

Microstructural Changes in White Matter in Prodromal and Clinical Parkinson's Disease

by

Lisa Ohlhauser

B.Sc. (Hons.), University of British Columbia, 2014

A Thesis Submitted in Partial Fulfillment  
of the Requirements for the Degree of

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**Supervisory Committee**

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**Departmental Member**

## Abstract

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**Background:** Parkinson's disease (PD) is a neurodegenerative disorder that causes distinct motor impairments (i.e., resting tremor, bradykinesia, rigidity, postural instability) and affects approximately one percent of the global population over the age of 60 years. Currently, there is no cure and diagnosis remain challenging due to the lack of well validated biomarkers. Prodromal PD is a phase that predates the onset of motor symptoms but includes brain changes and nonmotor symptoms, such as rapid eye movement sleep behaviour disorder (RBD) and hyposmia. Diffusion tensor imaging (DTI) provides non-invasively acquired metrics of microstructural changes in white matter and subcortical tissue and has potential as a biomarker for PD. To date, most DTI studies have focused on the clinical phase of PD. Investigating potential biomarkers in the prodromal phase of the disease is key for early diagnosis and treatment. This study had two primary objectives: (1) to investigate how white matter microstructure changes in different phases of PD progression, and (2) to investigate how sleep and motor symptoms relate to white matter microstructure in different phases of PD.

**Methods:** All study data were downloaded from the Parkinson's Progression Markers Initiative database. Subjects included 21 healthy controls (mean age=68.17±4.69; 6 female), 20 individuals with prodromal PD (14 with RBD and 6 with hyposmia) (mean age=67.95±5.90; 6 female), and 17 individuals with clinical PD (mean age=67.69±5.97; 6 female) (at baseline and one-year later). Tract based spatial statistics were used to

determine between group differences in fractional anisotropy (FA) and mean diffusivity (MD) at the whole brain level and in a region of interest (ROI), the substantia nigra. The relationship between sleep or motor symptoms and DTI metrics were investigated within each group.

**Results:** There were no differences between the groups in age, education level, or cognitive scores. Clinical PD had significantly higher motor symptoms than healthy controls or prodromal PD, and this significantly increased from baseline to one-year later. Between group comparisons showed increased MD (reflecting increased neurodegeneration) in prodromal PD relative to clinical PD (both at baseline and one-year later), while there were no group differences between either prodromal or clinical PD and healthy controls at the whole brain level or within the ROI. Increased motor symptoms were associated with neurodegeneration (i.e., decreased FA and increased MD) for healthy controls, while increased sleep symptoms were associated with decreased MD for clinical PD.

**Conclusion:** This was the first to study of white matter microstructure differences in a mixed prodromal PD group relative to clinical PD. The detected early brain changes may support an RBD subtype of PD with overall different pattern of neurodegeneration. However, these results are preliminary and future studies must be conducted to clarify and expand upon the microstructural differences between prodromal and clinical PD, ideally with longitudinal follow-up.

*Keywords:* Parkinson's disease, diffusion tensor imaging, prodromal, biomarker, rapid eye movement sleep behaviour disorder,

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**Dedication**

*To Keith, Louanne, Jon, Devan, and LRRR*

## Chapter 1

### Parkinson's Disease: A Brief Overview

Parkinson's disease (PD) is a neurodegenerative disorder that leads to a complex presentation of motor, nonmotor, and emotional symptoms for approximately one percent of the global population over the age of 60 (Tysnes & Storstein, 2017). Recently, the International Parkinson and Movement Disorder Society (MDS) published official clinical diagnostic criteria for PD to aid in both clinical conceptualization and research. According to these criteria, diagnosis should be based on the presence of hallmark motor symptoms - bradykinesia, postural instability, rigidity, and resting tremor - along with the exclusion of other related motor disorders, infection, and neurological damage (Wirdefeldt, Adami, Cole, Trichopoulos, & Mandel, 2011). Unfortunately, there is no objective assessment measure for PD. This is reflected in the time it takes to be diagnosed with PD, especially for younger individuals. In Canada, the time from symptom onset to diagnosis was measured at an average of approximately seven years for those diagnosed at younger than 50 years, two years for those between ages 50 and 64, and one year for those aged 65 to 79 years (Wong, Gilmour, & Ramage-Morin, 2014). Additionally, once diagnosed, there is no cure for PD or avenue for slowing disease progression, although treatments can manage symptoms. Given these current issues, a well-validated objective biological marker for PD could help to reduce the time to diagnosis, provide the opportunity for earlier treatment, and help to track the progression of the disease. In order to identify individuals with PD at the earliest time point, it is essential to understand the prodromal signs of PD.

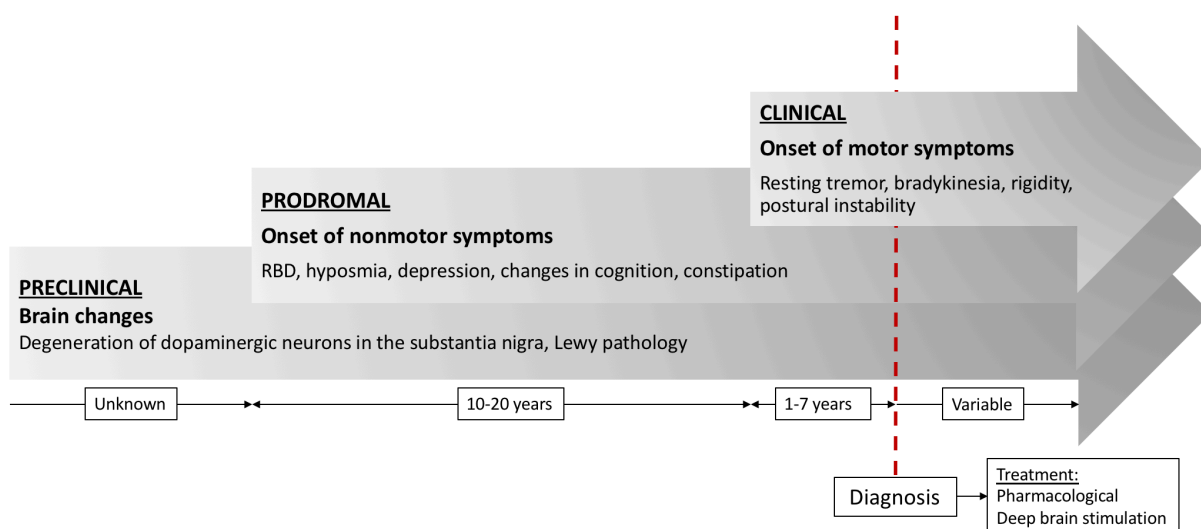
## **Prodromal Signs of Parkinson's Disease: Nonmotor Symptoms**

Although PD has classically been considered a motor disorder, over the last 20 years, there has been increasing recognition of nonmotor symptoms that can co-occur with and even predate the disease. Specifically, rapid eye movement (REM) sleep behaviour disorder (RBD), impaired olfaction, depression, changes in cognition (especially deficits in executive function), and constipation may collectively form a prodromal phase of PD. Although, on an individual level their sensitivity and specificity may vary (Postuma et al., 2012), these prodromal symptoms are quite prevalent in PD. A recent multi-site study found that, on average, approximately eight different nonmotor symptoms co-occurred with PD and virtually all (98.6%) cases experienced at least one nonmotor symptom (Barone et al., 2009). Nonmotor symptoms such as sleep disorders, mood disturbances, and impaired cognition, are particularly associated with reduced well-being and poorer quality of life (Duncan et al., 2014). Unfortunately, many nonmotor symptoms of PD go unrecognized and untreated. This may be due to the overshadowing of prominent motor symptoms of PD, overlapping symptomology, and under-reporting of nonmotor symptoms by patients who may not associate the symptoms with PD or may be too embarrassed to discuss these symptoms (Chaudhuri et al., 2010). This is particularly problematic since many nonmotor symptoms of PD are treatable.

One of the most prevalent prodromal markers of PD is RBD. Idiopathic RBD is characterized by loss of atonia during REM sleep and subsequent movement and dream enactment. Estimates of RBD co-occurring with PD range from 20-70% and there is evidence that approximately 80% of those with idiopathic RBD will eventually convert to PD or other synuclein related neurodegenerative disorder (Postuma et al., 2015). RBD

has been found to predate motor symptoms by an average of 12 to 14 years and is associated with poorer outcomes including visual hallucinations, visual color perception deficit, and cognitive impairments (Postuma et al., 2012). Further, RBD has also been associated with more severe motor deficits in the clinical phase of PD (Mahajan et al., 2014). RBD is one of the strongest prodromal signs of PD and other synucleinopathies and represents an early opportunity for disease modifying interventions when they become available (Postuma et al., 2012).

In line with this mounting evidence for a prodromal phase of PD based on nonmotor symptoms, the MDS task force redefined PD and proposed three phases of the disease: (a) preclinical PD, where there are no evident symptoms or signs, but there is evidence of PD-specific pathology supported by molecular or imaging markers (yet to be defined); (b) prodromal PD, where early nonmotor symptoms and signs are present, but are yet insufficient to define disease; and (c) clinical PD, where diagnosis of PD based on presence of classical motor signs (see *Figure 1*) (Stern, Lang, & Poewe, 2012). The MDS has also produced research criteria for the prodromal phase of PD that utilizes a naïve Bayesian classifier to combine prior risk based on age with various prodromal risk markers to estimate an individual's probability of prodromal PD, where >80% indicates "probable" and 30-80% indicates "possible" prodromal PD, respectively (Berg et al., 2015). Redefining PD into the preclinical, prodromal, and clinical phases provides an opportunity to elucidate the etiology and progression of the disease and represents an important step towards the development of early biomarkers of the disease.



*Figure 1.* The three phases of Parkinson's disease: preclinical, prodromal (also called premotor), and clinical. Diagnosis is made in the clinical phase one to seven years after the onset of motor symptoms. RBD=rapid eye movement sleep behaviour disorder.

## Parkinson's Disease: Underlying Neural Changes

In terms of underlying changes in the brain, PD has some clear known neuropathological findings including Lewy pathology and damage to dopaminergic neurons within the substantia nigra. The Braak model provides a theory of PD progression based on findings from postmortem brain studies. Together, these neuronal changes and model of PD progression can aid research in PD pathology.

**Lewy pathology.** Lewy pathology consists of insoluble intraneuronal inclusions composed primarily of the misfolded protein, alpha-synuclein, that can aggregate as Lewy neurites within axons and dendrites or Lewy bodies within cell bodies of neurons (Dickson, 2012). Findings based primarily on post-mortem studies show that the accumulation of alpha-synuclein within neurons and synapses may start a cascade of events that eventually leads to axonal damage, dysfunctional connectivity, and onset of

nonmotor and motor symptoms of PD (Bellucci et al., 2016). Detecting these brain changes before motor symptoms appear is an important goal for implementing disease modifying treatments at the earliest stages.

**Dopaminergic neurons in the substantia nigra.** In particular, PD is associated with the degeneration of neurons in the substantia nigra in the midbrain that produce the neurotransmitter dopamine. The resultant loss of dopamine is thought to disrupt the communication of neurons within the cortico-basal ganglia thalamocortical circuit, which leads to the hypokinetic motor symptoms of bradykinesia and rigidity (Weingarten, Sundman, Hickey, & Chen, 2015). Importantly, there is evidence from neuroimaging and post-mortem brain studies of extensive cell damage to this area before the onset of motor symptoms. Various studies have used regression and back extrapolation of neuronal counts and disease duration to estimate that 30-70% of dopaminergic neurons in the substantia nigra and 50-70% of striatal dopaminergic terminals have already degenerated by the time the first motor symptoms appear (reviewed in Cheng, Ulane, & Burke, 2010). Further, emerging evidence from neuroimaging, postmortem neurochemical studies, and genetic animal models suggest that the axons involved in the dopaminergic system may degenerate first (Tagliaferro & Burke, 2016).

**Theory of Progression: The Braak Model.** In 2003, Braak and colleagues developed a staging system based on post-mortem brain tissue that provided evidence for the formation of the six-stage progression of PD (Braak et al., 2003). This scheme of PD progression is based on findings of the Lewy pathology and degeneration of dopaminergic neurons in the substantia nigra, the hallmark neuropathological findings of PD. The Braak model proposes that Lewy pathology progresses temporally and spatially

in a caudal to rostral fashion starting with the peripheral nervous system (autonomic neurons), olfactory system (olfactory bulb, anterior olfactory nucleus), and the medulla (dorsal motor nuclei of vagal and glossopharyngeal nerves) in Stage 1; to the pons (locus ceruleus, magnocellular portions of the reticular formation, posterior raphe nuclei) and spinal cord gray matter in Stage 2; to proliferating further in the pons (pedunculopontine nucleus), midbrain (substantia nigra pars compacta), basal forebrain (magnocellular nuclei including nucleus basalis of Meynert), and limbic system (central subnucleus of amygdala) in Stage 3; continuing to advance into the limbic system (accessory cortical and basolateral nuclei of amygdala, interstitial nucleus of stria terminalis, ventral claustrum), thalamus (intralaminar nuclei), and temporal cortex (anteromedial temporal mesocortex, CA3 region of hippocampus) in Stage 4; and developing further in multiple cortical regions (insular cortex, association cortical areas, primary cortical areas) in Stage 5 and 6 (Braak et al., 2003). This model of PD progression aligns appropriately with the prodromal phase of PD (Stage 1 and 2), onset of motor symptoms due to nigrostriatal dopamine deficiency (Stage 3), and continued motor and nonmotor symptoms that correspond with the clinical phase of PD progression (Stage 4-6) (Kalia & Lang, 2015). The Braak model may prove as a useful guide for PD biomarker research.

Further, the Braak model theorizes that PD progresses in this manner due to the vulnerability of certain cell types in the brain stem and cortical regions. Though not fully understood, projection neurons that have poorly or incompletely myelinated axons that are disproportionately long to their somata are more prone to develop lesions. Projection neurons that have short axons and those that are highly myelinated are more resistant to pathology (Braak, Rub, Gai, & Del Tredici, 2003). The Braak model of caudal to rostral

spread of Lewy pathology within the brain aligns with the regional distribution of selectively vulnerable projection neurons.

