Journal of the International Neuropsychological Society (2016), **22**, 956–967.

doi:10.1017/S1355617716000503

Biomarkers in Prodromal Parkinson Disease: a Qualitative Review

Christine A. Cooper, AND Lama M. Chahine

Parkinson's Disease and Movement Disorders Center, Department of Neurology, University of Pennsylvania, Philadelphia, Pennsylvania

(RECEIVED January 18, 2016; FINAL REVISION March 23, 2016; ACCEPTED May 4, 2016)

Abstract

Background: Over the past several years, the concept of prodromal Parkinson disease (PD) has been increasingly recognized. This term refers to individuals who do not fulfill motor diagnostic criteria for PD, but who have clinical, genetic, or biomarker characteristics suggesting risk of developing PD in the future. Clinical diagnosis of prodromal PD has low specificity, prompting the need for objective biomarkers with higher specificity. In this qualitative review, we discuss objectively defined putative biomarkers for PD and prodromal PD. **Methods:** We searched Pubmed and Embase for articles pertaining to objective biomarkers for PD and their application in prodromal cohorts. Articles were selected based on relevance and methodology. **Key Findings:** Objective biomarkers of demonstrated utility in prodromal PD include ligand-based imaging and transcranial sonography. Development of serum, cerebrospinal fluid, and tissue-based biomarkers is underway, but their application in prodromal PD has yet to meaningfully occur. Combining objective biomarkers with clinical or genetic prodromal features increases the sensitivity and specificity for identifying prodromal PD. **Conclusions:** Several objective biomarkers for prodromal PD show promise but require further study, including their application to and validation in prodromal cohorts followed longitudinally. Accurate identification of prodromal PD will likely require a multimodal approach. (*JINS*, 2016, *22*, 956–967)

Keywords: Pre-clinical, Parkinsonism, Risk, Incipient neurodegeneration, Lewy body disorders, Alpha-synuclein

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder that has traditionally been defined clinically, based on the presence of specific motor deficits. However, motor symptoms develop at a stage of advanced neuronal loss, when there is approximately 60–80% striatal dopaminergic denervation (Bernheimer, Birkmayer, Hornykiewicz, Jellinger, & Seitelberger, 1973; Fearnley & Lees, 1991). Based on the latter finding, combined with clinical and biomarker data, it is evident that PD pathology begins long before onset of the motor symptoms that constitute the diagnostic criteria for PD (Hughes, Daniel, Kilford, & Lees, 1992).

In 2003, Braak and colleagues proposed a pathologic staging system for PD based on observations that Lewy body pathology appears to progress in a caudal to rostral direction (Braak et al., 2003). This hypothesis accounts for the well-documented non-motor symptoms that appear years before motor symptoms. These observations, along with identification of genetic mutations with varying degrees of penetrance that may cause or increase risk of PD, have led to the emergence of the concept of prodromal PD. The term prodromal PD encompasses individuals who do not fulfill motor diagnostic criteria for PD (Hughes et al., 1992), but who have characteristics suggesting that they are at risk of developing PD in the future. The goals of identifying such individuals are to inform counseling, and so that therapies to halt or slow neurodegeneration can be instituted early on, when they become available.

Since the inception of the idea of prodromal PD almost a decade ago (Siderowf & Stern, 2008; Stephenson, Siderowf, & Stern, 2009), significant progress has been made in defining this concept. Intensive work has focused on non-motor symptoms seen in the prodromal state (Table 1). These have been reviewed elsewhere in detail (Berg et al., 2015; Chahine et al., 2015; Postuma et al., 2012), and include hyposmia (Gaenslen et al., 2014; Liepelt et al., 2011; Noyce et al., 2014; Ponsen, Stoffers, Twisk, Wolters, & Berendse, 2009; Ponsen, Stoffers, Wolters, Booij, & Berendse, 2010; Ross, Abbott, Petrovitch, Tanner, & White, 2012), constipation (Gaenslen, Swid, Liepelt-Scarfone, Godau, & Berg, 2011; Postuma, Gagnon, Pelletier, & Montplaisir, 2013;

Correspondence and reprint requests to: Lama Chahine, Penn Neurological Institute, 330 S. 9th Street, Philadelphia, PA 19107. E-mail: lama.chahine@uphs.upenn.edu

Table 1. Non-motor symptoms in prodromal PD

Non-motor symptoms in prodromal PD
Hyposmia
Constipation
REM sleep behavior disorder
Excessive daytime sleepiness
Autonomic symptoms
Depression

Note. Reviewed in Berg et al., 2015; Chahine et al., 2015; Postuma et al., 2012.

Ross et al., 2012), rapid eye movement sleep behavior disorder (RBD) (Boeve et al., 2013; Gaenslen et al., 2011, 2014; Iranzo et al., 2014; Noyce et al., 2014; Postuma et al., 2009; Schenck, Boeve, & Mahowald, 2013), excessive daytime sleepiness (EDS) (Ross et al., 2012), autonomic symptoms (Gaenslen et al., 2011; Postuma et al., 2013), and depression (Gaenslen et al., 2011, 2014).

Non-motor signs and symptoms constitute a key part of the first formal criteria proposed by the Movement Disorders Society task force for definition of prodromal PD (Berg et al., 2015). These criteria also incorporate environmental and genetic factors that are known to contribute to PD risk (Siderowf & Lang, 2012; Siderowf & Stern, 2008; Stern & Siderowf, 2010). While these aspects of prodromal PD are of great importance, two major limitations for their use in isolation for the detection of prodromal PD are recognized. First, clinical findings and environmental exposures lack specificity when applied in isolation to identify prodromal PD. For example, constipation and hyposmia are ubiquitous, particularly among older adults, and in only a subset of individuals do they portend PD. Second, while there are well-validated measures of PD motor and non-motor manifestations that are applicable to the prodromal PD state, many of these are subjective and/or operator-dependent. For example, assigning numeric values on a scale to physical examination findings *via* direct observation by the examiner entails a substantial subjective component that can be reduced but not eliminated with proper training on scale administration. Therein lies the need for more specific and objective biomarkers that, when combined with clinical findings, genotype, and environmental exposures, maximize accurate detection of individuals with prodromal PD.

While the concept of prodromal PD is in its infancy, several promising objective biomarkers for prodromal PD have emerged. The majority of these were first identified and have been most extensively studied in the clinically diagnosed PD population. Data on their utility in prodromal PD is emerging from their application in longitudinal observational studies of prodromal cohorts. In light of recent developments, the aim of this review is to discuss the objectively defined components of the MDS criteria for prodromal PD (Berg et al., 2015), namely imaging and biospecimen biomarkers. We also illustrate the utility of such biomarkers in the context of clinically and/or genetically defined prodromal traits (Table 2).

METHODS

Articles pertaining to two main areas were identified: (i) objective components of the prodromal PD criteria (imaging and biospecimens), and (ii) application of objective PD biomarkers to clinically or genetically defined prodromal PD cohorts. Articles for this qualitative review were identified from two main sources: (i) keyword-based searches of Pubmed and Embase databases, with selection of articles based on relevance, and (ii) key citations included within relevant articles were also selected and reviewed. Key words used included several different combinations of the terms "Parkinson", "prodromal", "biomarker", and "premotor".

Studies investigating objective (e.g., imaging or biospecimens) markers for potential PD or prodromal PD diagnosis in humans were included. Studies investigating clinical symptoms or exam findings were excluded in light of the working definition of an objective marker as applied in this review. Other exclusion criteria were non-English language publications and studies strictly reporting on non-human data. Where available, we included results of meta-analyses examining the utility of the biomarkers in question, particularly meta-analyses that shed light on potential biomarkers that have yielded conflicting results in different studies, given the ability of well-done meta-analyses to synthesize data from conflicting reports to inform broader conclusions on existing evidence.

RESULTS

Imaging

Radionucleotide imaging

Loss of dopaminergic cells in the substantia nigra, with resulting dopaminergic denervation of the striatum, is the hallmark of PD and is known to occur early in the pathologic process (Bernheimer et al., 1973; Hughes et al., 1992). Imaging to capture the integrity of the striatal dopamine system thus has strong potential and biological plausibility in identifying prodromal PD. Various radionucleotide ligands have been applied. One of the most widely studied and available is an iodinated ligand of the dopamine transporter (DAT), a presynaptic membrane protein. DAT binding is decreased in PD due to degeneration of the presynaptic dopaminergic projections from the substantia nigra compacta (SNc) to the striatum.

