Pathophysiological mechanisms of non-motor features and their role on the pathogenic process in Parkinson's disease

Thesis submitted in fulfilment of the degree of Doctor of Philosophy

Reta Lila Weston Institute of Neurological Studies

Queen Square Brain Bank for Neurological Disorders

UCL Queen Square Institute of Neurology

University College London

Eduardo De Pablo Fernández

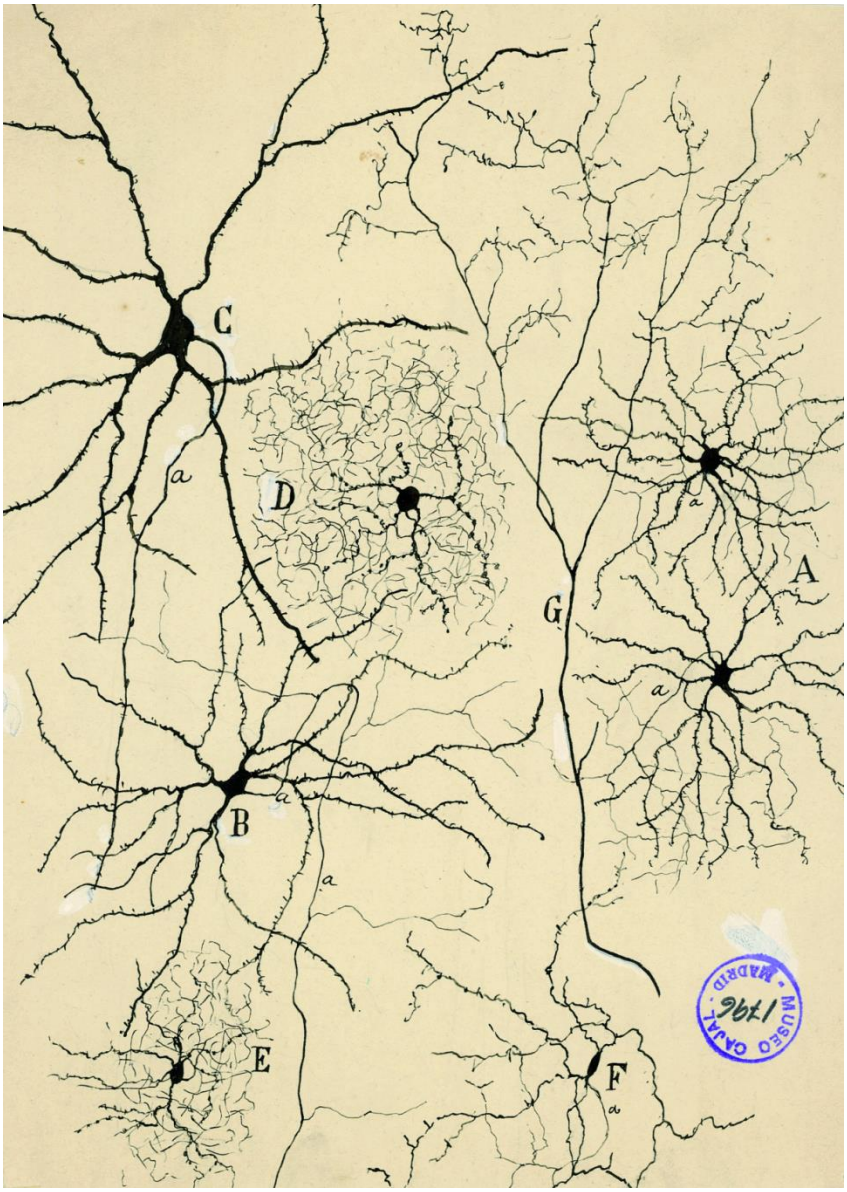
2019

I, Eduardo De Pablo Fernández confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:

Date:

A mis padres por su esfuerzo, sacrificio y apoyo.



Like the entomologist hunting for brightly coloured butterflies, my attention was drawn to the flower garden of the grey matter, which contained cells with delicate and elegant forms, the mysterious butterflies of the soul, the beatings of whose wings may someday (who knows?) clarify the secret of mental life.

Ramón y Cajal

Image courtesy of the Cajal Institute, Cajal Legacy, Spanish National Research Council (CSIC).

Abstract

In the first section, clinical and neuropathological studies were conducted to evaluate the underlying pathophysiological mechanisms of non-motor features in Parkinson's disease (PD) including hypothalamic dysfunction, circadian abnormalities and constipation.

A clinico-pathological study showed an early and progressive involvement by Lewy pathology of the paraventricular, infundibular and supraoptic nuclei of the hypothalamus. Neuropathological involvement spared dopaminergic structures and did not correlate with non-motor clinical features potentially associated to hypothalamic dysfunction.

