS

Despite the success of symptomatic treatment, there is no known therapy that can slow or reverse the progression of PD. The disease progresses relentlessly and eventually leads to severe motor and cognitive disability in advanced stages (8). In a recent study based in the United States, it was estimated that 630,000 individuals had diagnosed PD, and the national economic burden exceeded $14.4 billion in the United States in 2010. With increasing life expectancies, the prevalence of PD is expected to double by the year 2040 (3). Neuroprotective strategies that can modify the course of PD, therefore, are critically needed. The research of this dissertation had two main goals. The first goal was to identify imaging metrics that could be used to gauge cumulative PD progression in an objective and quantitative manner. The second goal was to evaluate potentially neuroprotective factors (with focus on cholesterol) using imaging markers of progression, rather than relying solely on traditional clinical evaluations.

Structural markers of PD progression

In *Chapter 2*, I explored longitudinal structural metrics of a broad range of brain structures in order to evaluate how brain atrophy might evolve over time and relate to known pathologies in PD. Many studies had compared PD and control subjects using cross-sectional designs, but none had systematically characterized brain structural changes in relation to progression through various disease stages (33-41). The spatiotemporal pattern of Lewy pathology has been characterized extensively in PD, and is thought to begin in the periphery, and the progress to the brainstem and eventually higher level cortical structures as disease progresses (2). Although striatal structures are

directly downstream of the primary site of pathology in PD, 60-80% of the dopaminergic cells in the substantia nigra are known to be lost by the time patients reach the clinic for disease diagnosis. The putamen, in particular, is affected most severely by nigrostriatal terminal losses in PD (4). In agreement with this prior knowledge, putamen atrophy was rapid in the earliest stages of PD, but reached a floor after roughly five years. In contrast to the early pathology known to occur in the nigrostriatal system, the cortex is thought to be affected later in disease progression. Consistent with this notion, cortical volume declined more steadily than other metrics of brain structural changes in PD, particularly in the later stages. Cortical volume, furthermore, was correlated with several clinical metrics including disease duration, cognitive, and motor function in PD. This suggested that cortex structure, overall, might serve as a marker of cumulative PD progression, although the exact cortical features that were most prominently associated with disease progression remained uncharacterized. Because PD related pathological changes are heterogeneous and dynamic, this chapter underscored the importance of investigating PD biomarkers in stage-dependent manner. This approach will guide our understanding of stage-dependent pathological changes during the full course of the disease process and facilitate the proper choice of biomarkers in future clinical trials in PD.

In *Chapter 3*, I examined the relationships between cortical folding (gyrification) and PD progression. Cortical folding had been relatively un unexplored by past studies, except one cross-sectional study suggested diffuse loss of gyrification without investigating disease stages (34) and one study correlated lower cortical gyrification with a composite score of PD progression that included dementia (79). To my knowledge, the study in *Chapter 3* was the first to investigate cortical gyrification in non-demented PD patients, and utilized a design based on stages of PD

progression. Cortical gyrification index of several cortical areas (overall, supramarginal, superior frontal, inferior parietal, postcentral, and precentral) were shown to correlate closely with disease progression. These findings were encouraging because such metrics could offer a method in clinical trials to gauge the effects of potentially neuroprotective agents in an objective manner. Gyrification index of these areas, accordingly, were utilized subsequently to gauge the effects of reportedly neuroprotective factors in Section 3.

Subcortical white matter and its relation to and cortex atrophy in PD

In *Chapter 4*, I investigated the relationships between the diffusion characteristics of subcortical white matter and overlying cortical gray matter structure. While gray matter atrophy in PD is well established (33-41), the extent of white matter involvement is controversial (167). Some previous studies have suggested that PD patients have worse white matter diffusion characteristics especially in advanced stages of disease and cognitive impairment (167, 195-201). Other studies have found weaker evidence of white matter in Parkinson's disease (202-205). I found that while there are white matter diffusion changes in PD, they are not as strong as changes in gray matter structure. Most studies have considered cortical gray and white matter separately in PD. I found robust associations between gray matter volume and white matter diffusion properties in PD. These findings suggest that cortical gray matter atrophy and poorer subcortical white matter diffusion characteristics are related in PD, which is consistent with anatomic knowledge that subcortical white matter tracts contain myelinated axons of cortico-cortical and cortico-subcortical projections (168). Thus, gray and white matter degeneration in PD should not be assumed to be

independent biologically. Furthermore, the associations were in contrast with those found in control subjects, suggesting that PD subjects have limited neuroplastic compensatory capacity.