**Biomarkers for PD.** The Braak model provides insights into the brain regions impacted by PD pathology at the earliest points of the disease. However, for utility in early diagnosis, it is important that biological markers of PD be established in vivo. To date, several types of biomarkers have been explored including genetic markers (e.g., specific gene testing, next-generation sequencing), biochemical markers (e.g., specific proteins like alpha-synuclein in cerebrospinal fluid, saliva, urine, serum, plasma), pathological markers (e.g., colonic biopsy, skin biopsy), clinical markers (e.g., olfactory impairment, RBD), and neuroimaging based markers (e.g., positron emission tomography; PET, single photon emission computed tomography; SPECT, transcranial sonography, magnetic resonance imaging; MRI) (Kalia & Lang, 2015). In particular, MRI has several strengths in its potential for PD biomarker research. Specifically, it is widely available, non-invasive, and easily repeatable. Diffusion tensor imaging (DTI) is an imaging method that uses MRI to quantify diffusion of water molecules and can provide more specific information about microstructural properties of white matter and subcortical tissue (Soares, Marques, Alves, & Sousa, 2013). Given that the earliest brain changes in PD may involve more extensive damage to axons rather than cell bodies, DTI could prove particularly useful for detecting early signs of neurodegeneration and has potential as an in vivo biomarker for PD.

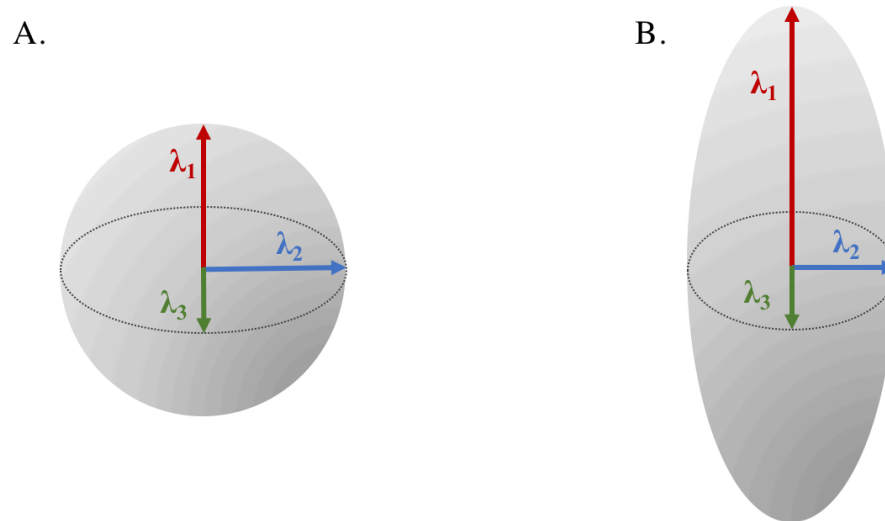
## Diffusion Tensor Imaging: How Does It Work?

Diffusion describes the random motion of molecules due to kinetic energy and can be described as “isotropic” or “anisotropic”. Isotropic diffusion refers to unrestricted diffusion, which occurs when there are few structural barriers, such as in cerebrospinal fluid. Anisotropic diffusion refers to restricted diffusion, which occurs when physical barriers are present, such as along tubular structures such as axons.

Essentially, DTI is a specific type of MRI acquisition. MRI is based on the principle of nuclear magnetic resonance, which capitalizes on the abundance of hydrogen atoms (i.e., single protons) largely present in human tissue in water molecules. DTI is a method of quantifying the molecular diffusion of water molecules measured by diffusion weighted imaging, which can be used to infer in vivo information about specific properties of various structures and tissue within the brain.

Specifically, DTI relies on the application of magnetic field gradients in different directions to estimate the strength and direction of diffusion of water within each voxel. The signal measured in a given voxel depends on strength (b-value) and direction of gradients, as well as on the local tissue microstructure (e.g., presence of barriers to diffusion, such as bundles of axons). If diffusion of protons has occurred in a certain direction, the signal will be attenuated, with more attenuation indicating more diffusion in the direction of the applied gradient. A minimum of six (and more typically 30) gradient directions are used to calculate the overall diffusion of water molecules within a voxel in an ellipsoid shape using a 3 x 3 symmetric matrix (i.e., a tensor model) (Basser, Mattiello, & LeBihan, 1994). Each voxel contains eigenvalues ( $\lambda_1, \lambda_2, \lambda_3$ ) and their

respective eigenvectors ( $V_1, V_2, V_3$ ) which measure the magnitude of and direction of diffusion (see *Figure 2*).



*Figure 2.* Diffusion tensor imaging model with A) isotropic diffusion, where eigenvalues are equal ( $\lambda_1 = \lambda_2 = \lambda_3$ ), and B) anisotropic diffusion, where eigenvalues are unequal.

To date, several DTI metrics using eigenvalues have been developed that provide information about the overall diffusion properties within each voxel. The most commonly reported metrics are fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Please see Table 1 for a brief description and common interpretation of each DTI metric. Fractional anisotropy provides an estimate of the overall direction of diffusion. In isotropic diffusion, all eigenvalues are equal in magnitude (i.e.,  $\lambda_1 = \lambda_2 = \lambda_3$ ) which results in an FA value of 0. In anisotropic diffusion, the eigenvalues are unequal which results in FA values closer to 1 (see *Figure 2*).

Table 1

*Diffusion Tensor Imaging Metrics*

	Metric			
	Fractional Anisotropy	Mean Diffusivity	Radial Diffusivity	Axial Diffusivity
Abbreviation	FA	MD	RD	AD
Mathematic equation	$\frac{\sqrt{1 - \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{2}}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$	$\frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$	$\frac{\lambda_2 + \lambda_3}{2}$	$\lambda_1$
Description	Degree of elongation of the diffusion tensor ellipsoid	Average of the diffusivity values of the three axes of the diffusion ellipsoid	Average of the diffusivities in the axes perpendicular to the major axes of diffusion	Diffusivity in the direction of maximum diffusion in the voxel
Common Interpretation	Summary of microstructural integrity	Inverse measure of membrane density	Increased in white matter with demyelination	Variable with white matter changes
Direction typically associated with neuro-degeneration <sup>1</sup>	Decreases	Increases	Variable	Variable
Sensitive to:	Wide range of pathologies	Cellularity, edema, and necrosis	Myelin loss	Axonal injury
Limitations	Crossing fibres with high integrity can have low FA	Large variability in measurement; crossing fibers	Voxels with crossing fibres can increase RD	Voxels with crossing fibers can increase AD

*Note.*  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$ =reflect the longest, middle, and shortest eigenvalues in a diffusion tensor imaging model (see *Figure 2*). <sup>1</sup>Decreased FA and increased MD associated with decreases in physical barriers to diffusion. Information adapted from Alexander, Lee, Lazar, & Field, 2007; Van Hecke, Emsell, & Sunaert, 2016.

## **Diffusion Tensor Imaging: Clinical Phase of Parkinson's Disease**

Numerous studies have used DTI to investigate PD compared to healthy controls. When results are examined across the whole brain, findings have been mixed. For example, at the whole brain level, some studies have found significant increases in MD in PD patients compared to healthy controls (Duncan et al., 2016), while others failed to observe significant differences in MD or other metrics, such as FA (Zhang et al., 2016).

A priori theory has often been utilized in DTI studies to focus on specific brain regions of interest (ROIs) (Soares, Marques, Alves, & Sousa, 2013). Given loss of dopaminergic neurons in the substantia nigra, many DTI studies of PD have focused on this area. While many studies report reductions in FA or increases in MD and RD in the substantia nigra in PD compared to healthy controls (Chan et al., 2007; Du et al., 2012; Jiang, Shi, Niu, Xie, & Yu, 2015; Kamagata et al., 2016; Langley et al., 2016; Nagee et al., 2016; Péran et al., 2010; Perea et al., 2013; Rolheiser et al., 2011; Scherfler et al., 2013; Schuff et al., 2015; Skorpil, Söderlund, Sundin, & Svenningsson, 2012; Zhan et al., 2012; Zhang et al., 2015; Vaillancourt et al., 2009; Gattellaro et al., 2009; Rolheiser et al., 2011), others have found no difference in substantia nigra FA values between PD and healthy controls (Chan et al., 2014; Menke, Jbabdi, Miller, Matthews, & Zarei, 2010; Prakash, Sitoh, Tan, & Au, 2012; Schwarz et al., 2013), and a few have even found a slight increase (Lenfeldt et al., 2013; Wang et al., 2011). The variability in findings could be attributed to several factors, including small sample size, heterogeneity of sample characteristics and variable DTI acquisition methods.

There have been several meta-analyses that have examined FA in the substantia nigra in PD compared to healthy controls. The results of these meta-analyses and other

notable DTI studies are summarized in Table 2 at the end of Chapter 1. The first meta-analysis contained nine studies and found a large main effect size for lowered FA in the substantia nigra in the PD groups, Hedges'  $g = -0.639$ , 95% confidence interval  $-0.860$  to  $-0.417$ ,  $p < 0.0001$ , meaning that collectively, FA in the PD groups were .639 standard deviations below the healthy control groups. They concluded, "DTI may be a promising biomarker in parkinsonian syndromes and have a future role in differential diagnosis" (Cochrane & Ebmeier, 2013). The second meta-analysis contained 11 studies and did not find reductions in FA in the substantia nigra for PD compared to healthy controls concluding, "usage of nigral FA changes as a biomarker of PD is neither reliable nor useful at this point in time" (Schwarz et al., 2013). Finally, a third meta-analysis of 23 studies found a small decrease in FA values in PD compared to healthy controls, however, the authors noted, "the discriminatory capability of this metric for establishing a diagnosis of PD using this metric alone was low" (Hirata et al., 2017). Notably, these meta-analyses found considerable heterogeneity of findings across individual studies ( $I^2=86-91.0\%$ ), which may be reflective of significant variability in sample characteristics (e.g., severity of disease state, types of therapy), technical aspects of image acquisition and preprocessing (e.g., resolution of MRI, software), and anatomical ROIs of the substantia nigra (e.g., rostral, middle, caudal) making the overall effect of FA of the substantia nigra difficult to determine.

Some DTI studies have refrained from using ROIs and have continued to study microstructural changes at the whole brain level. These studies show changes outside of the substantia nigra, suggesting that important information can be missed when utilizing solely an ROI approach. For example, one study found increases in MD in PD relative to

controls bilaterally in frontal and parietal subcortical tracts, including forceps minor, cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract, corpus callosum, and internal capsule (Duncan et al., 2016) and another found increases in MD and decreases in FA in the genu of the corpus callosum and in the superior longitudinal fasciculus (Gattellaro et al., 2009). A recent meta-analysis of 39 DTI studies that compared clinical PD to healthy controls at the whole brain level revealed structural differences between the groups in five cerebral regions: the substantia nigra, the corpus callosum, the cingulate and temporal cortices, and the corticospinal tract. The first four areas showed an overall effect of lower FA and higher MD in clinical PD relative to healthy controls. Interestingly, the meta-analysis found that the corticospinal tract showed the opposite trend of increased FA and decreased MD in clinical PD relative to healthy controls, which was indicative that this area may be undergoing possible brain reorganization (Atkinson-Clement, Pinto, Eusebio, & Coulon, 2017). These studies suggest that microstructural changes outside of the substantia nigra are also of importance.

Given the heterogeneity of research methods and mixed findings observed to date, studies with consistent metrics and large sample sizes are needed to clarify the utility of DTI as a neuroimaging biomarker for PD. The Parkinson's Progression Markers Initiative (PPMI) is a comprehensive observational, international, multi-center study designed to identify PD progression biomarkers (<http://www.ppmi-info.org/>). The study is open-access and provides clinical, molecular, and imaging data for several cohorts of PD patients (e.g., de novo, genetic, scans without evidence of dopaminergic deficits, prodromal) and healthy controls to researchers worldwide. Recently, studies have

emerged using this sample. Given the inconsistencies findings of white matter alterations across studies, one of the largest DTI studies to date used PPMI data to investigate various indices of white matter microstructure at the unbiased whole brain level between groups of PD patients and healthy controls (Wen et al., 2016). No differences in AD were found; however, greater FA and lower MD and RD were found in callosal (corpus callosum and forceps minor), projection, and association fibres in a PD group relative to healthy controls. The study also found that severity of motor dysfunction was inversely correlated with FA and positively correlated with MD and RD in the PD groups compared to healthy controls.

### **Relationship Between DTI Findings and Rapid Eye Movement Sleep Behaviour Disorder**

In addition to investigating the differences in microstructural DTI based metrics in PD and healthy controls, there have also been studies that have examined the relationship between changes in the brain and symptoms of PD. Notably, several studies have used DTI to investigate nonmotor symptoms, such as RBD, in the clinical phase of PD. These findings are important to consider in the context of future DTI research in the prodromal phase of PD, especially for RBD, since it is a frequent and highly specific marker for future conversion to clinical PD.