Using single-photon emission computerized tomography (SPECT) imaging, radionucleotide ligand binding to DAT in PD patients was found to be decreased and correlated with severity of motor impairment (Huang et al., 2001; Seibyl et al., 1995, 1998). Cognitive impairment and behavioral symptoms (psychosis and depression) also correlated with decreased DAT SPECT binding in PD patients (Ravina et al., 2012). The sensitivity of DAT SPECT for PD diagnosis in a group of subjects with a parkinsonian syndrome is estimated to be 92% in comparison to a gold standard of movement disorder expert

https://doi.org/10.1017/S1355617716000503 Published online by Cambridge University Press

Table 2. Putative prodromal PD biomarkers

Biomarker	Tested in prodromal cohort? (yes/no)	Type of prodromal cohort tested (reference):	PD cohort (references)
Imaging			
DAT SPECT	Y	RBD (Iranzo et al., 2010); hyposmic (Ponsen et al. 2009); PARS (Jennings et al., 2014).	(Huang et al., 2001; Jennings et al., 2004; Seibyl et al.; 1995, Seibyl et al., 1998)
FDOPA-PET	Y	RBD with depression (Wing et al., 2015), parkin carriers (Walter et al., 2004), PINK1 carriers (Khan et al., 2002)	(Wing et al., 2015)
DBTZ-PET	Y	RBD (Albin et al., 2000)	
FDG-PET	Y	RBD (Wu et al., 2014), SNCA carriers (Nishioka et al., 2004)	(Eidelberg et al., 1994; Wu et al., 2014)
MRI - neuromelanin sequence	N		(Ohtsuka et al., 2013; Sasaki et al., 2006)
MRI - DTI	Y	RBD (Scherfler et al., 2011)	(Cochrane and Ebmeier, 2013)
MRI - DKI	N		(Wang et al., 2011)
Transcranial sonography	Y	RBD (Iranzo et al., 2010), hyposmia (Berg et al., 2013; Tunc et al., 2015), G2019S LRRK2 carriers (Bruggemann et al., (2011)), parkin carriers (Walter et al., 2004)	(Berg et al., 1999; Berg et al., 2001; Berg et al., 2013; Tunc et al., 2015)
Cardiac scintography	Y	RBD (Kashihara et al., 2010; Miyamoto et al., 2006)	(Orimo et al., 2005; Quattrone et al., 2008; Sawada et al., 2009)
Blood			
Alpha-synuclein	N		(El-Agnaf et al., 2006; Foulds et al., 2011; Malek et al., 2014; Wang et al., 2015)
DJ-1	N		(Lin et al., 2012; Shi et al., 2010; Waragai et al., 2007)
Serum uric acid	Y	G2019S LRRK2 carriers (Johansen et al., 2009)	(Asherio et al., 2009; Davis et al., 1996; de Lau et al., 2005; Moccia et al., 2014; Moccia et al., 2015; Schwarzschild et al., 2008; Weisskopf et al., 2007)
Serum APOE A1	Y	PARS (Qiang et al., 2013)	(Qiang et al., 2013)
Inflammatory markers	N		(Brodacki et al., 2008; Chahine et al., 2013; Chen et al., 2008; Dursun et al., 2015)
Metabolomics	N		(Ahmed et al., 2009; Bogdanov et al., 2008)
gene expression patterns	Y	G2019S LRRK2 (Chikina et al., 2015)	(Chikina et al., 2015; Scherzer et al., 2007)
CSF			
Alpha-synuclein	N		(Gao et al., 2015; Mollenhauer et al., 2015; Zhou et al., 2015)
DJ-1	N		(Herbert et al., 2014; Hong et al., 2010)
tau (total and phosphorylated)	N		(Jimenez-Jimenez et al., 2014; Kang et al., 2013)
NFL	N		(Constantinescu et al., 2010; Hall et al., 2012; Sako et al., 2015)
ccf-mtDNA	N		(Pyle et al., 2015)
Nrf2	N		(Loeffler et al., 2015)
dopamine and serotonin metabolites	N		(Jimenez-Jimenez et al., 2014)
NMR metabolomics	N		(Ohman et al., 2015)
Tissue			
Intestinal alpha-synuclein	Y	RBD (Sprenger et al., 2015)	(Braak et al., 2006; Gold et al., 2013; Lebouvier et al., 2008; Pouclet et al., 2012; Shannon et al., 2012; Visanji et al., 2014; Visanji et al., 2015)
Skin alpha-synuclein	N		(Donadio et al., 2014; Rodriguez-Leyva et al., 2014)
Salivary gland alpha-synuclein	N		(Beach et al., 2013; Folgoas et al., 2013; Tredici et al., 2010)

diagnosis at 6-month follow-up (Jennings et al., 2004). However, while decreased DAT SPECT binding is highly specific for a neurodegenerative parkinsonian disorder, it does not reliably distinguish between the various neurodegenerative parkinsonian disorders [e.g., PD, multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and dementia with Lewy bodies (DLB)].

Decreased DAT binding on imaging has been demonstrated in several clinically defined prodromal cohorts and longitudinal studies, suggesting that it indicates a high likelihood of developing PD motor symptoms. For example, in a cohort of RBD patients without a diagnosable neurodegenerative parkinsonian disorder at baseline, 40% (17 of 43) showed decreased striatal DAT binding (Iranzo et al., 2010). After an average follow-up of 21 months, 8 of 43 RBD patients developed a parkinsonian neurodegenerative disorder (PD or DLB), with 6 of these 8 having had decreased DAT binding at baseline testing (Iranzo et al., 2010). In another study, first-degree asymptomatic relatives of PD patients underwent smell testing and DAT SPECT imaging. Decreased DAT binding was demonstrated in a greater number of hyposmic *versus* normosmic individuals (Ponsen et al., 2010). Among the 40 hyposmic individuals, 5 developed PD on 5-year follow-up; all 5 had decreased DAT binding on baseline imaging (Ponsen et al., 2010).

In another cohort of individuals with hyposmia and first-degree relatives with PD, the PARS cohort, 11% of hyposmic individuals showed decreased DAT binding in comparison to 1% of normosmic individuals (Jennings et al., 2014). When additional clinical features, namely male sex and constipation, accompanied hyposmia in this cohort, 43% of this group showed a DAT deficit (Jennings et al., 2014). These studies highlight the utility of DAT imaging in identifying prodromal PD, particularly in combination with clinical data such as RBD or hyposmia.

PD-related changes in cerebral metabolism due to striatonigral denervation can also be imaged with fluorodopa (^{18}F-DOPA) positron emission tomography (PET). Similar to studies performed with SPECT, ^{18}F-DOPA PET imaging identifies decreased ^{18}F-DOPA uptake in the caudate and putamen of PD patients. This has also been demonstrated in individuals with features of prodromal PD, namely RBD and depression, in comparison to healthy controls (Wing et al., 2015). Similarly, using the ligand [^{11}C]dihydrotetrabenazine, which binds to vesicular monoamine transporter 2, PET showed decreased striatal binding in a group of subjects with RBD in comparison to healthy controls (Albin et al., 2000).

Preliminary studies of PET imaging in genetically defined prodromal individuals suggests this imaging modality may be of utility in this group as well. For example, in a study of seven asymptomatic *parkin* mutation carriers and seven PD patients with *parkin* mutation, ^{18}F-DOPA PET was abnormal in three of the asymptomatic *parkin* carriers (compared to all of the manifesting *parkin* PD patients) (Walter et al., 2004). Similarly, asymptomatic carriers of *PINK1* mutations show decreased uptake of ^{18}F-DOPA PET in comparison to controls (Khan et al., 2002). Longitudinal follow-up is needed to clarify the predictive value of baseline PET imaging, alone or in combination with other prodromal markers, in individuals genetically predisposed to PD, particularly among those with mutations that have incomplete penetrance.

^{18}F-fluorodeoxyglucose (^{18}FDG) PET imaging can be used to identify metabolic network patterns. In PD, ^{18}FDG PET shows increased lentiform nucleus and thalamic metabolic activity, with decreased lateral frontal, paracentral, inferior parietal, and parieto-occipital activity. This pattern discriminates early stage symptomatic PD patients from healthy controls (Eidelberg et al., 1994). In a study comparing individuals with RBD, PD, and healthy controls, the PD-associated metabolic network activity was increased in the RBD patients in comparison to the controls (Wu et al., 2014). In contrast, in a study of asymptomatic carriers of *SNCA* duplication (an established genetic cause of PD with incomplete penetrance) (Nishioka et al., 2006), no abnormalities in smell or changes in occipital lobe metabolism on ^{18}FDG PET were found (Nishioka et al., 2009), illustrating the importance of considering the reduced penetrance and phenotypic heterogeneity of mutations associated with PD, particularly in the prodromal phase.

Magnetic resonance imaging

While conventional clinical magnetic resonance imaging (MRI) sequences are not of utility in detecting prodromal PD, several more advanced MRI techniques show promise in this regard. A neuromelanin-sensitive MRI sequence on 3.0 Tesla MRI was designed to visualize neuromelanin-containing nuclei with greater detail. In PD, this imaging technique shows attenuation in the lateral SNc and locus coeruleus (LC) (Ohtsuka et al., 2013; Sasaki et al., 2006). This attenuation discriminated early (median duration 1.5 years, Hoehn & Yahr stage 2) and late PD (median duration 12 years, Hoehn & Yahr stage 4) from healthy controls, but did not differ between the early and late PD groups (Ohtsuka et al., 2013). The sensitivity and specificity for discriminating early PD from healthy controls was 73% and 87% in the lateral SNc, and 82% and 90% in the LC (Ohtsuka et al., 2013). The higher sensitivity and specificity of attenuation in the LC compared to the SNc fits with Braak's PD staging, in which LC involvement defines Braak stage 2, and SNc involvement defines Braak stage 3 (Braak et al., 2003). This modality has yet to be studied in prodromal PD, to our knowledge.