Although some evidence has suggested that axonal myelination may serve a protective role against Lewy pathology in PD (146, 163), I did not find strong evidence to support this hypothesis. While the diffusion-based surrogate of myelination (radial diffusivity) was associated with higher cortical volumes in PD at baseline, so was the more general surrogate of white matter integrity (axial diffusivity) (187-189). Longitudinal changes in cortical volume, however, seemed to have stronger associations with axial diffusivity than radial diffusivity. Although I could not absolutely confirm or reject the notion that axonal myelination modulates cortical atrophy in PD, future research could employ more precise techniques to reflect myelination properties and perhaps and higher temporal resolutions or longer follow-up in the study design.

Lastly, previous studies have hypothesized that changes in cortical gyrification in PD (see *Chapter 3*) could be due to changes in subcortical white matter. The exact dynamics of cortical gyrification in human development are quite controversial, but are thought to involve tangential forces of rapidly expanding cortical gray matter constrained by underlying white matter (164). The mechanisms for lost gyrification in neurodegenerative processes, however, remain unexplored. Interestingly, cortical gyrification showed minimal associations with subcortical white matter diffusion characteristics. The lack of strong associations suggest that cortical gyrification might, indeed, be a separate process in the cortex that is driven by cellular processes in the gray matter rather than an indirect effect of subcortical white matter changes. The lack of robust associations between cortical gyrification with underlying white matter changes could explain partially why

cortical gyrification seems to be a more robust measurement of intrinsic PD progression. In contrast with volume, cortical gyrification could be relatively resistant to changes occurring in the underlying white matter due to aging or other factors (212). Further investigation of the relevant mechanisms of cortical gyrification change in PD are warranted.

Cholesterol and PD progression

Although many factors have been postulated as potentially neuroprotective in PD (9), only a handful have yielded relatively consistent association of either lower PD risk or slower progression (e.g. plasma cholesterol, cigarette smoking, and caffeine) (10-12). Of these, plasma cholesterol is particularly easy to quantify. The relationships between cholesterol and PD are controversial and could possibly be mediated by other mechanisms. In *Chapter 5*, I reviewed the existing literature regarding topics that are related to both PD and cholesterol (i.e. cardiovascular, genetic, cholesterol-associated metabolites, cholesterol-modifying drugs, inflammation). While the exact biological mechanisms underlying the PD-cholesterol relationship are still unknown, there are several worthwhile avenues for further investigation. For example, there is evidence suggesting that genetic loci involved in cholesterol metabolism are associated with PD risk (297) and that cholesterol metabolism in PD brains is altered (301). In this review, I gave special attention to the topic of cholesterol-modifying drugs and PD. Statins are among some of the most widely-used drugs, and recent guidelines will expand the use of these drugs in the coming years (223, 314). In the meta-analysis, I found that while the literature overall supported an association between statin usage and lower PD risk, there was also significant evidence of publication bias and many of those studies did not account for cholesterol levels before statin usage. Future studies

are warranted to elucidate the mechanisms of the PD-cholesterol relationship. Furthermore, the link between statins and PD risk deserves significant attention in future studies, since understanding this relationship may have important implications for individuals who are at higher risk for PD (i.e. rapid eye movement sleep behavioral disorder) (21).