To date, only a few studies have compared clinical PD with and without RBD using DTI (see Table 2). One study found decreased FA and increased MD in PD with RBD relative to those without RBD, but these results became non-significant with statistical correction (Ford et al., 2013). These results match other non-significant

findings (García-Lorenzo et al., 2013). However, another study which utilized diffusion MRI connectometry found significant differences in white matter density in PD patients with (PD-RBD) compared to PD without RBD using PPMI data (Ansari, Rahmani, Dolatshahi, Pooyan, & Aarabi, 2017). Instead of using tract-based spatial statistics or ROIs to assess discrete regions in the brain, diffusion connectometry measures the degree of connectivity between adjacent voxels within a white matter fibre defined by the spin distribution function (i.e., density of diffusing water) and then tracts only segments of fibre bundles in the entire brain that exhibits significant association with a specified study variable (Yeh, Badre, & Verstynen, 2016). This study utilized a metric of diffusion connectometry called quantitative anisotropy (QA), which is comparable to FA as it is a similar metric of anisotropy, but it is thought to be more sensitive to physiological conditions and “compactness” of fibre bundles. The study found that persons with PD-RBD had significant white matter changes in the left and right cingulum, inferior front occipital fasciculus, bilateral corticospinal tracts, and middle cerebellar peduncles, compared to persons with PD without RBD. These white matter pathways had significantly reduced QA in PD-RBD than PD without RBD in the left and right cingulum, left and right interior fronto-occipital fasciculus, left and right corticospinal tract, and the body, genu, and splenium of the corpus callosum. These results suggest that those with PD-RBD had significantly lower density of certain white matter tracts compared with PD without RBD, which could suggest more significant neurodegeneration. These groups were well matched and did not differ by age, sex, cognition, depression, motor dysfunction, Hoehn & Yahr Staging, or disease duration. Results from this study are novel and no other studies exist for comparison. Follow-up

research using this sample is needed to determine if the PD group will eventually also develop RBD and to determine if the two groups will differ in disease progression. Future research is needed to replicate these findings.

Altogether, these studies show how DTI can be used to investigate the microstructural changes associated with RBD in PD. Knowing that RBD can occur long before PD can be diagnosed, future research should investigate the relationship between this nonmotor symptom and DTI metrics in the prodromal phase of PD.

### **DTI: Prodromal Parkinson's Disease**

There are many challenges in studying prodromal PD, as longitudinal follow-up is essential to confirm progression to clinical PD. However, some DTI studies have been conducted using samples of patients with idiopathic RBD, since eventual conversion to PD is high (Postuma et al., 2012).

Of note, there have been four studies using DTI to compare microstructure between RBD patients and healthy controls (see Table 2) (Mangia et al., 2017; Rahayel et al., 2014; Scherfler et al., 2011; Unger et al., 2010). The first study found significant differences in the RBD patients compared to healthy controls, specifically there were significant increases in FA (in the internal capsule bilaterally and olfactory region), significant decreases in FA (in the fornix, right visual stream, and left superior temporal lobe); significant decreases in AD (bilaterally in the corona radiata and in parts of the brainstem, including the pons and right substantia nigra), and significant increases in RD (in the fornix, right visual stream, and the left superior temporal lobe) (Unger et al., 2010). The second study found no significant increases in FA or decreases in MD at the

whole brain level but did find significant decreases in FA (in the tegmentum of the midbrain and the rostral pons) and increases in MD (within the pontine reticular formation overlapping with altered FA cluster in the midbrain) (Scherfler et al., 2011). The third study found no differences in FA, MD, AD, or RD between RBD and controls (Rahayel et al., 2014). Finally, the fourth study did find increased MD in RBD relative to controls in the substantia nigra reticula, but this finding became non-significant when controlling for age (Mangia et al., 2017).

Taken together, the results of these studies, though mixed, suggest that DTI can be used to detect microstructural differences between healthy controls and RBD patients in brain areas related to PD etiology and progression (i.e., substantia nigra, brainstem, olfactory region). However, these studies contained small samples, did not include a longitudinal follow up, and did not include a PD group, so it is difficult to determine if the RBD groups truly represent prodromal PD and if this group will eventually progress to the clinical phase of the disease.

### **Future Directions of Parkinson's Disease Research Using DTI: Towards Earlier Findings**

Research findings suggest that DTI has potential as a neuroimaging biomarker of PD, but that further investigations are required. There has been increasing recognition that brain changes (e.g., degeneration of neurons in the substantia nigra, Lewy pathology) and nonmotor symptoms (e.g., RBD) occur long before the onset of hallmark motor symptoms that are used to diagnose PD, leading to the recent reframing of PD into three phases: preclinical (asymptomatic, but changes in pathology), prodromal (nonmotor

symptoms insufficient to diagnose the disease), and clinical (onset of motor symptoms and diagnosis). While most DTI studies have focused on the clinical phase of PD, few have focused on the prodromal phase of PD. Given the known substantial neurodegeneration in the clinical phase of PD, finding biological markers in the prodromal phase of the disease is key for early diagnosis and informing the development of disease modifying treatments.

As mentioned, the PPMI study is a comprehensive observational, international, multi-center study designed to identify PD progression biomarkers. The study includes a cohort of prodromal PD participants who have not yet been diagnosed with PD, but who have hyposmia (impaired olfaction) and/or RBD. The PPMI study represents an important opportunity to investigate microstructural changes in the prodromal phase of PD. Future studies should use the PPMI database to investigate microstructural changes in the brain in the prodromal and clinical phases of PD using DTI. Only a few studies have focused on the prodromal PD group and none to date have compared microstructural changes across multiple phases of PD using DTI, which would represent an important step in biomarker identification.

Table 2  
*Summary of Notable Diffusion Tensor Imaging Studies of Parkinson's Disease*

Meta-analyses of Substantia Nigra ROI Studies: PD vs. HC				
Study	# of Studies: Sample	FA	MD	Notes
Cochrane & Ebmeier, 2013	9:193 PD 195 HC	Decreased FA	NA	I <sup>2</sup> =9.53%
Schwarz et al., 2013	11: 297 PD 268 HC	NS	NS Uncorrected: increased MD	I <sup>2</sup> =86%
Hirata et al., 2017	23: 806 PD 626 HC	Small decrease in FA	NA	I <sup>2</sup> =91.9
Meta-analysis of Whole Brain Studies: PD vs. HC				
Study	Sample	FA	MD	Notes
Atkinson-Clement et al., 2017	Meta-analysis of 39 studies: 1087 PD 768 HC	Decreased FA in SN, CC, cingulate and temporal cortices Increased FA in CS tract and caudate nucleus	Increased MD in SN, CC, cingulate and temporal cortices Decreased MD in CS tract	I <sup>2</sup> =31.1-89.8%
Individual Studies: PD-RBD vs. PD no RBD				
Study	Sample	FA	MD	Notes
Ford et al., 2013	46 PD-RBD 78 PD	NS Uncorrected: decreased FA in R inf. fronto-occipital and LF, L inf. LF, R CS tract, L sup. LF; increased FA in the brainstem, localizing to the pontine tegmentum	NS Uncorrected: increased MD in R and L inf. LF	TBSS; RBD dx by questionnaire;
Garcia-Lorenzo et al., 2013	24 PD-RBD 12 PD	NS	NS	RBD dx by sleep study
Ansari et al., 2017	23 PD-RBD 31 PD	Increased QA	NA	Diffusion connectometry
Individual Studies: iRBD vs. HC				
Study	Sample	FA	MD	Notes
Unger et al., 2010	12 iRBD 12 HC	Increased FA in the internal capsule bilaterally and olfactory region; decreased FA in the fornix, R visual stream, and L sup. temporal lobe	NA	TBSS; RBD dx by PSG;
Scherfler et al., 2011	26 iRBD 14 HC	Decreased FA in the tegmentum of the midbrain and the rostral pons	Increased MD within the pontine reticular formation	RBD dx by PSG;
Rahayel et al., 2014	24 iRBD 42 HC	NS	NS	TBSS; RBD dx by PSG
Mangia et al., 2017	9 iRBD 9 PD 10 HC	NS	NS Uncorrected: Increased MD in the SN reticula	TBSS; RBD dx by questionnaire

*Note.* Findings presented with first group relative to second group mentioned. PD=Parkinson's disease, HC=healthy controls, RBD=rapid eye-movement sleep behaviour disorder, PD-RBD=PD subjects with RBD, FA=fractional anisotropy, MD=mean diffusivity, NA=not applicable, NS=non-significant findings, SN=substantia nigra, CC=corpus callosum, CS=corticospinal, inf.=inferior, sup.=superior, LF=longitudinal fasciculi, L=left, R=right, TBSS=tract based spatial statistics, PSG=polysomnography, QA=quantitative anisotropy, iRBD=idiopathic RBD. I<sup>2</sup>=heterogeneity index (higher values indicating increased heterogeneity).

## Chapter 2

Parkinson's disease (PD) is a common neurodegenerative disorder that affects one percent of the global population over the age of 60 (Tysnes & Storstein, 2017). Currently, diagnosis of PD is based on behavioural observation of distinct parkinsonian motor symptoms (i.e., resting tremor, bradykinesia or slowness of movement, postural instability, and rigidity) and the exclusion of other known causes of these symptoms. However, the diagnosis of PD remains challenging and can take years from the onset of symptoms due to the lack of well validated biomarkers (i.e., indicators of the pathological process).

Though the cause of PD is unknown, the disease is associated with progressive deterioration of dopamine producing neurons in the substantia nigra in the midbrain and Lewy pathology; it has been estimated that 30-70% of dopaminergic neurons in the substantia nigra degenerate before the first motor symptoms appear (Cheng, Ulane, & Burke, 2010). In particular, the resultant loss of the neurotransmitter dopamine is thought to disrupt the communication of neurons within the cortico-basal ganglia thalamocortical circuit, which leads to the hypokinetic motor symptoms of bradykinesia and rigidity (Weingarten, Sundman, Hickey, & Chen, 2015). Furthermore, studies on Lewy body pathology indicate that the accumulation of alpha-synuclein within neurons and synapses may start a cascade of events that eventually leads to axonal damage, dysfunctional connectivity, and onset of nonmotor and motor symptoms of PD (Bellucci et al., 2016). Detecting these brain changes at the earliest time point is important for improving our understanding of the disease process and ultimately, developing disease modifying treatments for PD before substantial neurodegeneration can occur.

With an aim to improve early diagnosis of PD, there has been an increasing focus on nonmotor symptoms that are thought to predate hallmark motor symptoms. One multisite study found that on average, approximately eight different nonmotor symptoms co-occurred with PD and virtually all (98.6%) cases experienced at least one nonmotor symptom (Barone et al., 2009).

Importantly, several nonmotor symptoms of PD have been identified as possible prodromal markers of the disease including rapid eye movement (REM) sleep behaviour disorder (RBD), impairments in olfaction, deficits in cognition, constipation, and mood disturbances (i.e., depression, anxiety). Of particular note is RBD, given that it is one of the most prevalent nonmotor symptom of PD and has one of the highest specificity as a prodromal marker (Postuma et al., 2012). Idiopathic RBD occurs in 20-70% of cases of PD and is characterized by loss of atonia during REM sleep and subsequent movement and dream enactment. There is evidence that approximately 80% of those with idiopathic RBD eventually convert to PD or other synuclein related neurodegenerative disorders (Postuma et al., 2015). Further, RBD has been found to predate motor symptoms by an average of 12 to 14 years and is associated with poorer outcomes in PD including visual hallucinations, visual color perception deficit, cognitive impairments and more severe motor deficits (Mahajan et al., 2014; Postuma et al., 2012). RBD has been identified as one of the strongest prodromal signs of PD and represents an early opportunity for disease modifying interventions when they become available (Postuma et al., 2012).

In light of these findings of early brain changes and prodromal symptoms of PD before the onset of motor symptoms (and subsequent diagnosis of disease), the International Parkinson and Movement Disorder Society (MDS) task force redefined PD

and proposed three phases of the disease: (a) preclinical PD, where there are no evident symptoms or signs, but there is evidence of PD-specific pathology supported by molecular or imaging markers (yet to be defined); (b) prodromal PD, where early nonmotor symptoms and signs are present, but are yet insufficient to define disease; and (c) clinical PD, where diagnosis of PD based on presence of classical motor signs (Stern, Lang, & Poewe, 2012). Given the substantial neurodegeneration found in the clinical phase of PD, it is likely that disease modifying treatments, when available, will be best implemented before this phase, stressing the importance of biomarker validation in the preclinical and prodromal phases of PD.

Neuroimaging methods have started to be explored as potential in vivo biomarkers for PD (Tuite, 2016). Magnetic resonance imaging (MRI) based approaches are ideal for biomarker detection, as they are non-invasive, widely available, and easily repeatable. Diffusion tensor imaging is a method of quantifying the molecular diffusion of water molecules measured by diffusion weighted MRI, which can be used to infer information about specific properties of various structures and tissue within the brain. (Soares, Marques, Alves, & Sousa, 2013). Diffusion describes the random motion of molecules due to kinetic energy and can be described as “isotropic” or “anisotropic”. Isotropic diffusion describes unrestricted diffusion when there are few structural barriers, such as in cerebrospinal fluid, whereas anisotropic diffusion describes restricted diffusion when physical barriers are present, for example, along long tubular structures such as axons. The most commonly reported DTI metrics are fractional anisotropy (FA) and mean diffusivity (MD). Fractional anisotropy is a measure of the overall direction of water diffusion within a voxel. Decreased FA is associated with isotropic diffusion and

has values closer to 0 and increased FA is associated with anisotropic diffusion and has values closer to 1. Mean diffusivity provides no information about direction of diffusion, but instead quantifies the average rate of diffusion within a voxel. In general, decreased FA and increased MD is associated with neurodegeneration when using DTI to examine white matter tracts; however, the interpretation of the metrics is not always straightforward. Nonetheless, these DTI metrics can be used to infer early signs of neurodegeneration and could act as an early in vivo biomarker for PD.

To date, most DTI studies have focused on the clinical phase of PD and compare those with clinical PD to healthy controls. Findings across individual studies have been mixed. At the whole brain level, some studies have found increases in MD in PD, while others have observed no significant differences in FA or MD (Duncan et al., 2016; Zhang et al., 2016). The largest meta-analysis to date included 39 DTI studies that compared clinical PD to healthy controls at the whole brain level and revealed structural differences between the groups in five cerebral regions: the substantia nigra, the corpus callosum, the cingulate and temporal cortices, and the corticospinal tract. The first four areas showed an overall effect of lower FA and higher MD in clinical PD relative to healthy controls. Interestingly, the meta-analysis found that the corticospinal tract showed the opposite trend of increased FA and decreased MD in clinical PD relative to healthy controls, which may suggest that this area may be undergoing possible brain reorganization (Atkinson-Clement, Pinto, Eusebio, & Coulon, 2017).