Another MRI modality, diffusion tensor imaging (DTI) has shown some potential in differentiating healthy controls from PD, with lower fractional anisotropy in the substantia nigra being the most consistent finding according to a recent meta-analysis (Cochrane & Ebmeier, 2013). In regards to application in prodromal cohorts, DTI in individuals with RBD in comparison to healthy controls shows changes in brainstem areas relevant to REM sleep, but not the substantia nigra, in a cross-sectional analysis (Scherfler et al., 2011). MRI with diffusion kurtosis imaging (DKI) can discriminate subjects with PD from healthy controls with higher

https://doi.org/10.1017/S1355617716000503 Published online by Cambridge University Press

sensitivity and specificity than DTI (Wang et al., 2011). While promising, the utility of MRI in prodromal PD remains to be defined.

Ultrasound

Transcranial sonography (TCS) at the temporal bone windows allows for assessment of abnormal intracranial iron deposition. This is of relevance in PD as increased iron deposition is seen in the substantia nigra in PD compared to HC (Sofic et al., 1988), and is involved in PD pathophysiology (Faucheux et al., 2003). Hyperechogenicity in the substantia nigra correlates with higher iron levels on postmortem studies (Berg et al., 2002). The prevalence of substantia nigra hyperechogenicity in PD patients is approximately 90% (Berg, Siefker, & Becker, 2001), compared to 9–19% (Berg et al., 1999, 2013) in community-dwelling older adults without PD.

TCS has been applied in both genetic and clinical prodromal cohorts and shows great promise as an imaging biomarker for the prodromal PD state. In a study of TCS in subjects with PD and healthy controls, the subset of controls with substantia nigra hyperechogenicity were also found to have decreased dopamine binding on ^{8}F-DOPA PET, suggesting they have increased risk of developing PD symptoms in the future (Berg et al., 1999). Substantia nigra hyperechogenicity on TCS in a community sample of older adults without PD has been found to have a sensitivity and specificity of 80% and 81% for development of PD over 3 years (Berg et al., 2013). In this same cohort, if both hyposmia and a family history of PD are present, sensitivity and specificity of substantia nigra hyperechogenicity for development of PD over 3 years increases to 80% and 91% (Berg et al., 2013).

In a study of RBD patients without parkinsonism, substantia nigra hyperechogenicity was found in 36% (14 of 30) of individuals with RBD, compared to 11% (16/149) of healthy controls (Iranzo et al., 2010). Five of the 14 individuals with RBD and substantia nigra hyperechogenicity went on to develop parkinsonism at 21 months follow-up (Iranzo et al., 2010). This study found combining substantia nigra hyperechogenicity on TCS and decreased DAT SPECT binding yielded a combined sensitivity of 100% and specificity 55% in identifying individuals with RBD who later developed parkinsonism (Iranzo et al., 2010).

The utility of combining easily obtained, low cost biomarkers with more specific and yet more logistically demanding ones was demonstrated in a population-based study that incorporated TCS. Four groups were identified: (i) idiopathic PD, (ii) presence of parkinsonian signs possibly due to a neurodegenerative parkinsonism, (iii) presence of non-specific motor abnormalities presumed to be due to non-neurologic etiologies (e.g., arthritis), and (iv) healthy controls (Tunc et al., 2015). Hyperechogenicity in the substantia nigra on TCS had a sensitivity of 76.6% and a specificity of 86.5% for PD diagnosis (Tunc et al., 2015). In comparison, hyposmia had a sensitivity of 68.1% and a specificity of 74.9% for PD diagnosis (Tunc et al., 2015). The combination of both hyperechogenicity in the substantia nigra and hyposmia yielded an excellent specificity of 97.7%, but sensitivity was reduced to 51.1% (Tunc et al., 2015).

With regard to application in cohorts with incompletely penetrant genetic mutations that cause PD, substantia nigra hyperechogenicity is not different between idiopathic PD and G2019S *LRRK2* PD patients; asymptomatic *LRRK2* carriers have less substantia nigra hyperechogenicity compared to PD patients, but more substantia nigra hyperechogenicity than controls (Brüggemann et al., 2011). Similarly, in a study of seven asymptomatic *parkin* mutation carriers and seven PD patients with *parkin* mutation, substantia nigra hyperechogenicity was found in all PD patients with *parkin* mutation and five parkin carriers (Walter et al., 2004).

Cardiac scintography

Cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigram is a nuclear imaging technique used to assess postganglionic sympathetic cardiac innervation. In PD, cardiac denervation due to vagal nerve nucleus involvement is expected to occur early, at Braak's stage 1 (Braak et al., 2003). The primary assessment of a cardiac scintigram is the ratio of heart-to mediastinum (H/M) MIBG accumulation at early (20 min) and late (4 hr) intervals. In PD, the sensitivity and specificity for early MIBG scintigram ratios is 81% and 85%; for late ratios it is 84% and 90% (Sawada et al., 2009). In early PD, defined in this study as disease duration less than 3 years, sensitivity decreases to 76% and 74%, respectively, for early and late H/M accumulation (Sawada et al., 2009).

The utility of cardiac MIBG scintography in prodromal PD is not fully defined. MIBG uptake was reduced in a group of individuals with RBD compared to controls (Kashihara, Imamura, & Shinya, 2010; Miyamoto et al., 2006). Application of MIBG in other prodromal groups has not been reported, to our knowledge. Of note, in patients with PD due to known genetic causes (*parkin,* DJ-1, *PINK1,* and G2019S *LRRK2* gene mutation), cardiac MIBG scintigram often shows preserved MIBG accumulation ratios (Orimo et al., 2005; Quattrone et al., 2008). If similar results are seen in asymptomatic carriers of these mutations, this would suggest that cardiac MIBG scintigram may not be useful in genetically defined prodromal PD.

Biospecimens

Blood

Given that abnormal alpha-synuclein accumulation is a primary component of PD pathology, body fluid alpha-synuclein has long been sought after as a potential biomarker candidate. In the blood, the ratio of red blood cell (RBC) oligomeric/total alpha-synuclein is higher in PD patients than controls with a sensitivity of 79% and specificity of 65% (Wang, Yu, Li, & Feng, 2015). Elevated plasma alpha-synuclein oligomer levels have been found in PD patients compared to controls with a sensitivity of 53% and

https://doi.org/10.1017/S1355617716000503 Published online by Cambridge University Press

specificity 86% (El-Agnaf et al., 2006). However, the same group was later unable to replicate these results for total or oligomeric alpha-synuclein, normal or phosphorylated (Foulds et al., 2011). It is apparent from their work and others that the strain of alpha-synuclein measured and the techniques used largely influence the results of such studies (Malek et al., 2014).

DJ-1 is a protein related to oxidative stress and was initially linked to PD after familial cases of PD were found to be caused by a mutation in the gene encoding DJ-1. A study of PD and DLB patients showed elevated DJ-1 plasma levels compared to healthy controls (Waragai et al., 2007). This was contradicted in a subsequent study by a different group who looked at DJ-1 levels in platelet-free plasma, comparing PD patients to a group of healthy and Alzheimer's disease (AD) controls (Shi et al., 2010). Yet, a later study by the same group looked at DJ-1 isoforms in whole blood samples and found differences between these groups, with some of the differences only demonstrable in late-stage PD (Lin et al., 2012). Thus, similar to alpha-synuclein, serum DJ-1 is a potential biomarker of the prodromal PD state, but much remains to be learned before it can be applied.

Serum uric acid, an antioxidant, has consistently been found to be lower in PD patients compared to controls, and is inversely correlated with both PD risk and PD disease progression (Ascherio et al., 2009; Davis et al., 1996; de Lau, Koudstaal, Hofman, & Breteler, 2005; Schwarzschild et al., 2008; Weisskopf, O'Reilly, Chen, Schwarzschild, & Ascherio, 2007). Uric acid levels are also inversely correlated with the likelihood of non-motor symptoms in PD (Moccia et al., 2014, 2015). This has been one of the most consistent and replicated findings in regard to potential PD biomarkers. Preliminary studies show this may extend to the prodromal PD state. A metabolomics profiling study of asymptomatic carriers of the G2019S *LRRK2* mutation showed that they had significantly lower uric acid levels compared to controls (Johansen et al., 2009).

Apolipoprotein A1, a serum protein, is a major component of high-density lipoprotein (HDL) and functions in lipid metabolism. Decreased plasma apolipoprotein A1 levels correlate with increased PD risk and decreased age of PD onset, a finding that was subsequently replicated in an independent cohort (Qiang et al., 2013). Importantly, in a cohort of asymptomatic people at high-risk of PD (the PARS cohort), a group enriched for hyposmia, low plasma apolipoprotein A1 was associated with decreased binding on DAT SPECT (Qiang et al., 2013). This makes apolipoprotein A1 a prime candidate for a serum biomarker in prodromal PD.