In *Chapter 6,* I investigated the relationships between LDL-cholesterol and cognitive function PD. Cognitive decline is common in PD and is considered to be one of the most debilitating aspects of the disease, since patients having PD-related dementia can pose major unintended burdens to caregivers (8, 50). Thus, successful approaches to preserve cognitive function and prevent PD-related dementia would have important public health relevance. I found that higher LDL-cholesterol at baseline was associated with both preserved executive function and fine motor skill. These findings, taken together with the findings of *Chapter 7,* suggested that higher LDL-cholesterol may be associated with more favorable trajectories of both PD-related clinical symptoms and imaging metrics of progression.

In *Chapter 7,* I investigated the relationships between LDL-cholesterol and cortical gyrification. Past studies had suggested that higher LDL-cholesterol is associated with lower risk of PD (10, 16-18), but there has been a lack of studies investigating PD-related outcomes. A prospective study by our group suggested that higher LDL-cholesterol was associated with a trend of slower PD symptom progression (222). Similarly, another prospective by Postuma et al. (21) found that higher LDL-cholesterol was associated with lower risk of conversion to PD among patients with idiopathic rapid eye movement sleep behavioral disorder, a disorder thought to often represent a prodromal state of PD. Similarly, Mahlknecht et al. (20) found that higher LDL-

cholesterol was associated with later age of PD onset. To my knowledge, no other studies have examined LDL-cholesterol in relation to *in vivo* markers of PD progression. Interestingly, higher LDL-cholesterol was found to be associated with higher cortical gyrification at baseline but increased rate of gyrification loss over time. In other words, later-stage PD patients having higher LDL-cholesterol had similar patterns of gyrification and gyrification change as those in earlier stages of disease. These results suggested that higher LDL-cholesterol in PD was associated with delayed progression of neurodegeneration in the cortex. It is, however, unclear whether the effects of this delayed loss persist or are short-lived. Future studies with longer follow-up are needed to understand how LDL-cholesterol associates with the evolution of PD over time.

Summary & future directions

The research detailed in this dissertation has several clinical and scientific implications. First, structural imaging measurements of the cortex are useful as markers to gauge PD progression *in vivo*. In contrast to clinical measurements, these measurements are objective and repeatable. Second, subcortical white matter changes may be associated with atrophy of overlying cortical gray matter in PD, and there may be a suggestion of lost neuroplasticity in PD. Cortical gyrification, interestingly, is less associated with changes in underlying cortical white matter than cortical volume, suggesting that cortical gyrification could be more resistant to the loss of white-matter axons that is known to occur throughout the aging (212) or other disease process and might be a more sensitive measure of intrinsic PD progression. Third, higher plasma is associated with delayed loss of cortical gyrification and slower decline of cognitive and motor function in PD. These results are congruent with recent data suggesting delayed age of onset in association with

higher LDL-cholesterol in PD (20). Taken together, these results suggest that structural magnetic resonance imaging may be able to capture and track PD-related brain changes *in vivo*, providing markers to gauge the factors that are associated with disease progression.

Future studies should focus on further refinement of the imaging markers of PD progression. For example, there may be other metrics of cortical folding, such as surface complexity, that are more sensitive or specific to PD-related changes. In addition, although cortical gyrification seemed to be useful particularly in more advanced stages of PD, it may be desirable to measure progression in the earlier stages of disease. One potential avenue would be to explore composite markers using several imaging measurements (i.e. nigrostriatal and cortical structure) to gauge PD progression throughout the entire spectrum of PD severity (i.e. preclinical through advanced stages). Finally, the link between PD and cholesterol may be of high clinical relevance, especially since plasma cholesterol is highly modifiable, but the brain mechanisms underlying the reported beneficial associations are poorly understood. Future studies are warranted to investigate the underlying mechanisms, which may be particularly relevant for patients at high risk for PD.