Given the evidence of neurodegeneration within the substantia nigra in PD, many DTI studies have focused on structural differences in this area using region of interest (ROI) analyses. These studies have also been mixed with two meta-analyses showing

lower FA in PD compared to healthy controls (Cochrane & Ebmeier, 2013; Hirata et al., 2017), and one showing no between group differences in FA (Schwarz et al., 2013). The discrepancy in these results may be due to several factors including differences in image acquisition parameters, dopaminergic state during scanning (i.e., “on” or “off” state), and even the use of levo-dopa medication itself (Atkinson-Clement, Pinto, Eusebio, & Coulon, 2017). Nonetheless, the substantia nigra remains an important ROI in PD pathology.

While most DTI studies have focused on the clinical phase of PD, few have focused on the prodromal phase of PD. There are many challenges to the study of prodromal PD as it is not possible to confirm progression to clinical PD without longitudinal follow up. However, some DTI studies have been conducted using samples of patients with idiopathic RBD, since eventual conversion to PD is high (Postuma et al., 2012). Of particular note, there have been several studies using DTI to compare microstructure between individuals with RBD and healthy controls (Mangia et al., 2017; Rahayel et al., 2014; Scherfler et al., 2011; Unger et al., 2010). Taken together, the results of these studies suggest that DTI has potential for detecting microstructural differences between healthy controls and RBD in brain areas related to PD etiology and progression (i.e., substantia nigra, brain stem, olfactory region). However, these studies contained small samples and did not include a longitudinal follow up, so it is difficult to determine if the RBD groups truly represent prodromal PD and will eventually progress to the clinical phase of the disease.

Investigating potential biomarkers in the prodromal phase of PD is key for early diagnosis. The Parkinson Progression Markers Initiative (PPMI) is a comprehensive

observational longitudinal study that uses advanced imaging, biological sampling, and behavioural assessments to identify biomarkers of PD progression (Marek et al., 2011).

The PPMI database provides the opportunity to investigate microstructural changes in the brain during the prodromal and clinical phases of PD using DTI. The current study utilized data from the PPMI database with two main objectives: (1) to investigate how white matter microstructure changes in different phases of PD progression, and (2) to investigate how sleep and motor symptoms related to white matter microstructure in different phases of PD. These objectives were designed to answer the following research questions:

1. What are the microstructural differences in white matter between healthy controls and individuals with prodromal PD?
2. What are the microstructural differences in white matter between healthy controls and individuals with clinical PD?
3. What are the microstructural differences in white matter between individuals with prodromal and clinical PD?
4. What are the microstructural differences in white matter in individuals with clinical PD from baseline to one year later?
5. What is the relationship between white matter microstructure and PD symptoms (RBD and motor scores) in healthy controls?
6. What is the relationship between white matter microstructure and PD symptoms (RBD and motor scores) in prodromal PD?
7. What is the relationship between white matter microstructure and PD symptoms (RBD and motor scores) in clinical PD?

It was hypothesized that results would reveal a progressive deterioration in white matter microstructure (decreased FA and increased MD at the whole brain level and in an ROI of the substantia nigra) that is reflective of the phase of disease and that increased motor and sleep disorder symptoms would be associated with increased neurodegeneration (i.e., decreased FA and increased MD).

Table 3 displays the study objectives, research questions, and hypotheses. Given that only a few studies have focused on the prodromal PD group and none to date have compared microstructural changes across multiple phases of PD using DTI, the current study represents an important step in PD biomarker identification.

Table 3

*Study Objectives and Hypotheses*

<b>Objective 1: How does white matter microstructure change in different phases of PD progression?</b>					
Research Questions		Hypotheses			
<i>What are the microstructural differences between...</i>	Between Group Comparison	Whole-brain		Substantia nigra ROI	
		FA	MD	FA	MD
1. healthy controls and prodromal PD?	HC v. PPD	HC>PPD	HC<PPD	HC>PPD	HC<PPD
2. healthy controls and clinical PD?	HC v. PD1	HC>PD1	HC<PD1	HC>PD1	HC<PD1
	HC v. PD2	HC>PD2	HC<PD2	HC>PD2	HC<PD2
3. prodromal and clinical PD?	PPD v. PD1	PPD>PD1	PPD<PD1	PPD>PD1	PPD<PD1
	PPD v. PD2	PPD>PD2	PPD<PD2	PPD>PD2	PPD<PD2
4. clinical PD at baseline and one-year later?	PD1 v. PD2	PD1>PD2	PD1<PD2	PD1>PD2	PD1<PD2

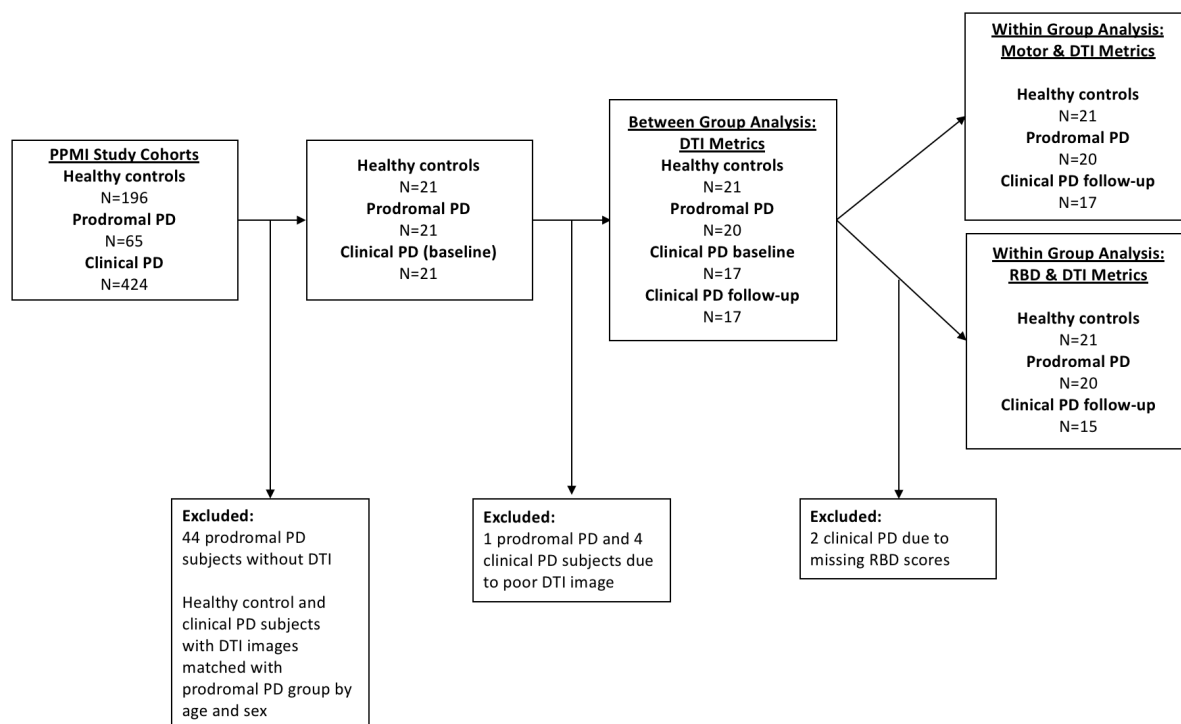
<b>Objective 2: How are sleep and motor symptoms related to white matter microstructure at different phases of PD?</b>					
Research Questions		Hypotheses			
<i>How is white matter microstructure related to sleep and motor symptoms within...</i>	Within Group Comparison	RBD symptoms		Motor symptoms	
		FA	MD	FA	MD
5. healthy controls?	HC	(-)	(+)	(-)	(+)
6. prodromal PD?	PPD	FA will decrease with increasing motor symptoms	MD will increase with increasing motor symptoms	FA will decrease with increasing motor symptoms	MD will increase with increasing motor symptoms
7. clinical PD?	PD2				

*Note.* PD=Parkinson's disease. HC=healthy controls. PPD=prodromal PD. PD1=Parkinson's disease at baseline. PD2=Parkinson's disease at one-year follow-up. RBD=Rapid eye movement sleep behaviour disorder. FA=fractional anisotropy. MD=mean diffusivity.

## Methods

### Participants

All data used for this study were obtained from the PPMI database. For up-to-date information on the study, visit [www.ppmi-info.org](http://www.ppmi-info.org). Participants were selected from three cohorts from the PPMI database and included 21 healthy control subjects (mean age=68.17±4.69; 6 female), 20 prodromal PD subjects (mean age=67.95±5.90; 6 female), and 17 subjects with PD at baseline (mean age=67.69±5.97; 6 female), and one year later (mean=68.85±6.02; 6 female). Control subjects were matched with clinical groups for age and sex, had no diagnosis of PD, and did not have a first degree relative with PD. Prodromal PD subjects were listed in PPMI as having a diagnosis of hyposmia and/or RBD. Fourteen prodromal PD subjects were confirmed to have RBD by polysomnography, while data for the other six prodromal subjects was unavailable. Sixteen out of the 20 prodromal subjects had a scoring of five or greater on the RBD Screening Questionnaire indicating RBD. PD subjects were de novo, meaning they had a diagnosis of PD for two years or less and were not taking PD medications at baseline. More specific study eligibility criteria are available on the PPMI website. Participant characteristics can be found in Table 3. Participants were first selected by availability of DTI data at the first-time point. Healthy controls and PD subjects were matched as closely as possible to prodromal PD subjects by age and sex since that group had the smallest pool of available data. A flow chart of participant selection can be found in Figure 3.



*Figure 3.* Flow diagram of participant selection and analysis. PPMI=Parkinson Progression Marker Initiative. PD=Parkinson’s disease. RBD=rapid eye movement sleep behaviour disorder.

## Measures

**DTI.** All images were acquired with a Siemens 3T TIM Trio scanner with a 12 channel Matrix head coil. Diffusion-weighted images were acquired with a single shot echo-planar imaging sequence, along 64 uniformly distributed directions using a b-value of 1000 s/mm<sup>2</sup> with a single b = 0 image (matrix = 116 × 116, isotropic resolution = 2 mm isotropic resolution, TR/TE = 900/88 ms). For more information regarding MRI acquisition, please see: <http://ppmi-info.org/>.

**REM Sleep Behaviour Disorder Questionnaire.** The REM Sleep Behaviour Disorder Questionnaire (RBDSQ) is a self-report questionnaire containing 10 yes or no items to assess sleep behavior. A bed partner was encouraged to provide addition

information if available, but this was not required. The items contained questions about the frequency and content of dreams, nocturnal movements and behavior, self-injuries and injuries of a bed partner during sleep, nocturnal motor behavior (e.g., nocturnal vocalizations, sudden limb movements, complex movements), awakenings, disturbed sleep, and presence of any neurological disorder. The items are totaled to a maximum of 13 points (some items have multiple parts), with higher scores indicating increased RBD symptoms. Total scores greater than or equal to 5 were used to indicate RBD (Stiasny-Kolster et al., 2007). Initial studies show the screener can accurately diagnose 66-88% of cases and has a sensitivity and specificity ranging from 68-96% and 56%-87%; respectively, suggesting the RBDSQ is a useful tool for diagnosis of RBD (Stiasny-Kolster et al., 2007; Stiasny-Kolster et al., 2015)

**MDS-UPDRS.** The Movement Disorder Society Unified Parkinson Disease Rating Scale (UPDRS) contains 65 items across four parts: Part I, Nonmotor Experiences of Daily Living; Part II, Motor Experiences of Daily Living; Part III, Motor Examination; and Part IV, Motor Complications (Goetz et al., 2004). Each item is rated on a five-point scale describing which are tailored to each item, but are generally based on this infrastructure: 0=normal, 1=slight, symptoms/signs with sufficiently low frequency or intensity sufficient to cause no impact on function; 2=mild, symptoms/signs of frequency or intensity sufficient to cause a modest impact on function; 3=moderate, symptoms/signs sufficiently frequent or intense to impact considerably; and 4=severe, symptoms/signs that prevent function. Scores are totaled to provide a summary of disease severity. Part III is particularly useful for identifying motor impairments in PD and was used for the data analysis.

## Data Analysis

**Image preprocessing.** Raw diffusion weighted images were downloaded from the PPMI website and converted from DICOM to NifTi files using dcm2nii converter from mricron (Rorden, 2016; <https://www.nitrc.org/projects/mricron>). FMRI Software Library (FSL) Version 5.0.10 was used for all image preprocessing and analysis (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004; Woolrich et al., 2009). First, to correct for eddy currents distortions and head movement, the Eddy Current Correction (ECC) tool was used (Andersson & Sotiropoulos, 2016). Next, the skull and other non-brain tissue were removed from the images using the Brain Extraction Tool (BET) (Smith, 2002); accuracy was confirmed with visual inspection.

**Image analysis.** Voxelwise statistical analysis of FA and MD data were carried out using Tract Based Spatial Statistics (TBSS). TBSS is a fully automated approach to objectively estimate the overall white matter tracts within the brain that are common to study subjects, which can then be compared statistically (Smith et al., 2006). First, FA images were created by fitting a tensor model to the raw diffusion data using DTIfit (Behrens et al., 2003; Johansen-Berg et al., 2004). All subjects' FA data were then aligned into a common space using the nonlinear registration which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was created and thinned using a threshold of 0.2 to create a mean FA skeleton which represents the centres of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. The previous steps were repeated for MD.

**Voxelwise statistical comparisons.** Between-group comparisons were conducted for FA and MD at the whole brain level and in a substantia nigra ROI (from NeuroVault; Gorgolewski et al., 2015; Keuken et al., 2014) for healthy controls, prodromal PD, and PD at baseline and one-year later. The relationship between each DTI metric (FA and MD ) and behavioural data (i.e., sleep behaviour and motor impairment) was also examined within each group using data from the RBDSQ and the UPDRS Part III.

All contrast files were created using FSL's GLM setup and group voxelwise comparisons were conducted using FSL's Randomise, a tool for nonparametric permutation inference on neuroimaging data (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). Randomise was conducted with 5000 permutations using threshold free cluster enhancement to correct for multiple comparisons (Smith & Nichols, 2009). For each contrast, Randomise produced a test static image that was overlaid onto its corresponding mean skeleton mask and standard brain image using FSLEyes, the FSL image viewer. Statistically significant group differences in FA and MD were identified with the ICBM-DTI-81 white matter label atlas and the JHU White Matter Tractography Atlas (Mori & Crain, 2006).