Several inflammatory markers have been proposed as biomarkers for PD. A few studies have found higher levels of interleukin-6 (IL-6) in PD patients compared to controls (Brodacki et al., 2008; Chen, O'Reilly, Schwarzschild, & Ascherio, 2008), although one study found lower IL-6 levels (Dursun et al., 2015). In a study of PD patients with *GBA* mutations, IL-8 was found to be elevated in a discovery and replication cohort, but not elevated in idiopathic PD patients without *GBA* mutation (Chahine et al., 2013).

In addition, metabolomics have been applied to identify potential serum PD biomarkers. A metabolomics study of 43 drug-naïve PD patients and 37 healthy controls showed increased plasma pyruvate (Ahmed, Santosh, Kumar, & Christlet, 2009). A different metabolomics study showed uric acid is decreased and glutathione is increased in a sample of 66 PD patients compared to 25 controls (Bogdanov et al., 2008).

Finally, differential gene expression patterns in PD have been pursued as a biomarker. Using a microarray on whole blood samples to look for a transcriptional mRNA PD signature, one group found a panel of expression of eight genes was associated with PD in comparison to a control group of healthy, AD, and PSP patients (Scherzer et al., 2007). This gene expression pattern was validated in a second PD sample; its positivity confers an odds of PDF of 5.1 (Scherzer et al., 2007). In a cross-sectional study of mRNA expression profiles in an Ashkenazi Jewish cohort of asymptomatic individuals and PD patients, mRNA expression pattern distinguished both PD disease state and LRRK2 genotype (Chikina et al., 2015).

Inflammatory markers, metabolomics, and gene expression studies have not yet been applied widely to prodromal cohorts. As serum biomarkers for PD are identified, their validation and replication in independent cohorts will be essential, as will be studies of their utility in prodromal PD.

Cerebrospinal fluid

As in the case of the search for potential PD biomarkers in the blood, the most studied biomarker candidate in the cerebrospinal fluid (CSF) is alpha-synuclein. Studies of CSF alpha-synuclein and alpha-synuclein oligomers have shown mixed results, possibly due to heterogeneity in collection and testing techniques, as well as the potential for contamination with blood, which can falsely elevate measured CSF alpha-synuclein levels (Malek et al., 2014). Biological factors may also play a role in the variability of the results, which were reviewed in depth by Mollenhauer et al. (2015). A recent meta-analysis of 12 studies found CSF alpha-synuclein levels significantly differed between PD, MSA, and controls, with no difference between PD and DLB or PSP (Zhou, Wen, Yu, Zhang, & Jiao, 2015). A different meta-analysis, of 17 studies, found no difference in CSF alpha-synuclein levels between PD, DLB, and MSA patients, yielding an estimated sensitivity and specificity of 88% and 40% (Gao et al., 2015). Given the role of alpha-synuclein in PD pathophysiology and the goal of targeting this protein to prevent PD, further CSF studies of alpha-synuclein are eagerly awaited.

As in the blood, DJ-1 protein is also present in CSF. A study comparing a group of PD patients to a control group of healthy individuals and AD patients found a sensitivity and specificity of DJ-1 in the CSF of 90% and 70%, respectively, for PD (Hong et al., 2010). In a study of PD, MSA, and healthy controls, DJ-1 levels were found to be highest in MSA, followed by PD, and then controls, discriminating MSA from PD with a sensitivity of 78% and specificity of

https://doi.org/10.1017/S1355617716000503 Published online by Cambridge University Press

78%; and discriminating PD from controls with a sensitivity of 81% and specificity of 52% (Herbert et al., 2014).

Studies on the utility of AD biomarkers in PD have yielded mixed results (Jiménez-Jiménez, Alonso-Navarro, García-Martín, & Agúndez, 2014). Attempts have been made to use CSF proteins in combination to improve diagnostic accuracy. For example, in a study comparing healthy controls to early PD patients (0.4 year median disease duration), only lower beta-amyloid 1–42 and phosphorylated tau levels were associated with PD diagnosis on multivariate regression, although all tested CSF biomarkers, including total tau, alpha-synuclein, and total tau/beta-amyloid 1–42 ratio, were slightly lower in PD patients compared to controls (Kang et al., 2013). Studies of these putative CSF biomarkers in prodromal PD cohorts have not been published to our knowledge.

Several other CSF biomarkers have been investigated as PD biomarkers and show promise. However, much remains to be learned about them and their specificity for PD *versus* their utility more as non-specific markers of neurodegeneration. For example, neurofilament light chain (NFL) levels are elevated in the CSF in some neurodegenerative diseases, but not in PD in comparison to healthy controls (Constantinescu, Rosengren, Johnels, Zetterberg, & Holmberg, 2010; Hall et al., 2012). A meta-analysis of 4 studies showed elevated NFL levels in MSA and PSP compared to PD (Sako, Murakami, Izumi, & Kaji, 2015). Similarly, cell-free circulating mitochondrial DNA (ccf-mtDNA) is reduced in CSF of PD patients compared to controls, but it is also decreased in AD (Pyle et al., 2015).

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a CSF protein, did not discriminate between PD and controls, but Nrf2 concentrations did correlate with motor scores in 1 study of LRRK2 positive PD subjects (Loeffler, Smith, Coffey, Aasly, & LeWitt, 2015).

There are inconsistent findings on the utility of dopamine and serotonin metabolites in discriminating PD from healthy controls, and for their potential for correlation with PD features (Jiménez-Jiménez et al., 2014). To our knowledge, these have not been studied in prodromal PD cohorts.

As with serum metabolomic studies, CSF metabolomics is a promising area as well. Nuclear magnetic resonance metabolomics uses spectroscopy to quantify metabolites in biofluids. A study in 10 PD patients and 10 healthy controls found lower CSF alanine, creatinine, and mannose levels in the PD patients sufficient to discriminate between the groups (Öhman & Forsgren, 2015). Further validation and replication in PD and prodromal PD is required.

Skin, colon, salivary glands

Multiple studies have found alpha-synuclein in several types of peripheral tissues (Malek et al., 2014). In 2006, Braak, de Vos, and Del Tredici reported alpha-synuclein in the enteric nervous system in post mortem examination of PD patients, which they proposed could reflect the entry point of the pathology to the vagus nerve, stage 1 of the Braak PD pathology staging system (Braak et al., 2003). Multiple studies have since reported increased colonic alpha-synuclein expression in PD and pre-motor PD in comparison to controls (Gold, Turkalp, & Munoz, 2013; Lebouvier et al., 2008; Pouclet et al., 2012; Shannon, Keshavarzian, Dodiya, Jakate, & Kordower, 2012).

In one study, colonic biopsies sampled from patients 2–5 years before the development of PD motor symptoms were found to exhibit alpha-synuclein pathology (Shannon et al., 2012). However, the possibility that colonic alpha-synuclein can discriminate PD and prodromal PD from controls came into question when a study of 22 PD patients and 11 controls found alpha-synuclein in the colon of all patients *and* controls (Visanji et al., 2015). The differing results from studies of colonic alpha-synuclein may be partially due to differences in collection techniques and staining (Visanji, Marras, Hazrati, Liu, & Lang, 2014), as demonstrated in a study comparing colonic alpha-synuclein levels in RBD, PD, and controls. It ultimately failed to show any significant differences between the groups, and provided further evidence that differences in biopsy depth, location, and staining technique affect results (Sprenger et al., 2015). Further studies of colonic alpha-synuclein with refinement and standardization of techniques should continue as it could hold great potential as a prodromal PD biomarker.

In the skin, alpha-synuclein has been found in idiopathic PD patients, but not in patients with other types of parkinsonism or controls (Donadio et al., 2014; Rodríguez-Leyva et al., 2014). In addition, salivary gland alpha-synuclein has been found to have high specificity for PD in most studies (Beach et al., 2013; Tredici, Hawkes, Ghebremedhin, & Braak, 2010), but not all (Folgoas et al., 2013).

Alpha-synuclein testing in peripheral tissues holds great promise for potential PD biomarkers. Once sampling and testing procedures have been refined, application to prodromal PD cohorts will be of great interest.

DISCUSSION

In the search for prodromal PD biomarkers, the length of longitudinal follow-up is one of the most important study design considerations. For example, in one of the first studies to report on longitudinal follow-up of prodromal PD (Ponsen et al., 2010, discusssed above), while some participants exhibited motor symptoms as early as 9 months, others first showed motor symptoms 52 months from baseline (Ponsen et al., 2010). A total of 12.5% of the cohort developed PD over 5 years (Ponsen et al., 2010), but it is likely additional members of the cohort would have phenoconverted on additional follow-up. The quest for prodromal markers that accurately capture at-risk individuals will thus require validation studies with follow-up of sufficient duration to identify those who will go on to develop motor symptoms consistent with PD diagnosis.