APPENDIX: OTHER PUBLISHED RESEARCH (ABSTRACTS ONLY)

Cortical thinning and cognitive impairment in PD without dementia

Zhang L., **Sterling N.W.**, Wang M., Lee E.Y., Eslinger P.J., Wagner D., Du G., Lewis M.M, Huang X. (2015). Cortical Thickness Thinning and Cognitive Impairment in Parkinson's Disease Without Dementia. Probabilistic Methods in Computational Neuroscience. Accepted July 29 2015.

[Role of NWS: Image processing, conceptualization, manuscript writing.]

Background and Purpose: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized clinically by motor dysfunction (bradykinesia, rigidity, tremor, and postural instability), and pathologically by the loss of dopaminergic neurons in the substantia nigra of basal ganglia. Growing literature supports that cognitive deficits may also be present in PD, even in non-demented patients. Gray matter (GM) atrophy has been reported in PD and may be related to cognitive decline. This study investigated cortical thickness in non-demented PD subjects and elucidated its relationships to cognitive impairment.

Methods: High-resolution T1-weighted brain MRI and comprehensive cognitive function tests were acquired from 71 non-demented PD and 48 control subjects matched for age, gender, and education (Controls). Cortical thickness was compared between groups was conducted using a flexible hierarchical Bayesian model in order to account for correlation between brain regions. Correlation analyses were performed among brain areas and cognitive domains, which showed significant group differences in the PD population after adjusting for multiple comparisons.

Results: Compared to Controls, PD subjects demonstrated significant age-adjusted cortical thinning predominantly in inferior and superior parietal areas and extended to superior frontal, superior temporal, and precuneus areas (posterior probability > 0.9). Cortical thinning was also

found in the left precentral and lateral occipital, and right postcentral, middle frontal, and fusiform regions (posterior probability > 0.9). PD patients showed significantly reduced cognitive performance in executive function, including set shifting (p=0.005) and spontaneous flexibility (p=0.02), which were associated with the above cortical thinning regions ($p < 0.05$).

Conclusions: Non-demented PD subjects demonstrated significant cortical thinning that was related to cognitive loss and may serve as a biomarker of PD cognitive decline.

History of smoking and olfaction in Parkinson's disease

Lucassen, E. B.*, **Sterling, N. W.***, Lee, E. Y., Chen, H., Lewis, M. M., Kong, L., & Huang, X. (2014). History of smoking and olfaction in Parkinson's disease. Movement Disorders. Accepted May 2014.

[Role of NWS: Co-lead author, conceptualization, statistical analysis, manuscript writing.]

Objective: Olfactory dysfunction is the most common pre-motor symptom in Parkinson's disease, and smoking is known to be associated with lower risk of PD. This study tested the hypothesis that smoking is associated with better olfaction in PD.

Methods: Smoking history was obtained from 76 PD subjects [22 with a history of smoking (smokers), 54 who never smoked (non-smokers)], and 70 Controls (17 smokers, 53 non-smokers). Olfaction was assessed using the 40-item University of Pennsylvania Smell Identification Test (UPSIT). The UPSIT scores between groups and subgroups were compared using analysis of covariance with adjustment for age, gender, and MAO-B inhibitor usage.

Results: Overall the UPSIT score was lower in PD compared to Controls (UPSIT scores: 21.54 vs. 33.45, $p<0.0001$). Among Controls, there was no significant difference in olfaction between smokers and non-smokers (UPSIT scores: 33.2 vs. 34.2, $p=0.95$). Among PD subjects, however, smokers scored significantly better regarding olfaction compared to non-smokers (UPSIT scores: 24.4 vs. 19.9, $p=0.02$).

Conclusions: These data suggest that history of smoking is associated with better olfaction among PD patients. The finding may be related to why smoking may be protective against PD. Further studies are needed to confirm this finding and investigate the underlying mechanism(s).

Microstructural changes in the substantia nigra of asymptomatic agricultural workers

Du, G., Lewis, M. M., **Sterling, N. W.**, Kong, L., Chen, H., Mailman, R. B., & Huang, X. (2014). Microstructural changes in the substantia nigra of asymptomatic agricultural workers. Neurotoxicology and teratology, 41, 60-64. Accepted Dec 2013.