## **Results**

### **Descriptive Statistics**

Demographic information for healthy controls, prodromal PD, PD at baseline and one year later are displayed in Table 4. Between group comparisons revealed no

significant differences in age, education, or total score on the Montreal Cognitive Assessment (MoCA; a brief screener of cognitive function). PD at baseline had significantly higher motor scores on the UPDRS-III than healthy controls and prodromal PD. The mean rating of each motor symptom on the UPDRS-III for healthy controls, prodromal PD, and clinical PD are displayed in *Figure 4*. These scores also significantly increased within the PD group between baseline and one year later. The prodromal group had significantly higher RBDSQ scores than the healthy control and PD groups (both at baseline and one year later). Specific RBD symptoms experienced by healthy controls, prodromal PD, and clinical PD are displayed in *Figure 5*.

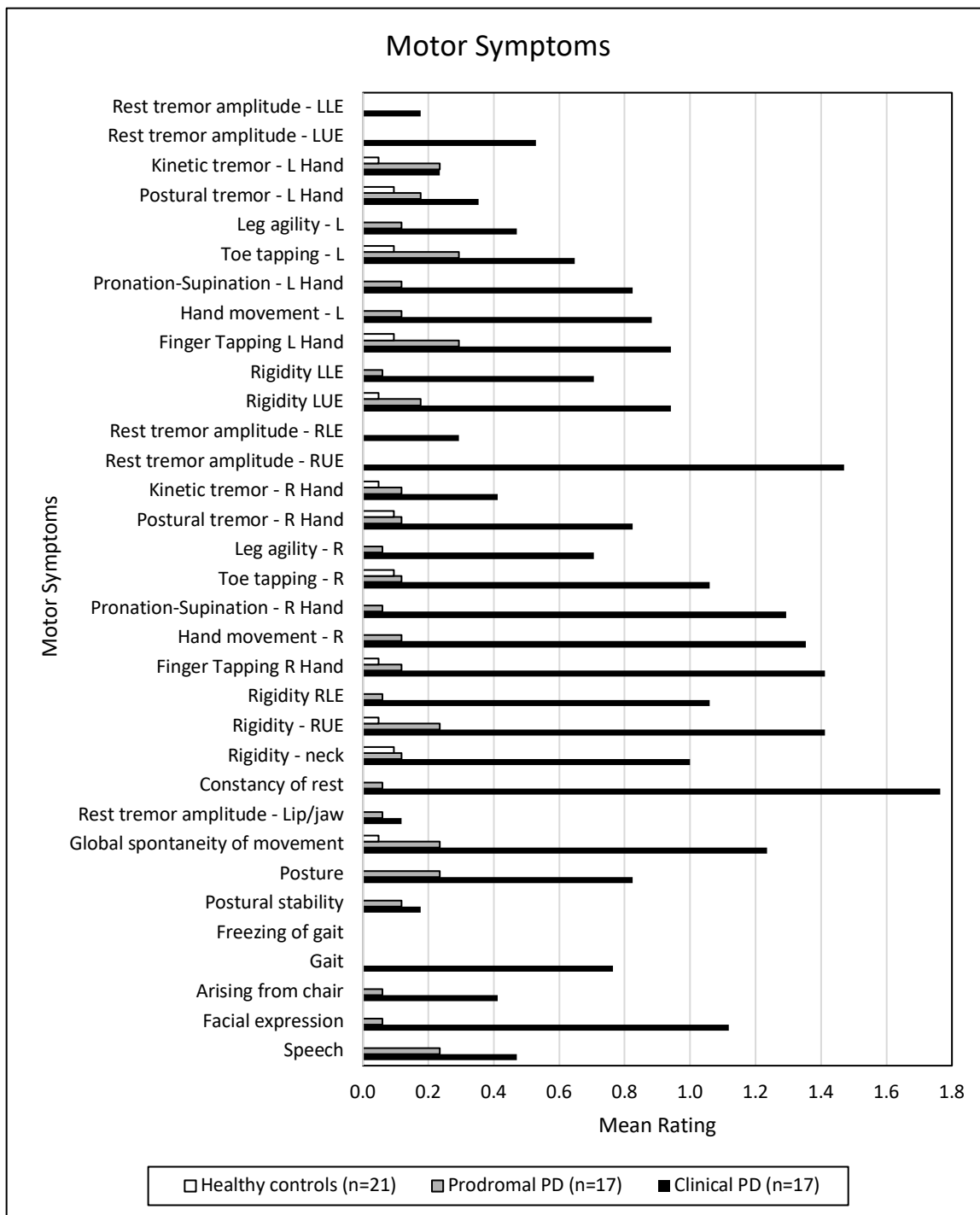
Table 4

*Participant Demographics*

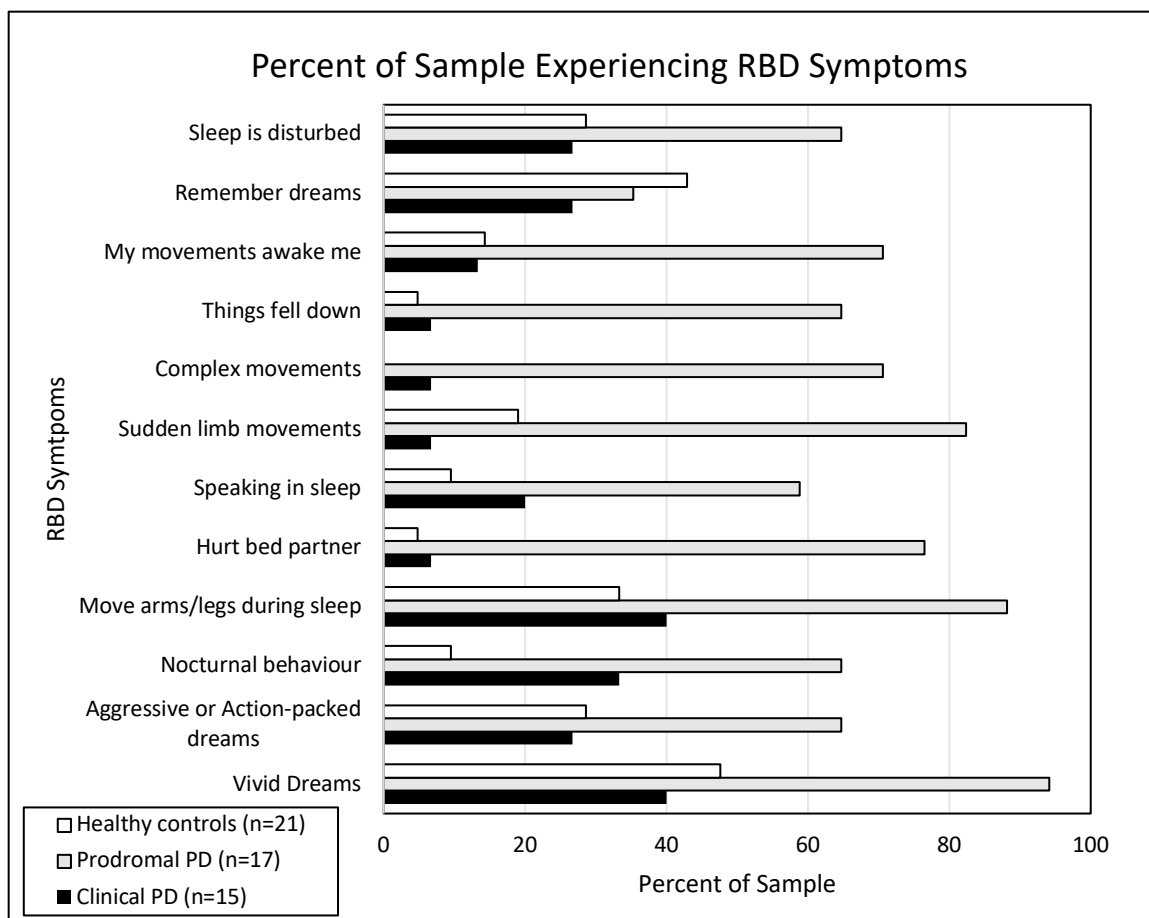
Variable	Controls	Prodromal PD	Clinical PD	
			Baseline	One-year follow-up
Number (female)	21 (6)	20 (6)	17 (6)	17 (6)
+RBD by PSG	-	14 <sup>a</sup>	-	-
+RBDSQ	5	16	8	4 <sup>b</sup>
Mean (SD)				
Age	68.17 (4.69)	67.97 (5.90)	67.69 (5.97)	68.84 (6.02)
Education	16.10 (2.70)	14.85 (3.10)	15.76 (2.37)	15.76 (2.37)
<b>UPDRS-III</b>	0.86 (2.03)	3.76 (4.54)	<b>18.24 (6.08)**</b>	<b>25.88 (11.99)**</b>
<b>RBDSQ</b>	2.76 (2.51)	<b>8.82 (4.35)**</b>	4.53 (3.17)	3.60 (2.72) <sup>b</sup>
MoCA	28.24 (1.04)	27.21 (2.26) <sup>c</sup>	27.06 (2.36)	26.38 (2.66) <sup>a</sup>

*Note.* RBD=Rapid Eye Movement Sleep Behaviour Disorder, PSG=Polysomnography, UPDRS-III=Unified Parkinson Disease Rating Scale Part III (Motor), RBDSQ = RBD Screening Questionnaire, MoCA=Montreal Cognitive Assessment. Only <sup>a</sup>14, <sup>b</sup>15, <sup>c</sup>18 scores available from database. PD one-year follow-up scores were used for correlation analyses for RBDSQ and UPDRS-III. Significant between group differences shown in bold for RBDSQ ( $F(3,66)=12.24, p=.000$ ) and UPDRS-III ( $F(3, 68)=52.74, p=.000$ ). \*\* $p<.01$ .





*Figure 4.* Mean severity of motor symptoms measured by the UPDRS-III. LLE=left lower extremity, LUE=left upper extremity, L=left, RLE=right lower extremity, RUE=right upper extremity, R=right. Symptoms rated on a Likert type scale where 0=normal, 1=slight, 2=mild, 3=moderate, and 4=severe impairment.



*Figure 5.* Percent of healthy controls, prodromal PD, and clinical PD (at one-year follow-up) sample groups that endorsed specific RBD symptoms on the RBD Screening Questionnaire. The prodromal PD group had significantly higher RBDSQ scores than the healthy control and PD groups (both at baseline and one year later). RBD=Rapid Eye Movement Sleep Behaviour Disorder.

**Results of Objective One: How does white matter microstructure change in different phases of PD progression?**

Table 5 shows the results of the between group comparisons of DTI metrics at the whole brain level and in the substantia nigra ROI.

Table 5

*Results of Between Group Comparisons in White Matter Microstructure*

Between Group Comparison	Whole-brain		Substantia nigra ROI	
	FA	MD	FA	MD
HC v. PPD	ns	ns	ns	ns
HC v. PD1	ns	ns	ns	ns
HC v. PD2	ns	ns	ns	ns
PPD v. PD1	ns	<b>PPD&gt;PD1*</b>	ns	ns
PPD v. PD2	ns	<b>PPD&gt;PD2*</b>	ns	ns
PD1 v. PD2	ns	ns	ns	ns
RBD v. PD1	ns	<b>RBD&gt;PD1*</b>	-	-
RBD v. PD2	ns	<b>RBD&gt;PD2*</b>	-	-

*Note.* Decreased FA and increased MD indicate neurodegeneration in white matter. FA=fractional anisotropy, MD=mean diffusivity, HC=healthy controls, PPD=prodromal PD, PD1=Parkinson's disease baseline, PD2=Parkinson's disease at one-year follow-up, RBD=Rapid Eye Movement Sleep Behaviour Disorder. ns=non-significant. \* $p<.05$ .

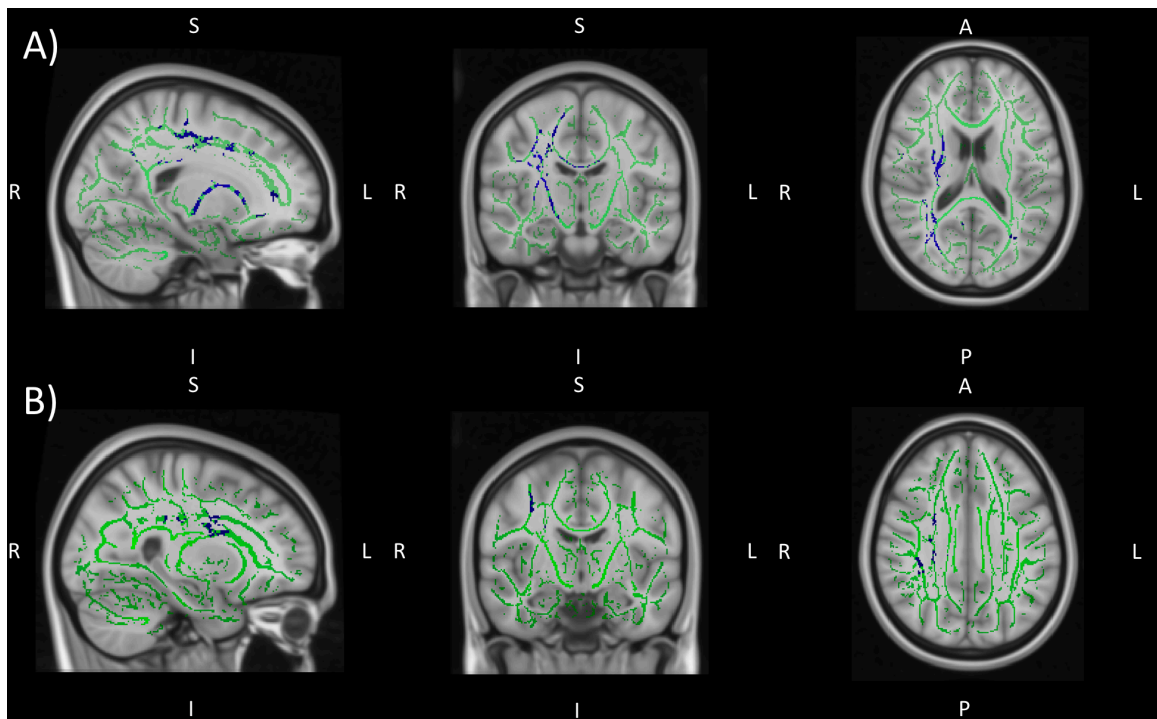
**Q1. Microstructural differences between healthy controls vs. prodromal PD.**

There were no significant differences in FA or MD between healthy controls or prodromal PD at the whole brain level or within the substantia nigra ROI.