Imaging markers are attractive given their potential to measure brain structure and/or function *in vivo* in a non-invasive manner. The majority of imaging modalities currently in use relies on degeneration of the SNc and its connections to the striatum. By Braak's PD staging criteria, pathology in the SNc starts in stage 3, and is severe in stage 4,

https://doi.org/10.1017/S1355617716000503 Published online by Cambridge University Press

affecting additional midbrain and basal forebrain nuclei (Braak et al., 2003). PD motor symptoms and diagnosis typically occurs in stage 4 (Braak et al., 2003). Many of the currently available imaging techniques rely on detecting abnormalities in structures involved at Braak's PD stages 3 and 4. It is important to keep this in mind as we look for potential prodromal biomarkers which correspond more closely to earlier Braak stages, 1 and 2. As alpha-synuclein is the protein that abnormally accumulates in PD, there is significant focus in the nuclear imaging field to develop new ligands to identify this protein *in vivo*. If an alpha-synuclein ligand is successfully developed, this has potential for diagnosing prodromal PD earlier than striatal DAT binding allows.

The majority of imaging studies show similar results between sporadic and genetic forms of PD, presumably due to the similar end effects, despite differences in etiology. Cardiac MIBG scintigram is an exception, as it has failed to consistently discriminate between healthy controls and genetic forms of PD (Orimo et al., 2005; Quattrone et al., 2008). This finding highlights the importance of considering which clinical or genetic features are used to define a given prodromal cohort. This is true in both genetically defined prodromal cohorts due to the reduced penetrance of several monogenic PD forms, and in clinical prodromal cohorts due to the lack of specificity of prodromal symptoms, such as constipation and hyposmia. In light of this, there is a recognized need to combine various biomarker modalities to improve detection of prodromal PD, but a balance needs to be found between the rigor of multimodal biomarker panels and their sensitivity (Tunc et al., 2015).

Limitations of imaging biomarkers often include at least one of the following: high cost, lengthy time commitment, or the need for experts trained in specialty imaging collection and analysis. Thus, biofluid and/or tissue biomarkers are of interest as well. Currently there are no routinely used biospecimen biomarkers for PD and there are fewer potential biomarker candidates that have been tested in prodromal PD. Differences in sampling and processing techniques are a limitation of past biospecimen studies, which has likely contributed to the conflicting results. Of the biospecimen studies discussed, serum uric acid has the most evidence for its potential as a PD/prodromal PD biomarker. Apolipoprotein A1 is overall less studied, but also has strong potential to be a PD/prodromal PD biomarker. The results of alpha-synuclein in all biospecimens are inconclusive at this time, but likely holds promise as well if technical aspects are refined.

Many of the candidate PD biomarkers are products of neuronal degeneration and inflammation. These would presumably be less elevated in prodromal states. Therefore, it is possible that proteins that could serve as a PD biomarker will not identify the prodromal state. Regardless, it is hoped that efforts aimed at identifying a biospecimen biomarker for PD will be successful as such biomarkers have the potential to be less costly and more time-efficient than current imaging biomarkers for PD.

CONCLUSIONS

Results of decades of clinical trials in neurodegenerative disorders suggest that any success at altering disease course in PD needs to occur at the earliest stages of the disease process. Significant advances have been made in describing several clinical signs/symptoms and genetic mutations that constitute the prodromal PD state. These, however, lack specificity and exhibit incomplete penetrance, respectively. This adds great complexity to the identification of the prodromal PD state, and necessitates the identification of objective, robust biomarkers of prodromal PD that exhibit a long lag time (time between positive biomarker and time to manifestation of motor symptoms of PD). Several modalities hold promise, but require extensive additional study, including imaging, serum/CSF, and tissue biomarkers. Studies investigating putative clinical, imaging, and biospecimen PD biomarkers in prodromal PD cohorts are eagerly awaited (Berg et al., 2013; Berg, Marek, Ross, & Poewe, 2012; Gaenslen et al., 2014; Jennings et al., 2014; Liepelt et al., 2011; Marek et al., 2011).

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency, commercial or not-for-profit sectors. Dr. Cooper has no conflicts of interest and no disclosures. Dr. Chahine has no conflicts of interest. Dr. Chahine (i) receives support from the NIH (P50 NS053488); (ii) receives support from the Michael J. Fox Foundation as site Principal Investigator of the Parkinson's Progression Marker's Initiative and (iii) receives royalties from Wolters Kluwel (for book authorship).

REFERENCES

Ahmed, S.S., Santosh, W., Kumar, S., & Christlet, H.T. (2009). Metabolic profiling of Parkinson's disease: Evidence of biomarker from gene expression analysis and rapid neural network detection. *Journal of Biomedical Science*, *16*(1), 63. http://doi.org/10.1186/1423-0127-16-63

Albin, R.L., Koeppe, R.A., Chervin, R.D., Consens, F.B., Wernette, K., Frey, K.A., & Aldrich, M.S. (2000). Decreased striatal dopaminergic innervation in REM sleep behavior disorder. *Neurology*, *55*(9), 1410–1412. http://doi.org/10.1212/WNL.55.9.1410

Ascherio, A., LeWitt, P.A., Xu, K., Eberly, S., Watts, A., Matson, W.R., ... Parkinson Study Group DATATOP Investigators. (2009). Urate as a predictor of the rate of clinical decline in Parkinson disease. *Archives of Neurology*, *66*(12), 1460–1468. http://doi.org/10.1001/archneurol.2009.247

Beach, T.G., Adler, C.H., Dugger, B.N., Serrano, G., Hidalgo, J., Henry-Watson, J., ... Arizona Parkinson's Disease Consortium. (2013). Submandibular gland biopsy for the diagnosis of Parkinson disease. *Journal of Neuropathology and Experimental Neurology*, *72*(2), 130–136. http://doi.org/10.1097/NEN.0b013e3182805c72

Berg, D., Becker, G., Zeiler, B., Tucha, O., Hofmann, E., Preier, M., … Lange, K.W. (1999). Vulnerability of the nigrostriatal system as detected by transcranial ultrasound. *Neurology*, *53*(5), 1026–1026. http://doi.org/10.1212/WNL.53.5.1026

Berg, D., Godau, J., Seppi, K., Behnke, S., Liepelt-Scarfone, I., Lerche, S., … PRIPS study group. (2013). The PRIPS study: Screening battery for subjects at risk for Parkinson's disease. *European Journal of Neurology*, *20*(1), 102–108. http://doi.org/10.1111/j.1468-1331.2012.03798.x

Berg, D., Marek, K., Ross, G.W., & Poewe, W. (2012). Defining at-risk populations for Parkinson's disease: Lessons from ongoing studies. *Movement Disorders*, *27*(5), 656–665. http://doi.org/10.1002/mds.24985

Berg, D., Postuma, R.B., Adler, C.H., Bloem, B.R., Chan, P., Dubois, B., … Deuschl, G. (2015). MDS research criteria for prodromal Parkinson's disease. *Movement Disorders*, *30*(12), 1600–1611. http://doi.org/10.1002/mds.26431

Berg, D., Roggendorf, W., Schröder, U., Klein, R., Tatschner, T., Benz, P., … Becker, G. (2002). Echogenicity of the substantia nigra: Association with increased iron content and marker for susceptibility to nigrostriatal injury. *Archives of Neurology*, *59*(6), 999–1005.

Berg, D., Siefker, C., & Becker, G. (2001). Echogenicity of the substantia nigra in Parkinson's disease and its relation to clinical findings. *Journal of Neurology*, *248*(8), 684–689.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences*, *20*(4), 415–455.

Boeve, B.F., Silber, M.H., Ferman, T.J., Lin, S.C., Benarroch, E.E., Schmeichel, A.M., … Dickson, D.W. (2013). Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. *Sleep Medicine*, *14*(8), 754–762. http://doi.org/10.1016/j.sleep.2012.10.015

Bogdanov, M., Matson, W.R., Wang, L., Matson, T., Saunders-Pullman, R., Bressman, S.S., & Beal, M.F. (2008). Metabolomic profiling to develop blood biomarkers for Parkinson's disease. *Brain*, *131*(2), 389–396. http://doi.org/10.1093/brain/awm304

Braak, H., Tredici, K.D., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*(2), 197–211. http://doi.org/10.1016/S0197-4580(02)00065-9

Brodacki, B., Staszewski, J., Toczyłowska, B., Kozłowska, E., Drela, N., Chalimoniuk, M., & Stepien, A. (2008). Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFα, and INFγ concentrations are elevated in patients with atypical and idiopathic parkinsonism. *Neuroscience Letters*, *441*(2), 158–162. http://doi.org/10.1016/j.neulet.2008.06.040

Brüggemann, N., Hagenah, J., Stanley, K., Klein, C., Wang, C., Raymond, D., … Saunders-Pullman, R. (2011). Substantia nigra hyperechogenicity with LRRK2 G2019S mutations. *Movement Disorders*, *26*(5), 885–888. http://doi.org/10.1002/mds.23644

Chahine, L.M., Qiang, J., Ashbridge, E., Minger, J., Yearout, D., Horn, S., … Chen-Plotkin, A. (2013). Clinical and biochemical differences in patients having Parkinson disease with vs without GBA mutations. *JAMA Neurology*, *70*(7), 852–858. http://doi.org/10.1001/jamaneurol.2013.1274