[Role of NWS: Image processing, manuscript writing.]

Parkinson's disease (PD) is marked by the loss of dopamine neurons in the substantia nigra (SN). Although the exact etiology is unknown, sporadic PD is hypothesized to be a result of genetic susceptibility interacting with environmental insult. Epidemiological studies suggest that pesticide exposure is linked to higher PD risk, but there are no studies demonstrating SN changes with chronic pesticide exposure in human subjects. High resolution T2-weighted magnetic resonance imaging (MRI) and diffusion tensor (DTI) images were obtained from 12 agricultural workers with chronic pesticide exposure, 12 controls, and 12 PD subjects. Neither controls, nor pesticide-exposed subjects, had any parkinsonian symptoms. Exposure history to pesticides was assessed by a structured questionnaire. DTI measures in the SN, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), were obtained for all subjects and compared among groups. Compared to controls, PD patients showed the expected significant changes in all DTI measurements in the SN. The pesticides-exposed subjects, compared to controls, had significantly lower FA values ($p=0.022$, after multiple comparisons correction), but no significant differences in RD, MD, or AD measures. The study is the first to demonstrate microstructural changes in the SN of human subjects with chronic pesticide exposure. The changes detected by MRI may mark “one of the hits” leading to PD, and underlie the increased risk of PD in pesticide users found in epidemiological studies. Further human studies assisted by these imaging markers may be useful in understanding the etiology of PD.

Dopaminergic modulation of arm swing during gait among Parkinson's disease patients

Sterling N.W. Cusumano J.P., Shaham N., Piazza S.J., Liu G., Kong L., Du G., Lewis M.M., Huang X. Dopaminergic modulation of arm swing during gait among Parkinson's disease patients. Journal of Parkinson's Disease. Accepted Nov 2014.

[Role of NWS: First author, data collection, data processing, statistical analysis, manuscript writing.]

Background: Reduced arm swing amplitude, symmetry, and coordination during gait have been reported in Parkinson's disease (PD), but the relationship between dopaminergic depletion and these upper limb gait changes remains unclear. This study investigated the effects of dopaminergic drugs on arm swing velocity, symmetry, and coordination in PD.

Methods: Forearm angular velocity was recorded in 16 PD and 17 control subjects (Controls) during free walking trials. Angular velocity amplitude of each arm, arm swing asymmetry, and maximum cross-correlation were compared between control and PD groups, and between OFF- and ON-medication states among PD subjects.

Results: Compared to Controls, PD subjects in the OFF-medication state exhibited lower angular velocity amplitude of the slower- ($p=0.0018$), but not faster- ($p=0.2801$) swinging arm. In addition, PD subjects demonstrated increased arm swing asymmetry ($p=0.0046$) and lower maximum cross-correlation ($p=0.0026$). Following dopaminergic treatment, angular velocity amplitude increased in the slower- ($p=0.0182$), but not faster- ($p=0.2312$) swinging arm among PD subjects. Furthermore, arm swing asymmetry decreased ($p=0.0386$), whereas maximum cross-correlation showed no change ($p=0.7436$). Pre-drug angular velocity amplitude of the slower-

swinging arm was correlated inversely with the change in arm swing asymmetry (R=-0.73824, p=0.0011).

Conclusions: This study provides quantitative evidence that reduced arm swing and symmetry in PD can be modulated by dopaminergic replacement. The lack of modulations of bilateral arm coordination suggests that additional neurotransmitters may also be involved in arm swing changes in PD. Further studies are warranted to investigate the longitudinal trajectory of arm swing dynamics throughout PD progression.

Multimodal MRI effectively distinguishes Parkinson's disease from atypical Parkinsonism

[This paper is in the final preparation stages for submission.]

[Role of NWS: Image processing, conceptualization, manuscript writing.]