**Q2. Microstructural differences between healthy controls vs. clinical PD.**

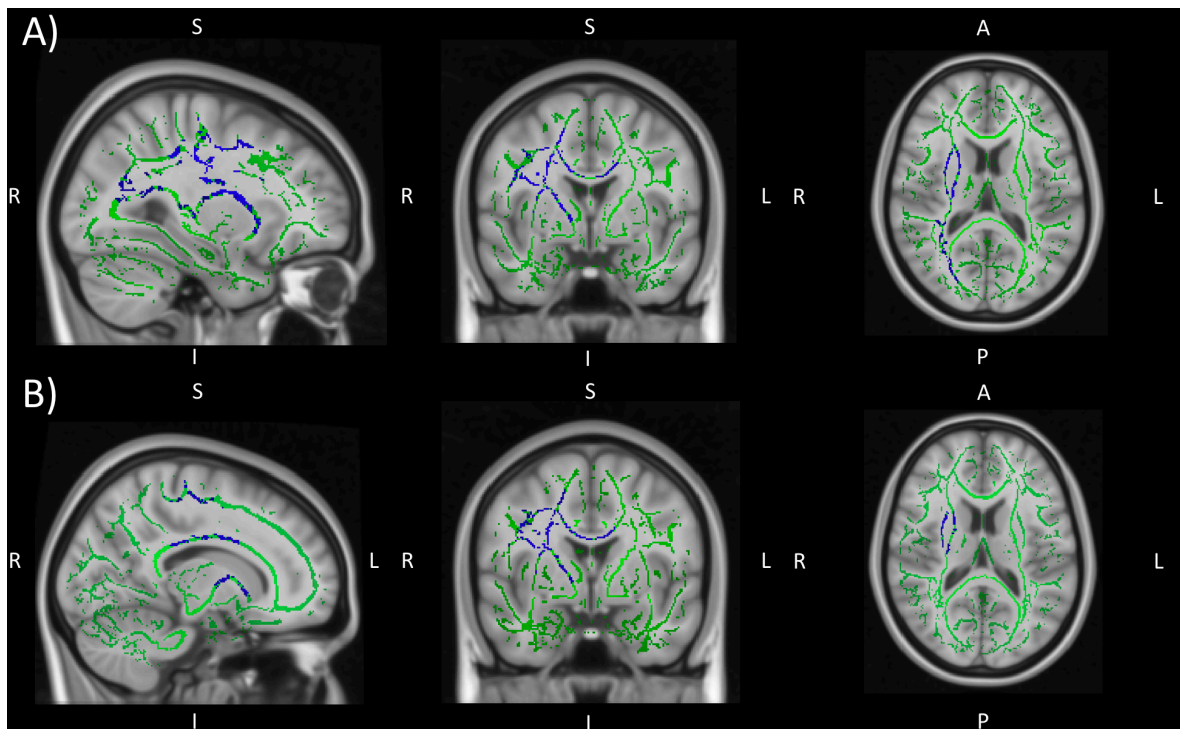
There were no significant differences in FA or MD between healthy controls and clinical PD (at baseline or the one-year follow-up) at the whole brain level or within the substantia nigra ROI.

**Q3. Microstructural differences between prodromal and clinical PD.** At the whole brain level, there were no significant differences in FA between prodromal PD and clinical PD (at baseline or the one-year follow-up). However, prodromal PD showed significantly increased MD than the PD group, both at baseline and one year later (see *Figure 6*). There were no significant differences in FA or MD between prodromal PD and clinical PD in the substantia nigra ROI.



*Figure 6.* Prodromal PD vs. clinical PD. From left to right: sagittal, coronal, and axial slices of the standard MNI\_152\_T1\_1mm with the mean FA skeleton (green) showing increased MD (blue) in prodromal PD relative to clinical PD at A) baseline, and B) one year later. Panel A shows increased MD in the corpus callosum, in the right limb of the internal and external capsule, right superior and inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus, right cortical spinal tract, right forceps major, right corona radiata, right tapetum and left posterior thalamic radiation. Panel B shows increased MD in the right corona radiata, right superior longitudinal fasciculus, corpus callosum, right cortical spinal tract, and right external and internal capsule ( $p < .05$ ).

**Post-hoc tests: Microstructural differences between RBD+ prodromal PD and clinical PD.** To determine if RBD could account for the group differences between prodromal and clinical PD, the whole-brain analyses were conducted a second time using only subjects with RBD confirmed by polysomnography in the prodromal group (RBD+ prodromal PD). Our results showed significant increases in MD in the RBD+ prodromal group relative to the clinical PD group, both at baseline and the one-year follow-up (see *Figure 7*).



*Figure 7.* RBD+ From left to right: sagittal, coronal, and axial slices of the standard MNI\_152\_T1\_1mm with the mean FA skeleton (green) showing increased MD (blue) in RBD+ prodromal PD subjects with clinical PD at A) baseline, and B) one-year later ( $p < .05$ ).

#### Q4. Microstructural differences in clinical PD from baseline to one-year

**later.** There were no significant differences in FA or MD between clinical PD at baseline and the one-year follow-up at the whole brain level or within the substantia nigra.

#### Results of Objective Two: How are sleep and motor symptoms related to white matter microstructure at different phases of PD?

Within each group, the relationship between sleep disorder symptoms (i.e., RBDSQ scores) and motor symptoms (i.e., UPDRS-III scores) and each DTI metric (i.e., FA and MD) and were examined separately while controlling for age. Higher scores on the behavioural measures indicate increasing severity of symptoms. The results of the within group analyses comparing FA and MD with sleep and motor symptoms are displayed in Table 6.

Table 6

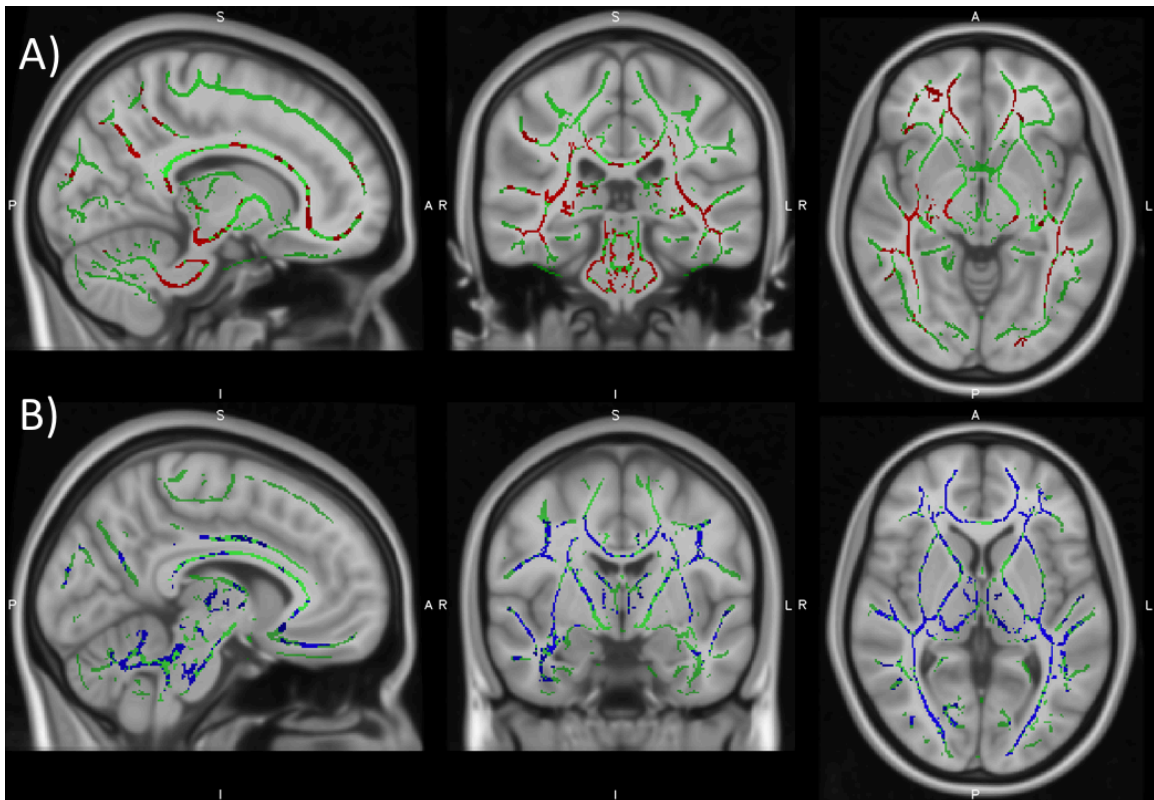
*Relationship Between DTI Metrics and Behavioural Symptoms*

Group	RBDSQ		UPDRS-III	
	FA	MD	FA	MD
HC	ns	ns	<b>Sig (-)</b>	<b>Sig (+)</b>
PPD	ns	ns	ns	ns
PD2	ns	<b>Sig (-)</b>	ns	ns

*Note.* RBD=rapid eye movement sleep behaviour disorder questionnaire score. Motor=Unified Parkinson's Disease Rating Scale Part III. HC=healthy control. PPD=prodromal PD. PD2=clinical PD at one-year follow-up. Sig=significant at  $p<.05$ . ns=non-significant. Decreased FA and increased MD indicate neurodegeneration. Higher RBDSQ and UPDRS-III scores reflect increased severity of sleep disorder and motor symptoms.

### Q5. Relationship between sleep disorder, motor symptoms, and DTI metrics

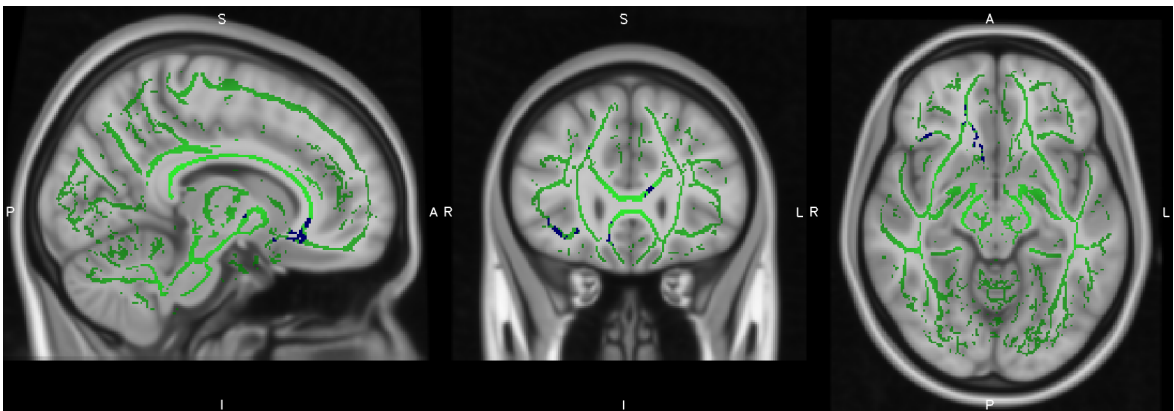
**in healthy controls.** For healthy controls, increased sleep disorder symptoms as measured by the UPDRS-III were not significantly associated with FA or MD. Increased motor symptoms as measured by the UPDRS-III was significantly associated with increased MD and decreased FA in the healthy control group. See *Figure 8*.



*Figure 8.* In healthy controls, a) decreased FA (red) and b) increased MD (blue) were associated with increased motor symptoms as measured by the UPDRS-III in the corpus callosum (genu, body, and splenium), left and right internal capsule, left and right cerebellar peduncle, right and left posterior thalamic radiation, left and right fornix, corona radiata, right and left corticospinal tract, pontine crossing tract, right and left sagittal stratum (including the inferior longitudinal fasciculi and inferior fronto-occipito fasciculi).

**Q6. Relationship between sleep disorder, motor symptoms, and DTI metrics in prodromal PD.** For prodromal PD, there were no significant relationships between sleep disorder symptoms or motor symptoms and FA or MD.

**Q7. Relationship between sleep disorder, motor symptoms, and DTI metrics in clinical PD.** For clinical PD, increased sleep disorder symptoms were associated with decreased MD in the genu of the corpus callosum, the right anterior corona radiata, the right posterior limb of the internal capsule, and the right superior longitudinal ( $p < .05$ , see *Figure 9*).



*Figure 9.* For clinical PD, decreased MD (blue) was significantly associated with increased RBDSQ scores (reflecting higher symptoms of RBD) in the genu of the corpus callosum, the right anterior corona radiata, the right posterior limb of the internal capsule, and the right superior longitudinal fasciculus (blue),  $p < .05$ . Results are displayed on the mean FA skeleton (green) projected onto the standard MNI\_152\_T1\_1mm structural MRI image.

A summary of the study objectives, research questions, and results are displayed below in Table 7.