Chahine, L.M., Weintraub, D., Hawkins, K.A., Siderowf, A., Eberly, S., Oakes, D., … PARS Investigators. (2015). Cognition in individuals at risk for Parkinson's: Parkinson associated risk syndrome (PARS) study findings. *Movement Disorders*, *31*, 86–94. http://doi.org/10.1002/mds.26373

Chen, H., O'Reilly, E.J., Schwarzschild, M.A., & Ascherio, A. (2008). Peripheral inflammatory biomarkers and risk of Parkinson's disease. *American Journal of Epidemiology*, *167*(1), 90–95. http://doi.org/10.1093/aje/kwm260

Chikina, M.D., Gerald, C.P., Li, X., Ge, Y., Pincas, H., Nair, V.D., … Sealfon, S.C. (2015). Low-variance RNAs identify Parkinson's disease molecular signature in blood. *Movement Disorders*, *30*(6), 813–821. http://doi.org/10.1002/mds.26205

Cochrane, C.J., & Ebmeier, K.P. (2013). Diffusion tensor imaging in parkinsonian syndromes: A systematic review and meta-analysis. *Neurology*, *80*(9), 857–864. http://doi.org/10.1212/WNL.0b013e318284070c

Constantinescu, R., Rosengren, L., Johnels, B., Zetterberg, H., & Holmberg, B. (2010). Consecutive analyses of cerebrospinal fluid axonal and glial markers in Parkinson's disease and atypical Parkinsonian disorders. *Parkinsonism & Related Disorders*, *16*(2), 142–145. http://doi.org/10.1016/j.parkreldis.2009.07.007

Davis, J.W., Grandinetti, A., Waslien, C.I., Ross, G.W., White, L.R., & Morens, D.M. (1996). Observations on serum uric acid levels and the risk of idiopathic Parkinson's disease. *American Journal of Epidemiology*, *144*(5), 480–484.

de Lau, L.M.L., Koudstaal, P.J., Hofman, A., & Breteler, M.M.B. (2005). Serum uric acid levels and the risk of Parkinson disease. *Annals of Neurology*, *58*(5), 797–800. http://doi.org/10.1002/ana.20663

Donadio, V., Incensi, A., Leta, V., Giannoccaro, M.P., Scaglione, C., Martinelli, P., … Liguori, R. (2014). Skin nerve α-synuclein deposits: A biomarker for idiopathic Parkinson disease. *Neurology*, *82*(15), 1362–1369. http://doi.org/10.1212/WNL.0000000000000316

Dursun, E., Gezen-Ak, D., Hanağası, H., Bilgiç, B., Lohmann, E., Ertan, S., … Yılmazer, S. (2015). The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *Journal of Neuroimmunology*, *283*, 50–57. http://doi.org/10.1016/j.jneuroim.2015.04.014

Eidelberg, D., Moeller, J.R., Dhawan, V., Spetsieris, P., Takikawa, S., Ishikawa, T., … Przedborski, S. (1994). The metabolic topography of parkinsonism. *Journal of Cerebral Blood Flow and Metabolism*, *14*(5), 783–801. http://doi.org/10.1038/jcbfm.1994.99

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

Faucheux, B.A., Martin, M.-E., Beaumont, C., Hauw, J.-J., Agid, Y., & Hirsch, E.C. (2003). Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. *Journal of Neurochemistry*, *86*(5), 1142–1148. http://doi.org/10.1046/j.1471-4159.2003.01923.x

Fearnley, J.M., & Lees, A.J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain*, *114*(5), 2283–2301. http://doi.org/10.1093/brain/114.5.2283

Folgoas, E., Lebouvier, T., Leclair-Visonneau, L., Cersosimo, M.-G., Barthelaix, A., Derkinderen, P., & Letournel, F. (2013). Diagnostic value of minor salivary glands biopsy for the detection of Lewy pathology. *Neuroscience Letters*, *551*, 62–64. http://doi.org/10.1016/j.neulet.2013.07.016

Foulds, P.G., Mitchell, J.D., Parker, A., Turner, R., Green, G., Diggle, P., … Allsop, D. (2011). Phosphorylated α-synuclein can

be detected in blood plasma and is potentially a useful biomarker for Parkinson's disease. *FASEB Journal*, *25*(12), 4127–4137. http://doi.org/10.1096/fj.10-179192

Gaenslen, A., Swid, I., Liepelt-Scarfone, I., Godau, J., & Berg, D. (2011). The patients' perception of prodromal symptoms before the initial diagnosis of Parkinson's disease. *Movement Disorders*, *26*(4), 653–658. http://doi.org/10.1002/mds.23499

Gaenslen, A., Wurster, I., Brockmann, K., Huber, H., Godau, J., Faust, B., … Berg, D. (2014). Prodromal features for Parkinson's disease–baseline data from the TREND study. *European Journal of Neurology*, *21*(5), 766–772. http://doi.org/10.1111/ene.12382

Gao, L., Tang, H., Nie, K., Wang, L., Zhao, J., Gan, R., … Wang, L. (2015). Cerebrospinal fluid alpha-synuclein as a biomarker for Parkinson's disease diagnosis: A systematic review and meta-analysis. *The International Journal of Neuroscience*, *125*(9), 645–654. http://doi.org/10.3109/00207454.2014.961454

Gold, A., Turkalp, Z.T., & Munoz, D.G. (2013). Enteric alpha-synuclein expression is increased in Parkinson's disease but not Alzheimer's disease. *Movement Disorders*, *28*(2), 237–240. http://doi.org/10.1002/mds.25298

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

Herbert, M.K., Eeftens, J.M., Aerts, M.B., Esselink, R.A.J., Bloem, B.R., Kuiperij, H.B., & Verbeek, M.M. (2014). CSF levels of DJ-1 and tau distinguish MSA patients from PD patients and controls. *Parkinsonism & Related Disorders*, *20*(1), 112–115. http://doi.org/10.1016/j.parkreldis.2013.09.003

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

Huang, W.-S., Lin, S.-Z., Lin, J.-C., Wey, S.-P., Ting, G., & Liu, R.-S. (2001). Evaluation of early-stage Parkinson's disease with 99mTc-TRODAT-1 imaging. *Journal of Nuclear Medicine*, *42*(9), 1303–1308.

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

Iranzo, A., Fernández-Arcos, A., Tolosa, E., Serradell, M., Molinuevo, J.L., Valldeoriola, F., … Santamaría, J. (2014). Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: Study in 174 patients. *PloS One*, *9*(2), e89741. http://doi.org/10.1371/journal.pone.0089741

Iranzo, A., Lomeña, F., Stockner, H., Valldeoriola, F., Vilaseca, I., Salamero, M., … Sleep Innsbruck Barcelona (SINBAR) group. (2010). Decreased striatal dopamine transporter uptake and substantia nigra hyperechogenicity as risk markers of synucleinopathy in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A prospective study [corrected]. *The Lancet. Neurology*, *9*(11), 1070–1077. http://doi.org/10.1016/S1474-4422(10)70216-7

Jennings, D.L., Seibyl, J.P., Oakes, D., Eberly, S., Murphy, J., & Marek, K. (2004). (123i) β-cit and single-photon emission computed tomographic imaging vs clinical evaluation in parkinsonian syndrome: Unmasking an early diagnosis. *Archives of Neurology*, *61*(8), 1224–1229. http://doi.org/10.1001/archneur.61.8.1224

Jennings, D., Siderowf, A., Stern, M., Seibyl, J., Eberly, S., Oakes, D., … PARS Investigators. (2014). Imaging prodromal Parkinson disease The Parkinson Associated Risk Syndrome Study. *Neurology*, *83*(19), 1739–1746. http://doi.org/10.1212/WNL.0000000000000960

Jiménez-Jiménez, F.J., Alonso-Navarro, H., García-Martín, E., & Agúndez, J.A.G. (2014). Cerebrospinal fluid biochemical studies in patients with Parkinson's disease: Toward a potential search for biomarkers for this disease. *Frontiers in Cellular Neuroscience*, *8*, 369. http://doi.org/10.3389/fncel.2014.00369

Johansen, K.K., Wang, L., Aasly, J.O., White, L.R., Matson, W.R., Henchcliffe, C., … Bogdanov, M. (2009). Metabolomic profiling in LRRK2-related Parkinson's disease. *PLoS ONE*, *4*(10), e7551. http://doi.org/10.1371/journal.pone.0007551

Kang, J.-H., Irwin, D.J., Chen-Plotkin, A.S., Siderowf, A., Caspell, C., Coffey, C.S., … Parkinson's Progression Markers Initiative. (2013). Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. *JAMA Neurology*, *70*(10), 1277–1287. http://doi.org/10.1001/jamaneurol.2013.3861

Kashihara, K., Imamura, T., & Shinya, T. (2010). Cardiac 123I-MIBG uptake is reduced more markedly in patients with REM sleep behavior disorder than in those with early stage Parkinson's disease. *Parkinsonism & Related Disorders*, *16*(4), 252–255. http://doi.org/10.1016/j.parkreldis.2009.12.010