Background and Objectives: Distinguishing Parkinson's disease (PD) from atypical parkinsonism [PDism, e.g., progressive nuclear palsy (PSP) and multiple system atrophy-parkinsonian subtype (MSA-P)] in the early stages can be challenging clinically. Although PD and PDism both cause basal ganglia (BG) dysfunction, the underlying pathologies and their effects on various regions is different. I hypothesized that multimodal MRI could capture these differences and assist differential diagnoses.

Methods: High resolution T1- and T2-weighted, diffusion tensor (DTI), and transverse relaxation rate (R2*) MRI images were acquired in 16 Control, 16 PD, 15 PSP, and 11 MSA-P subjects. Regions of interest (ROIs) in BG and related structures were segmented automatically on T1- and T2-weighted images, and co-registered to DTI and R2* maps, respectively. DTI fractional anisotropy (FA) and mean diffusivity (MD), and R2* values were compared and their contributions in discriminating among groups were determined with regularized logistic regression models and receiver operator curve analyses.

Results: Differences among groups were found in substantia nigra, putamen, global pallidus, and caudate for DTI, and substantia nigra and red nucleus for R2*. Using regularized logistic regression, these measures discriminated Controls from PD (sensitivity=88%, specificity=88%), PD from PDism (sensitivity=100%, specificity=81%), although the discrimination was not significant between PSP and MSA-P.

Conclusions: Multimodal MRI measures are effective in discriminating among Controls, PD, and PDism. Future studies are warranted to confirm these results and determine if this can improve clinical diagnosis in the early stages of these diseases.

Master of Science Thesis

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Education

Institution and Location	Degree	Year	Field of Study
Pennsylvania State University, Hershey, PA	[M.D.]	[2018]	Medicine (in progress)
Pennsylvania State University, Hershey, PA	Ph.D.	2016	Biomedical Sciences
Pennsylvania State University, Hershey, PA	M.S.	2013	Public Health Sciences
Messiah College, Mechanicsburg, PA	B.S.	2011	Biochem. & Molec. Biology

Publications

1. **Sterling N.W.** et al. Plasma LDL-cholesterol is associated with preserved executive and fine motor functions in Parkinson's disease. Aging & Disease. Accepted Nov 8 2015.
2. **Sterling N.W.** et al. Stage-dependent loss of cortical gyrification as Parkinson's disease "unfolds." Neurology. In press, accepted Oct 15 2015.
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4. Tucker, C., Han, Y., Black H.; Wang-Chien; Lewis, M.M.; **Sterling, N.W**.; Huang, X.. A Data Mining Methodology For Predicting Early Stage Parkinson's Disease Using Non-Invasive, High Dimensional Gait Sensor Data. IIE Transactions on Healthcare Systems Engineering. In press, accepted Sept 12 2015.
5. Tucker C., Behoora I., Black-Nembhard H., Lewis M.M., **Sterling N.W.**, Huang X. Machine Learning Classification of Medication Adherence in Patients with Movement Disorders Using Non-Wearable Sensors. Computers in Biology and Medicine. Accepted Aug 12 2015.
6. Zhang L., **Sterling N.W.**, Wang M., Lee E.Y., Eslinger P.J., Wagner D., Du G., Lewis M.M, Huang X. Cortical Thickness Thinning and Cognitive Impairment in PD Without Dementia. Probabilistic Methods in Computational Neuroscience. Accepted July 29 2015.
7. **Sterling N.W.** et al. Dopaminergic modulation of arm swing during gait among Parkinson's disease patients. Journal of Parkinson's Disease. Accepted Nov 2014.
8. Lucassen, E. B.*, **Sterling, N. W.***, Lee, E. Y., Chen, H., Lewis, M. M., Kong, L., & Huang, X. (2014). History of smoking and olfaction in PD. *Mov. Disord*. Accepted May 2014.
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10. **Sterling N.W.*,** Du G.*, Lewis M.M., Dimaio C., Kong L., Eslinger P.J., Styner M., Huang X. Striatal shape in PD. *Neurobiology of aging*, 34(11), 2510-2516. Accepted May 22 2013.

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