Table 7  
*Study Objectives, Research Questions, and Results*

<b>Objective One: How does white matter microstructure change in different phases of PD progression?</b>		
Research Questions	Result	Brain Regions
1. What are the microstructural differences between healthy controls and prodromal PD?	No differences	NA
2. What are the microstructural differences between healthy controls and clinical PD?	No differences	NA
3. What are the microstructural differences between prodromal and clinical PD?	<b>Increased MD in prodromal vs. PD1 and PD2</b>	corpus callosum, right limb of the internal and external capsule, right superior and inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus, right cortical spinal tract, right forceps major, right corona radiata, right tapetum, left posterior thalamic radiation
4. What are the microstructural differences after one year of clinical PD?	No differences	NA
<b>Objective Two: How is white matter microstructure related to sleep and motor symptoms at different phases of PD?</b>		
5. What are the relationships between white matter microstructure and PD symptoms in healthy controls?	<b>Increased motor symptoms associated with decreased FA and increased MD</b>	corpus callosum; bilaterally in the internal capsule, cerebellar peduncle, posterior thalamic radiation, fornix, corona radiata, corticospinal tract, pontine crossing tract, right and left sagittal stratum (including the inferior longitudinal fasciculi and inferior fronto-occipito fasciculi).
	No relationship with sleep dx symptoms	
6. What are the relationships between white matter microstructure and PD symptoms in prodromal PD?	No relationship with sleep dx or motor symptoms	NA
7. What are the relationships between white matter microstructure and PD symptoms in clinical PD?	<b>Increased sleep dx symptoms associated with decreased MD</b>	genu of the corpus callosum, right anterior corona radiata, right posterior limb of the internal capsule, right superior longitudinal fasciculus
	No relationship with motor symptoms	

*Note.* In general, decreased FA and increased MD are associated with increased diffusion due to loss of physical barriers and are thought to represent neurodegeneration. However, interpretation of DTI metrics is variable and can depend on other factors.

## Discussion

The current study represents one of the first to compare both prodromal and clinical PD groups to healthy controls using DTI. There were two primary objectives: (1) to investigate how white matter microstructure differs in phases of PD progression, and (2) to investigate how sleep and motor symptoms relate to white matter microstructure in different phases of PD. It was hypothesized that decreased white matter integrity would be observed in the groups of higher expected disease state (i.e., increased FA and decreased MD in clinical PD vs prodromal PD). However, contrary to our hypothesis, we observed increased MD, a marker of neurodegeneration, in the prodromal PD group compared to clinical PD, both at baseline and one year later. Follow-up tests comparing RBD positive prodromal subjects to clinical PD also showed significantly increased MD. In the substantia nigra ROI, no differences in FA or MD were observed between any groups. It was also hypothesized that increasing motor and sleep disorder symptoms would be associated with increased neurodegeneration (i.e., decreased FA and increased MD). When examining the relationship between DTI metrics and symptoms of RBD as measured by the RBDSQ, a significant negative relationship between MD and RBD was found for the clinical PD group, with decreased MD being associated with increased RBD symptoms. Significant relationships between DTI metrics and motor symptom severity as measured by the UPDRS-III were found only for the healthy control group, in which decreased FA and increased MD were significantly associated with increased motor symptoms. These results and their context in the extant literature are discussed next.

## **White matter microstructural changes in PD progression**

### **Q1. Healthy controls vs. prodromal PD.**

Despite observing increased MD in prodromal PD versus clinical PD, there were no differences in white matter microstructure between prodromal PD and controls. To date, DTI studies comparing idiopathic RBD and controls have been mixed: some have also found no differences in FA or MD (Mangia et al., 2017; Rahayel et al., 2014); one found increased MD in mesencephalic tegmentum and decreased FA in the mesencephalic tegmentum, pontine tegmentum and formation reticularis (Scherfler et al., 2011); and another found significant increases in FA in the internal capsule and olfactory region and decreased FA in the fornix, the right visual stream, and the left superior temporal lobe (Unger et al., 2010). To date, there have not been any DTI studies of subjects with hyposmia versus controls to allow for comparison. To the author's knowledge, this is the first DTI study to include a mixed sample of prodromal PD subjects (with RBD and hyposmia). As such, these results are highly preliminary and more research using mixed prodromal PD groups are needed. The closest DTI study for comparison that examined similar groups found no group differences in FA or MD between controls, idiopathic RBD, and early PD (Mangia et al., 2017), while we found increased MD in the prodromal group relative to clinical PD in the corpus callosum, right limb of the internal and external capsule, right superior and inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus, right cortical spinal tract, right forceps major, right corona radiata, right tapetum.

Though the prodromal sample was largely RBD+, including a sample of mixed RBD/hyposmia was a conscious decision to be more representative of prodromal PD

presentation. Many studies have restricted their prodromal groups to include only those with idiopathic RBD as the eventual conversion to PD is high. However, new research criteria have emerged to help conceptualize prodromal PD to include several other risk factors such as hyposmia, depression, changes in cognition, and constipation (Berg et al., 2015). Further, utilizing RBD only prodromal samples may underrepresent women as RBD has a higher male to female ratio of 5:1, compared to the estimated 2:1 ratio in clinical PD (Barber, Klein, Mackay, & Hu, 2017). Future research must carefully consider inclusion and exclusion criteria for prodromal PD samples and ideally include longitudinal follow-up.

**Q2. Healthy controls vs. clinical PD.** Despite a recent meta-analysis showing overall microstructural differences between clinical PD and controls in several white matter tracts, we found no differences at the whole brain level or within the substantia nigra ROI (Atkinson-Clement, Pinto, Eusebio, & Coulon, 2017). However, many individual studies included in the meta-analysis also failed to find any significant differences between PD and controls. This raises an important question about the clinical utility of and replicability of DTI. Certainly, research using DTI has contributed to our understanding of PD pathology and progression; but clinically, DTI has not reliably discerned clinical PD from healthy controls, limiting its applications as a diagnostic neuroimaging biomarker for PD at this time. Nonetheless, some studies have found significant differences between PD and healthy control groups, suggesting that perhaps specific acquisition methods and data analyses techniques require further optimization. Indeed, heterogeneity in sample characteristics and imaging parameters across individual studies has been high as evidenced by the high heterogeneity indices of several meta-

analyses (see Table 2 in Chapter 1). The recent whole-brain meta-analysis identified several significant variables that influenced DTI metrics finding FA to be negatively correlated with field strength and MD to be negatively correlated with field strength, PD duration, number of DTI gradient directions, repetition time (i.e., TR), and echo time (i.e., TE). Future research should continue to investigate factors that influence DTI metrics with the goal to standardize and optimize acquisition methods for imaging in PD.

**Q3. Prodromal vs. clinical PD.** Our findings revealed significantly increased MD in the prodromal PD group relative to the clinical PD group. It is possible that the prodromal PD group represents a group with earlier, but more severe white matter degeneration – possibly due to RBD. Notably, the clinical PD group had relatively low rates of RBD that were unconfirmed, while the prodromal PD group was largely composed of polysomnography confirmed RBD (14 out of 20). Congruent with this, we found significantly higher RBD scores in the prodromal PD group compared to the PD group, which suggests that group differences could be due to RBD specifically. It is also possible that PD with RBD should be considered as a subtype of the PD, because clinically they have a more severe disease prognosis with higher probabilities of developing dementia and psychosis, a greater disease burden, and a higher risk of mortality (Kim et al., 2018).

To date, there have been few DTI studies that have compared PD with and without RBD. One study found decreased FA and increased MD in PD with RBD relative to PD without RBD, but these results became non-significant with statistical correction (Ford et al., 2013) and match other non-significant findings (García-Lorenzo et al., 2013). Another study using a different method of analysis called diffusion connectometry found

significantly reduced quantitative anisotropy (a metric similar to FA that measures the “compactness” of fibre bundles) in PD with RBD compared to PD without RBD bilaterally in the cingulum, inferior fronto-occipital fasciculi, corticospinal tract, and the body, genu, and splenium of the corpus callosum (Ansari, Rahmani, Dolatshahi, Pooyan, & Aarabi, 2017). The latter results are similar to ours in that subjects with RBD are displaying more significant white matter neurodegeneration, also within similar brain regions.

Our significant findings in follow-up tests of increased MD in RBD positive prodromal PD also supports the inference of poorer white matter microstructure in those with RBD relative to clinical PD. However, it is unknown at this time whether our subjects are truly prodromal of PD, or if they are stable, or prodromal for another alpha-synucleinopathy, such as multiple system atrophy or dementia with Lewy bodies, as RBD can also predate these diseases. As such, another possible interpretation of our findings is that the prodromal group may not necessarily have a poorer white matter microstructure, but prodromal PD may have a different pattern or profile of neurodegeneration than the clinical PD group (perhaps with some areas of overlap that would not lead to visualization of group differences). This is an area of research that requires further investigation, ideally with longitudinal rather than cross-sectional methods.

Of note, the areas of increased MD in prodromal PD relative to clinical PD were for the most part lateralized to the right side, with the exception of areas of increased within the corpus callosum. In clinical PD, motor symptoms typically emerge on one side of the body and findings from neuroimaging studies have found asymmetric neurodegeneration in brain regions that are contralateral to the side of motor symptoms

onset (Riederer et al., 2018). It is possible that the differences between prodromal and clinical PD are due to early asymmetric neurodegeneration or reorganization.

Unfortunately, we did not take note of side of motor symptom onset in our findings.

More research, that is longitudinal and takes lateralization of symptoms into account is needed to clarify the observed differences between prodromal and clinical PD.

**Q4. Clinical PD baseline vs. one-year later.** There were no differences in FA or MD between PD at baseline to one year later, at the whole brain level or within the substantia nigra ROI. Others have successfully monitored clinical PD progression over time and have observed significant increases in MD, RD, AD, and decreased in FA over three years (Guimarães et al., 2017), and have found MD to be a correlate of cognitive function and predictor of motor impairment in PD over 18 months (Minett et al., 2018). A study using PPMI data also found PD to be associated with higher rates of FA decreases and RD and AD increases in the substantia nigra, midbrain, and thalamus over one year (Zhang et al., 2016). However, in addition to having a much larger sample size, Zhang et al.'s method of analysis was different than ours in that they examined the group differences in rates of change in DTI metrics over time rather than group differences in DTI metrics at a single time point. Clinically, our PD group had a significant increase in motor symptoms as measured by the UPDRS-III from baseline to the one-year follow-up. Nonetheless, these findings stress the importance of ongoing longitudinal follow-up in order to characterize microstructural changes related to PD over time.

**Between group differences in the substantia nigra.** For the substantia nigra ROI, we found no difference among controls, prodromal PD, or clinical PD. Previous research focusing on the substantia nigra has been mixed; three meta-analyses supported

increased FA in PD compared to controls (Deng, Wang, Yang, Li, & Yu, 2018; Cochrane & Ebmeier, 2013; Hirata et al., 2017), while another revealed no significant differences in FA (Schwarz et al., 2013), which was similar to our results. Variability in technical aspects of individual studies, as well as large anatomical variability among ROIs may explain these mixed results. Currently, there is no standard method of delineating the substantia nigra. For example, our ROI consisted of the mean FA skeleton within the substantia nigra and as such it was specific to our sample characteristics and included a much smaller area than most other studies. It has also been suggested that age associated iron deposits in the basal ganglia may be exacerbated in PD and may cause alterations in FA that diminish observable differences in this area between PD and controls (Schwarz et al., 2013). Further, the substantia nigra is largely composed of grey matter structures which may make it difficult to measure using DTI. Perhaps more conclusive DTI findings in the substantia nigra ROI can be found as imaging acquisition parameters are optimized and ROI delineation becomes more standardized.

### **Relationship between white matter microstructure and sleep and motor symptoms at different phases of PD**

**Q 5-7.** Our findings revealed that FA and MD were significantly related to motor symptoms in healthy controls, but not within the prodromal or clinical PD groups. In healthy controls, decreased FA and increased MD were found bilaterally in the internal capsule, cerebellar peduncle, posterior thalamic radiation, fornix, corticospinal tract, sagittal stratum (including the inferior longitudinal fasciculi and inferior fronto-occipito fasciculi); and in the corpus callosum (genu, body, and splenium), the corona radiata, and

pontine crossing tract. The controls showed the expected relationship between DTI metrics and motor symptoms in areas related to motor function (i.e., fewer motor concerns were related to greater white matter integrity in motor tracts). However, contrary to hypotheses, DTI metrics in prodromal and clinical PD were unrelated to motor symptoms. It has been theorized that the brain may reorganize as it compensates for the loss of dopamine in early PD (Brotchie & Fitzer-Attas, 2009). Although this process is not yet fully understood, it could help to explain that lack of relationship in the prodromal and clinical PD groups. Given the variability in PD progression rates, it is possible that some individuals in our sample may be undergoing a reorganization process (which would likely be reflected in increased FA and decreased MD), while others may be in an attenuated compensation stage (i.e., decreased FA and increased MD). This variability in compensation patterns could “wash out” our ability to detect a group level relationship between DTI metrics and motor symptoms. Future research should examine the relationship between motor symptoms and DTI metrics in greater detail.

We also found a significant relationship with RBDSQ scores and MD in clinical PD, but there was no such relationship with RBDSQ scores and DTI metrics in healthy controls or in individuals with prodromal PD. For clinical PD, increased sleep disorder symptoms were associated with decreased MD in the in the genu of the corpus callosum, the right anterior corona radiata, the right posterior limb of the internal capsule, and the right superior longitudinal fasciculus in individuals with clinical PD. These latter results were surprising and initially suggest that more severe sleep disorder symptoms were associated with less neurodegeneration. However, there are a few alternative explanations for this unexpected finding. In clinical PD, it is possible that some of these tracts undergo

a reorganization or compensation process, especially since our sample was recently diagnosed and was still in the early stages of clinical PD. Other studies have found greater integrity in specific white matter networks in PD compared to controls related to compensation (Atkinson-Clement, Pinto, Eusebio, & Coulon, 2017). Another possibility may have to do with the RBDSQ questionnaire itself. The questionnaire may not be the most sensitive to increasing severity of RBD symptoms as it was designed to discriminate RBD positive and RBD negative individuals with a cut-off score (Stiasny-Kolster et al., 2015). This interpretation may also help explain the lack of findings in across healthy controls and prodromal PD.

### **Limitations**

Our study had several limitations. First, our study sample size was low, and we performed many groups comparisons, which though corrected for, may have limited statistical power. Further, DTI metrics have different sample size requirements to obtain certain statistical power. In a study comparing the number of subjects required for DTI metrics to obtain a statistical power of 0.9 at an effect size of 10% in specific white matter tracts, MD consistently required the smallest sample size of less than 20 subjects, while FA and several other metrics consistently required more than 20 subjects (De Santis, Drakesmith, Bells, Assaf, & Jones, 2014). This could explain why we found more differences in MD than in FA throughout our analyses. Nonetheless, our sample size is comparable to other neuroimaging studies that have found significant group differences.