Khan, N.L., Valente, E.M., Bentivoglio, A.R., Wood, N.W., Albanese, A., Brooks, D.J., & Piccini, P. (2002). Clinical and subclinical dopaminergic dysfunction in PARK6-linked parkinsonism: An 18F-dopa PET study. *Annals of Neurology*, *52*(6), 849–853. http://doi.org/10.1002/ana.10417

Lebouvier, T., Chaumette, T., Damier, P., Coron, E., Touchefeu, Y., Vrignaud, S., … Neunlist, M. (2008). Pathological lesions in colonic biopsies during Parkinson's disease. *Gut*, *57*(12), 1741–1743. http://doi.org/10.1136/gut.2008.162503

Liepelt, I., Behnke, S., Schweitzer, K., Wolf, B., Godau, J., Wollenweber, F., … Berg, D. (2011). Pre-motor signs of PD are related to SN hyperechogenicity assessed by TCS in an elderly population. *Neurobiology of Aging*, *32*(9), 1599–1606. http://doi.org/10.1016/j.neurobiolaging.2009.10.004

Lin, X., Cook, T.J., Zabetian, C.P., Leverenz, J.B., Peskind, E.R., Hu, S.-C., … Zhang, J. (2012). DJ-1 isoforms in whole blood as potential biomarkers of Parkinson disease. *Scientific Reports*, *2*, 954. http://doi.org/10.1038/srep00954

Loeffler, D.A., Smith, L.M., Coffey, M.P., Aasly, J.O., & LeWitt, P.A. (2015). CSF Nrf2 and HSPA8 in Parkinson's disease patients with and without LRRK2 gene mutations. *Journal of Neural Transmission (Vienna, Austria: 1996)*. http://doi.org/10.1007/s00702-015-1479-0

Malek, N., Swallow, D., Grosset, K.A., Anichtchik, O., Spillantini, M., & Grosset, D.G. (2014). Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease - A systematic review. *Acta Neurologica Scandinavica*, *130*(2), 59–72. http://doi.org/10.1111/ane.12247

Marek, K., Jennings, D., Lasch, S., Siderowf, A., Tanner, C., Simuni, T., … Taylor, P. (2011). The Parkinson Progression Marker Initiative (PPMI). *Progress in Neurobiology*, *95*(4), 629–635. http://doi.org/10.1016/j.pneurobio.2011.09.005

Miyamoto, T., Miyamoto, M., Inoue, Y., Usui, Y., Suzuki, K., & Hirata, K. (2006). Reduced cardiac 123I-MIBG scintigraphy in idiopathic REM sleep behavior disorder. *Neurology*, *67*(12), 2236–2238. http://doi.org/10.1212/01.wnl.0000249313.25627.2e

Moccia, M., Picillo, M., Erro, R., Vitale, C., Longo, K., Amboni, M., … Pellecchia, M.T. (2014). Is serum uric acid related to non-motor

symptoms in de-novo Parkinson's disease patients? *Parkinsonism & Related Disorders*, *20*(7), 772–775. http://doi.org/10.1016/j.parkreldis.2014.03.016

Moccia, M., Picillo, M., Erro, R., Vitale, C., Longo, K., Amboni, M., … Pellecchia, M.T. (2015). Presence and progression of non-motor symptoms in relation to uric acid in de novo Parkinson's disease. *European Journal of Neurology*, *22*(1), 93–98. http://doi.org/10.1111/ene.12533

Mollenhauer, B., Parnetti, L., Rektorova, I., Kramberger, M.G., Pikkarainen, M., Schulz-Schaeffer, W.J., … Schlossmacher, M.G. (2015). Biological confounders for the values of cerebrospinal fluid proteins in Parkinson's disease and related disorders. *Journal of Neurochemistry*, http://doi.org/10.1111/jnc.13390

Nishioka, K., Hayashi, S., Farrer, M.J., Singleton, A.B., Yoshino, H., Imai, H., … Hattori, N. (2006). Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. *Annals of Neurology*, *59*(2), 298–309. http://doi.org/10.1002/ana.20753

Nishioka, K., Ross, O.A., Ishii, K., Kachergus, J.M., Ishiwata, K., Kitagawa, M., … Hattori, N. (2009). Expanding the clinical phenotype of SNCA duplication carriers. *Movement Disorders*, *24*(12), 1811–1819. http://doi.org/10.1002/mds.22682

Noyce, A.J., Bestwick, J.P., Silveira-Moriyama, L., Hawkes, C.H., Knowles, C.H., Hardy, J., … Schrag, A. (2014). PREDICT-PD: Identifying risk of Parkinson's disease in the community: Methods and baseline results. *Journal of Neurology, Neurosurgery, and Psychiatry*, *85*(1), 31–37. http://doi.org/10.1136/jnnp-2013-305420

Öhman, A., & Forsgren, L. (2015). NMR metabonomics of cerebrospinal fluid distinguishes between Parkinson's disease and controls. *Neuroscience Letters*, *594*, 36–39. http://doi.org/10.1016/j.neulet.2015.03.051

Ohtsuka, C., Sasaki, M., Konno, K., Koide, M., Kato, K., Takahashi, J., … Terayama, Y. (2013). Changes in substantia nigra and locus coeruleus in patients with early-stage Parkinson's disease using neuromelanin-sensitive MR imaging. *Neuroscience Letters*, *541*, 93–98. http://doi.org/10.1016/j.neulet.2013.02.012

Orimo, S., Amino, T., Yokochi, M., Kojo, T., Uchihara, T., Takahashi, A., … Mizuno, Y. (2005). Preserved cardiac sympathetic nerve accounts for normal cardiac uptake of MIBG in PARK2. *Movement Disorders*, *20*(10), 1350–1353. http://doi.org/10.1002/mds.20594

Ponsen, M.M., Stoffers, D., Twisk, J.W.R., Wolters, E.C., & Berendse, H.W. (2009). Hyposmia and executive dysfunction as predictors of future Parkinson's disease: A prospective study. *Movement Disorders*, *24*(7), 1060–1065. http://doi.org/10.1002/mds.22534

Ponsen, M.M., Stoffers, D., Wolters, E.C., Booij, J., & Berendse, H.W. (2010). Olfactory testing combined with dopamine transporter imaging as a method to detect prodromal Parkinson's disease. *Journal of Neurology, Neurosurgery, & Psychiatry*, *81*(4), 396–399. http://doi.org/10.1136/jnnp.2009.183715

Postuma, R.B., Aarsland, D., Barone, P., Burn, D.J., Hawkes, C.H., Oertel, W., & Ziemssen, T. (2012). Identifying prodromal Parkinson's disease: Pre-motor disorders in Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, *27*(5), 617–626. http://doi.org/10.1002/mds.24996

Postuma, R.B., Gagnon, J.-F., Pelletier, A., & Montplaisir, J. (2013). Prodromal autonomic symptoms and signs in Parkinson's disease and dementia with Lewy bodies. *Movement Disorders*, *28*(5), 597–604. http://doi.org/10.1002/mds.25445

Postuma, R.B., Gagnon, J.F., Vendette, M., Fantini, M.L., Massicotte-Marquez, J., & Montplaisir, J. (2009). Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. *Neurology*, *72*(15), 1296–1300. http://doi.org/10.1212/01.wnl.0000340980.19702.6e

Pouclet, H., Lebouvier, T., Coron, E., Des Varannes, S.B., Neunlist, M., & Derkinderen, P. (2012). A comparison between colonic submucosa and mucosa to detect Lewy pathology in Parkinson's disease. *Neurogastroenterology & Motility*, *24*(4), e202–e205. http://doi.org/10.1111/j.1365-2982.2012.01887.x

Pyle, A., Brennan, R., Kurzawa-Akanbi, M., Yarnall, A., Thouin, A., Mollenhauer, B., … Hudson, G. (2015). Reduced CSF mitochondrial DNA is a biomarker for early-stage Parkinson's disease. *Annals of Neurology*. http://doi.org/10.1002/ana.24515

Qiang, J.K., Wong, Y.C., Siderowf, A., Hurtig, H.I., Xie, S.X., Lee, V.M.-Y., … Chen-Plotkin, A.S. (2013). Plasma Apolipoprotein A1 as a Biomarker for Parkinson's Disease. *Annals of Neurology*, *74*(1), 119–127. http://doi.org/10.1002/ana.23872

Quattrone, A., Bagnato, A., Annesi, G., Novellino, F., Morgante, L., Savettieri, G., … Condino, F. (2008). Myocardial 123metaiodobenzylguanidine uptake in genetic Parkinson's disease. *Movement Disorders*, *23*(1), 21–27. http://doi.org/10.1002/mds.21701

Ravina, B., Marek, K., Eberly, S., Oakes, D., Kurlan, R., Ascherio, A., … Shoulson, I. (2012). Dopamine transporter imaging is associated with long-term outcomes in Parkinson's disease. *Movement Disorders*, *27*(11), 1392–1397. http://doi.org/10.1002/mds.25157

Rodríguez-Leyva, I., Calderón-Garcidueñas, A.L., Jiménez-Capdeville, M.E., Rentería-Palomo, A.A., Hernandez-Rodriguez, H.G., Valdés-Rodríguez, R., … Castanedo-Cázares, J.P. (2014). α-Synuclein inclusions in the skin of Parkinson's disease and parkinsonism. *Annals of Clinical and Translational Neurology*, *1*(7), 471–478. http://doi.org/10.1002/acn3.78

Ross, G.W., Abbott, R.D., Petrovitch, H., Tanner, C.M., & White, L.R. (2012). Pre-motor features of Parkinson's disease: the The Honolulu-Asia Aging Study experience. *Parkinsonism & Related Disorders*, *18*(Suppl. 1), S199–S202. http://doi.org/10.1016/S1353-8020(11)70062-1

Sako, W., Murakami, N., Izumi, Y., & Kaji, R. (2015). Neurofilament light chain level in cerebrospinal fluid can differentiate Parkinson's disease from atypical parkinsonism: Evidence from a meta-analysis. *Journal of the Neurological Sciences*, *352*(1-2), 84–87. http://doi.org/10.1016/j.jns.2015.03.041

Sasaki, M., Shibata, E., Tohyama, K., Takahashi, J., Otsuka, K., Tsuchiya, K., … Sakai, A. (2006). Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport*, *17*(11), 1215–1218. http://doi.org/10.1097/01.wnr.0000227984.84927.a7

Sawada, H., Oeda, T., Yamamoto, K., Kitagawa, N., Mizuta, E., Hosokawa, R., … Kawamura, T. (2009). Diagnostic accuracy of cardiac metaiodobenzylguanidine scintigraphy in Parkinson disease. *European Journal of Neurology*, *16*(2), 174–182. http://doi.org/10.1111/j.1468-1331.2008.02372.x

Schenck, C.H., Boeve, B.F., & Mahowald, M.W. (2013). Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: A 16-year update on a previously reported series. *Sleep Medicine*, *14*(8), 744–748. http://doi.org/10.1016/j.sleep.2012.10.009

Scherfler, C., Frauscher, B., Schocke, M., Iranzo, A., Gschliesser, V., Seppi, K., … for the SINBAR (Sleep Innsbruck Barcelona) Group. (2011). White and gray matter abnormalities in idiopathic rapid eye movement sleep behavior disorder: A

diffusion-tensor imaging and voxel-based morphometry study. *Annals of Neurology*, *69*(2), 400–407. http://doi.org/10.1002/ana.22245

Scherzer, C.R., Eklund, A.C., Morse, L.J., Liao, Z., Locascio, J.J., Fefer, D., … Gullans, S.R. (2007). Molecular markers of early Parkinson's disease based on gene expression in blood. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 955–960. http://doi.org/10.1073/pnas.0610204104

Schwarzschild, M.A., Schwid, S.R., Marek, K., Watts, A., Lang, A.E., Oakes, D., … Ondrasik, J. (2008). Serum urate as a predictor of clinical and radiographic progression in Parkinson disease. *Archives of Neurology*, *65*(6), 716–723. http://doi.org/10.1001/archneur.2008.65.6.nct70003

Seibyl, J.P., Marek, K.L., Quinlan, D., Sheff, K., Zoghbi, S., Zea-Ponce, Y., … van Dyck, C. (1995). Decreased single-photon emission computed tomographic [123I]beta-CIT striatal uptake correlates with symptom severity in Parkinson's disease. *Annals of Neurology*, *38*(4), 589–598. http://doi.org/10.1002/ana.410380407

Seibyl, J.P., Marek, K., Sheff, K., Zoghbi, S., Baldwin, R.M., Charney, D.S., … Innis, R.B. (1998). Iodine-123-beta-CIT and iodine-123-FPCIT SPECT measurement of dopamine transporters in healthy subjects and Parkinson's patients. *Journal of Nuclear Medicine*, *39*(9), 1500–1508.

Shannon, K.M., Keshavarzian, A., Dodiya, H.B., Jakate, S., & Kordower, J.H. (2012). Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Movement Disorders*, *27*(6), 716–719. http://doi.org/10.1002/mds.25020

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

Siderowf, A., & Lang, A.E. (2012). Premotor Parkinson's disease: Concepts and definitions. *Movement Disorders*, *27*(5), 608–616. http://doi.org/10.1002/mds.24954

Siderowf, A., & Stern, M.B. (2008). Premotor Parkinson's disease: Clinical features, detection, and prospects for treatment. *Annals of Neurology*, *64*(S2), S139–S147. http://doi.org/10.1002/ana.21462

Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G.P., Hebenstreit, G., … Youdim, M.B. (1988). Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. *Journal of Neural Transmission*, *74*(3), 199–205.

Sprenger, F.S., Stefanova, N., Gelpi, E., Seppi, K., Navarro-Otano, J., Offner, F., … Poewe, W. (2015). Enteric nervous system α-synuclein immunoreactivity in idiopathic REM sleep behavior disorder. *Neurology*, *85*(20), 1761–1768. http://doi.org/10.1212/WNL.0000000000002126

Stephenson, R., Siderowf, A., & Stern, M.B. (2009). Premotor Parkinson's disease: Clinical features and detection strategies. *Movement Disorders*, *24*(S2), S665–S670. http://doi.org/10.1002/mds.22403

Stern, M.B., & Siderowf, A. (2010). Parkinson's at risk syndrome: Can Parkinson's disease be predicted? *Movement Disorders*, *25*(S1), S89–S93. http://doi.org/10.1002/mds.22719

Tredici, K.D., Hawkes, C.H., Ghebremedhin, E., & Braak, H. (2010). Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. *Acta Neuropathologica*, *119*(6), 703–713. http://doi.org/10.1007/s00401-010-0665-2

Tunc, S., Graf, J., Tadic, V., Brüggemann, N., Schmidt, A., Al-Khaled, M., … Kasten, M. (2015). A population-based study on combined markers for early Parkinson's disease. *Movement Disorders*, *30*(4), 531–537. http://doi.org/10.1002/mds.26100

Visanji, N.P., Marras, C., Hazrati, L.-N., Liu, L.W.C., & Lang, A.E. (2014). Alimentary, my dear Watson? The challenges of enteric α-synuclein as a Parkinson's disease biomarker. *Movement Disorders*, *29*(4), 444–450. http://doi.org/10.1002/mds.25789

Visanji, N.P., Marras, C., Kern, D.S., Al Dakheel, A., Gao, A., Liu, L.W.C., … Hazrati, L.-N. (2015). Colonic mucosal a-synuclein lacks specificity as a biomarker for Parkinson disease. *Neurology*, *84*(6), 609–616. http://doi.org/10.1212/WNL.0000000000001240

Walter, U., Klein, C., Hilker, R., Benecke, R., Pramstaller, P.P., & Dressler, D. (2004). Brain parenchyma sonography detects preclinical parkinsonism. *Movement Disorders*, *19*(12), 1445–1449. http://doi.org/10.1002/mds.20232

Wang, J.-J., Lin, W.-Y., Lu, C.-S., Weng, Y.-H., Ng, S.-H., Wang, C.-H., … Wai, Y.-Y. (2011). Parkinson disease: Diagnostic utility of diffusion kurtosis imaging. *Radiology*, *261*(1), 210–217. http://doi.org/10.1148/radiol.11102277

Wang, X., Yu, S., Li, F., & Feng, T. (2015). Detection of α-synuclein oligomers in red blood cells as a potential biomarker of Parkinson's disease. *Neuroscience Letters*, *599*, 115–119. http://doi.org/10.1016/j.neulet.2015.05.030

Waragai, M., Nakai, M., Wei, J., Fujita, M., Mizuno, H., Ho, G., … Hashimoto, M. (2007). Plasma levels of DJ-1 as a possible marker for progression of sporadic Parkinson's disease. *Neuroscience Letters*, *425*(1), 18–22. http://doi.org/10.1016/j.neulet.2007.08.010

Weisskopf, M.G., O'Reilly, E., Chen, H., Schwarzschild, M.A., & Ascherio, A. (2007). Plasma Urate and Risk of Parkinson's Disease. *American Journal of Epidemiology*, *166*(5), 561–567. http://doi.org/10.1093/aje/kwm127

Wing, Y.K., Lam, S.P., Zhang, J., Leung, E., Ho, C.L., Chen, S., … Ho, C.K.W. (2015). Reduced striatal dopamine transmission in REM sleep behavior disorder comorbid with depression. *Neurology*, *84*(5), 516–522. http://doi.org/10.1212/WNL.0000000000001215

Wu, P., Yu, H., Peng, S., Dauvilliers, Y., Wang, J., Ge, J., … Zuo, C. (2014). Consistent abnormalities in metabolic network activity in idiopathic rapid eye movement sleep behaviour disorder. *Brain*, *137*(12), 3122–3128. http://doi.org/10.1093/brain/awu290

Zhou, B., Wen, M., Yu, W.-F., Zhang, C.-L., & Jiao, L. (2015). The diagnostic and differential diagnosis utility of cerebrospinal fluid α-Synuclein levels in Parkinson's disease: A meta-analysis. *Parkinson's Disease*, *2015*, 567386. http://doi.org/10.1155/2015/567386