Second, our results may be difficult to generalize to sample characteristics. The clinical PD group was mixed, with some having positive RBDSQ scores and others

having negative scores. Most other studies have created separate homogenous groups that are either RBD positive or RBD negative. The prodromal PD group in the current study, consisted largely of individuals with idiopathic RBD, but also included participants with hyposmia. Though this was done purposefully to better represent prodromal PD, it also increased heterogeneity in this group. We did conduct follow-up analyses using only RBD positive prodromal subjects, which lowered our sample size but helped to confirm our results of increased MD in RBD+ prodromal PD relative to clinical PD. However, as mentioned previously, segregating the prodromal group by symptoms may not aptly represent prodromal PD and could in fact act to mischaracterize it by eliminating pathology from specific symptoms. In fact, another limitation is that the prodromal group also did not include subjects with prodromal markers other than hyposmia and RBD. PD is a heterogeneous disease both in the prodromal and clinical phases and future research should carefully consider subject characteristics when conceptualizing prodromal PD. Further, it is crucial to include longitudinal follow-up to confirm the conversion rate to clinical PD.

Third, though we followed the recommended processing pipeline for tract-based spatial statistics, the method has several known limitations. These include possible anatomic inaccuracies, potential problems analyzing small tubular structures due to inter-subject differences in its location and crossing or kissing fibres that can alter DTI metrics (Bach et al., 2014). Further, the interpretation of DTI metrics is not as simple as “increased MD equals neurodegeneration” as increased in MD can also be attributed to other factors, such as changes in partial volume effects due to characteristics in fiber bundles such as thickness, orientation, and curvature (Vos, Jones, Viergever, & Leemans,

2011). Altered metrics could also be due the reorganizing of white matter pathways as the brain compensates for damage elsewhere. In spite of these limitations, the results of this study show preliminary microstructural differences in white matter between prodromal and clinical PD using DTI.

### **Conclusions and future directions**

Prodromal PD is thought to be a phase that predates the onset of motor symptoms but includes brain changes and nonmotor symptoms. This study represents a comprehensive assessment of microstructural changes in white matter in the prodromal and clinical phases of PD. It is also the first DTI study to examine microstructural differences in a mixed prodromal PD group with hyposmia and RBD. Though we were able to conduct post-hoc tests using only RBD subjects, we were unable to conduct post-hoc tests using only hyposmia subjects due to their limited number.

Characterizing prodromal PD is challenging. While most DTI studies of prodromal PD subjects include homogenous samples of RBD positive subjects, such restrictions may fail to capture the pathology of other the risk factors involved in prodromal PD such as hyposmia, changes in cognition, depression, and constipation. Further, including only RBD positive subjects may overestimate the neurodegeneration involved in prodromal PD as there is evidence that RBD in clinical PD may represent a more severe sub-type of the disease.

Areas of increased MD in prodromal PD relative to clinical PD were largely lateralized to the right side. Asymmetries in neurodegeneration in clinical PD has been observed before and tends to coincide with the onset of motor symptoms on the

contralateral side (Riederer et al., 2018). These findings are preliminary and should be further explored as this could have important implication for future prognosis and rate of disease progression, as well as for directing treatment options (e.g., deep brain stimulation).

In this study, the RBDSQ was utilized as a measure of increasing RBD symptom severity; however, given that it is possible to use this measure with a cut off score (rather than as a continuous variable), future studies could compare RBD+ and RBD- individuals directly. This approach may help to better characterize the differences in white matter microstructure due to RBD. However, polysomnography remains the gold standard for RBD diagnosis and should be utilized whenever possible.

Future studies should build on these preliminary results to clarify and expand upon the microstructural differences between prodromal PD and clinical PD. Specifically, future studies on prodromal PD could be approached in two ways. First, prodromal PD could continue to be studied using homogenous samples of subjects with a single prodromal symptom. RBD is an ideal group due to its high specificity for PD. However, to the author's knowledge no DTI study has examined a prodromal PD group composed of subjects with only hyposmia. This is most likely due to hyposmia having low specificity for clinical PD; however, with large, multisite longitudinal studies, like PPMI, it will be easier to identify subjects that are truly prodromal for PD. This could help to identify specific structural changes related to specific prodromal symptoms through retrospective analysis of individuals that convert to PD (i.e., RBD, hyposmia, changes in cognition, depression, and constipation). Second, prodromal PD could be studied using more comprehensive samples with a mix of prodromal symptoms which could be

specifically characterized using already established likelihood ratios to estimate the probability of conversion to clinical PD. Likelihood ratios for many nonmotor symptoms and clinical risk factors have already been published; but so far, have gone unused (Berg et al., 2015). Nonetheless, longitudinal follow-up is crucial for confirming truly prodromal PD subjects and studying PD progression.

In summary, this DTI study represents an initial step in determining the microstructural changes in white matter as Parkinson's disease progresses from the prodromal to clinical phases of the disease. Future research using the above recommendations will aid in our understanding of the underlying microstructural changes in early PD progression. Ultimately, the goal is to identify and optimize the clinic utility of early objective biomarkers for PD in the preclinical phase, prior to the onset of significant motor and nonmotor symptoms, so that preventative interventions can be delivered at the earliest time point.

### Chapter 3

This master's thesis represents one of the most comprehensive assessments of the underlying microstructural changes in white matter from the prodromal to clinical phases of PD, to date. Our results showed increased MD in prodromal PD relative to clinical PD, suggesting either poorer white matter microstructure in prodromal PD relative to clinical PD or a different pattern of white matter degeneration altogether. Although not yet fully understood, these changes were largely lateralized to white matter tracts in the right hemisphere. However, our results also showed no differences in white matter microstructure between prodromal or clinical PD and healthy controls. In addition, we found a relationship for DTI metrics and motor symptoms in healthy controls, where increased motor symptoms were associated with decreased FA and increased MD; and a relationship for DTI metrics and sleep disorder symptoms in clinical PD, where increased sleep disorder symptoms were associated with decreased FA. These findings are preliminary and follow-up studies are needed. A more in-depth discussion of the study limitations and future directions for research follows.

#### **Limitations**

This study was impacted by some of the limitations inherent to the PPMI database. First, there were limitations in the characterization of the prodromal group. Unfortunately, not all participants completed every measure, making it difficult to assess the true prevalence of nonmotor prodromal symptoms in this group. Ideally, the prodromal PD group would have been classified by the probability of conversion to clinical PD based on risk factors and prodromal markers which were outlined in a recent

study (i.e., RBD, hyposmia, constipation, depression, changes in cognition) (Berg et al., 2015). The study by Berg et al. provided likelihood ratios of developing PD based on nonmotor symptoms and other risk factors. The likelihood ratios are then subsequently used to estimate the probability of developing clinical PD. Future studies could utilize available data could be used to estimate the probability of developing clinical PD for each prodromal PD subject. This was an original aim of this thesis; unfortunately, missing data from the PPMI made it impossible to utilize this method. Missing data from PPMI also made it difficult to compare scores for some variables between prodromal and clinical PD groups. For example, only some participants had completed the University of Pennsylvania Smell Test, so it was difficult to characterized hyposmia in all study groups. Related to this, it is unclear if the prodromal PD group was truly prodromal for PD. PPMI included subjects who either had RBD or hyposmia in its prodromal group. However, these markers can also predate other synucleinopathies like multiple system atrophy and dementia with Lewy bodies. Fortunately, data collection for PPMI is ongoing and longitudinal follow-up could help confirm conversion to clinical PD or related disorder.

Second, despite PPMI being a large multisite study, our sample size for the prodromal group was limited to 20 out of 65 subjects that had DTI data collected. Though DTI can find group differences with small samples, different DTI metrics require different minimum sample sizes to obtain the same statistical power. For example, MD can find group differences in samples smaller than 20 while retaining a statistical power of 0.9 at an effect size of 10%, while FA requires a sample larger than 20 to retain the

same statistical power (De Santis, Drakesmith, Bells, Assaf, & Jones, 2014). Increasing the samples size could help to clarify our findings.

Third, when using the PPMI database, researchers are limited to the measures that were chosen by the original study authors and the specific time points they were implemented. In the current study, the RBDSQ was used as a measure of sleep disorder symptoms severity. However, the questionnaire was originally designed as a screener for RBD and diagnosis of RBD was made when total scores were equal to or greater than 5. It is possible that scores beyond the cut-off on the RBDSQ are not particularly sensitive to increases in symptom severity. Another measure that may be a useful addition to the PPMI study is the RBD Severity Scale which can be completed in conjunction with a polysomnography and provides information about the severity of RBD symptoms (Sixel-Döring, Schweitzer, Mollenhauer, & Trenkwalder, 2011).

Despite these limitations, the PPMI database is a large, multisite, study that provides comprehensive and standardized biological, behavioural, and neuroimaging, on clinical PD and related cohorts (e.g., healthy controls, prodromal PD, genetic variants of PD). The database provides this data to researchers all over the world to collaboratively work towards biomarkers for PD. The study is comprehensive and includes data for biological (e.g., blood, saliva, cerebrospinal fluid), behavioural (e.g., cognitive, emotional, motor, sleep), and neuroimaging markers (e.g., MRI, DTI, fMRI). The study is especially advantageous for neuroimaging research. Due to cost, neuroimaging research is often plagued with low sample sizes which often results in research literature filled with multiple studies of varying acquisition parameters that may be difficult to compare. With PPMI, neuroimaging parameters are standardized and collected in large

numbers which increases statistical power to draw conclusions. Importantly, the study has already collected data for hundreds of participants and data collection will continue longitudinally. In particular, the inclusion of the prodromal PD cohort may aid in the identification of the earliest biomarkers for PD, which are crucial for diagnosis, tracking treatment progression, and development of effective treatments.

### **Does DTI Have Potential as a Biomarker for PD?**

In this study, we found no differences between healthy controls and prodromal or clinical PD. These findings add to a literature of inconsistent results that show that DTI cannot reliably distinguish clinical PD from controls. As such, its clinical use on an individual level to diagnose PD is limited. However, that does not mean that DTI is useless. Research using DTI has provided useful information about PD pathology and progression that helps to direct future research. Further, the inconsistency in findings may be due to the wide variety of image acquisition and data analysis methods. Reviewing previous work may provide information on how to best optimize DTI for clinical applications on an individual level. DTI has revealed several regions of interest that show an overall change due to PD. Though DTI studies have been somewhat inconsistent, results from such studies still provide valuable information, especially when examined in conjunction with behavioural symptoms of the disease. Additionally, biomarkers are needed for several different purposes (i.e., diagnosis, tracking progression) and it is unlikely that a single measure used in isolation will fulfill all purposes. It is more likely that a multimodal approach that takes multiple sources of information into account will be needed for clinical applications. Thankfully, many different biomarkers are currently

under investigation through the PPMI including biological (e.g., blood, saliva, cerebrospinal fluid), behavioural (e.g., cognitive, emotional, motor, sleep), and neuroimaging markers (e.g., MRI, DTI, fMRI). Further, PPMI employs a longitudinal design which will complement findings from previous cross-sectional studies.

### **Future Directions**

There are several avenues of future research prompted by the results from this thesis. This was the first DTI study to examine a mixed prodromal PD group with RBD and hyposmia. Future research should aim to further characterize and include mixed prodromal PD groups in comparison to healthy controls and clinical PD. It was an initial goal in this study to specifically characterize the prodromal PD cohort from PPMI to determine a specific probability of progression to clinical PD for each participant as proposed in a recent paper (Berg et al., 2015). Unfortunately, not every participant completed every measure, so it was difficult to accomplish this task. However, future prospective studies could attempt to characterize this or develop a screening questionnaire to establish probability of conversion to clinical PD. Future research must carefully balance the need for homogenous samples with the need to accurately represent prodromal PD pathology. Longitudinal follow-up is crucial for identifying truly prodromal PD populations.

The differences in MD between prodromal PD and clinical PD were seemingly lateralized to the right hemisphere of the brain. Asymmetry in neurodegeneration in PD has been observed before and coincides with the onset of motor symptoms on the contralateral side (Riederer et al., 2018). Clarifying the nature of these differences is

important for understanding the origins of PD and its progression. Future studies should consider the side of symptom onset and asymmetries in neuroimaging findings.

In this study, we examined DTI metrics with sleep disorder and motor symptoms within healthy controls, prodromal PD, and clinical PD. Future research should examine the relationship between DTI metrics and other variables of interest that are related to nonmotor symptoms such as cognitive changes (e.g., executive function, attention, memory), psychological symptoms (e.g., depression, anxiety), hyposmia, and constipation. Further, these variables should also be examined with each other. For example, RBD is a large risk factor for cognitive impairment (Jozwiak et al., 2017), but is this the first prodromal sign of PD? Does RBD contribute to cognitive and psychological impairment or are these factors caused by something else? Clarifying the relationship between DTI metrics and symptoms of PD could help us to better understand PD pathology and where to direct possible treatment strategies.

## **Conclusion**

In conclusion, this study aimed to investigate (1) how white matter microstructure changes with PD progression, and (2) how sleep and motor symptoms related to white matter microstructure at different phases of PD. Findings showed differences in white matter microstructure between prodromal and clinical Parkinson's disease. These findings represent an important step towards neuroimaging biomarkers in an earlier phase of PD. Future research should continue to investigate neuroimaging markers in the prodromal phase of PD with the goal for early biomarker identification and treatment. Behavioural symptoms, such as cognitive changes and emotional symptoms should also

continue to be investigated in conjunction with DTI metrics. Ultimately, our final goal remains to identify and optimize the clinical utility of early objective biomarkers for PD. Ideally these biomarkers will be implemented in the preclinical phase, prior to the onset of significant motor and nonmotor symptoms, so that preventative interventions can be delivered at the earliest time point to stop the progression of PD from ever beginning.

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