A neuropathological study of the circadian system demonstrated direct histological involvement of the suprachiasmatic nucleus of the hypothalamus likely responsible for circadian dysfunction in PD. Similar neuropathological involvement was seen in progressive supranuclear palsy whilst no neuropathological abnormalities were found in the suprachiasmatic nucleus and pineal gland in multiple system atrophy.

A comprehensive assessment of constipation in PD using clinical, radiological and electrophysiological techniques revealed heterogeneous multiple overlapping pathophysiological abnormalities involving slow colonic motility and anorectal dysfunction (rectal hyposensitivity and defecatory dyssynergia) with important clinical implications.

The second part of the thesis evaluated the influence of non-motor features on the pathogenic process and disease progression in PD through epidemiological and clinico-pathological studies.

A large retrospective record-linkage cohort study showed an increased risk of subsequent PD in individuals with type 2 diabetes, greater in younger participants or those with diabetic complications.

Autonomic dysfunction was independently associated with faster disease progression and reduced survival in a retrospective large cohort of autopsy-confirmed PD patients although these findings were not associated with more diffuse or severe neuropathology at post-mortem.

Finally, PD subtyping based on a combination of motor and non-motor features at diagnosis predicted disease progression and survival in a large cohort of pathology-proven patients with PD. Lewy pathology and Alzheimer's neuropathological changes showed different rates of progression among subtypes and, in addition to age, they constitute important determinants of clinical heterogeneity.

Impact statement

Despite the importance of non-motor features of PD as significant contributors to the clinical burden, little is known about their pathophysiology and only limited symptomatic therapies are available. This thesis provides a better understanding of the neuroanatomical structures and pathophysiology of the non-motor features in PD that will help advance the understanding of their underlying mechanisms and will ultimately provide the basis for new therapeutic targets.

A detailed clinico-pathological assessment showed an early and systematic involvement of the paraventricular, supraoptic, infundibular and suprachiasmatic hypothalamic nuclei in patients with PD that is likely to contribute to the non-motor symptomatology. In this regard, the suprachiasmatic nucleus was identified as the key neuroanatomical structure responsible for circadian dysfunction and contributor to the symptom fluctuations experienced by PD patients. As a consequence, the suprachiasmatic nucleus should be the therapeutic target of any future therapies aimed at the resynchronisation of the circadian function in PD.

A clinical, radiological and electrophysiological assessment of constipation showed that both slow transit and anorectal dysfunction contribute to this symptomatology in PD. These findings will increase awareness among clinicians of the need of a systematic assessment to elucidate pathophysiological mechanisms of refractory constipation as slow transit and anorectal dysfunction are treated differently. Improvement in the recognition and assessment will lead to a better symptomatic management of constipation in PD in clinical practice.

Non-motor features are important key determinants on PD clinical heterogeneity and this thesis provides further evidence of the influence that some non-motor features can exert on the pathogenesis and disease course.

In the largest cohort study to date, using a record-linkage of hospital admissions, type 2 diabetes was associated with an increased risk of subsequent PD suggesting a pathophysiological link between both conditions. Moreover, development of autonomic dysfunction was associated with worse disease progression and reduced survival in a group of patients with pathology-confirmed PD. These findings have important research and clinical implications and should stimulate further research into the pathogenic mechanisms linking these features. They also open up new therapeutic avenues as control of glucose metabolism with repurposing of diabetic drugs or a strict control of autonomic symptoms with already available treatments could potentially modify the disease course.

Finally, this thesis provides for the first time long-term clinical and neuropathological data on clinical PD subtypes incorporating non-motor features. PD subtyping using a combination of motor and non-motor features reliably estimated the disease progression and survival which is of fundamental value when discussing individual prognosis in clinical practice. The study also provided a better understanding on the contribution of different neuropathologies to the clinical heterogeneity supporting the idea that different pathophysiological mechanisms underlie heterogeneity among PD subgroups. Any therapeutic clinical trial should take these differences into consideration and a better understanding of these pathophysiological differences may lead eventually to more individualised treatments based on the underlying pathophysiological implications.

These findings have not only been disseminated in medical conferences and published in high impact neurology journals, but also have attracted the attention of general media given their important implications.

Publications related to this thesis

1. De Pablo-Fernández E, Courtney R, Holton JL, Warner TT. Hypothalamic α-synuclein and its relation to weight loss and autonomic symptoms in Parkinson's disease. Mov Disord. 2017 Feb;32(2):296-298. **(Chapter 2)**

2. De Pablo-Fernández E, Courtney R, Warner TT, Holton JL. A histologic study of circadian system in Parkinson disease, multiple system atrophy and progressive supranuclear palsy. JAMA Neurol. 2018 Aug 1;75(8):1008-1012. **(Chapter 3)**

3. De Pablo-Fernández E, Goldacre R, Pakpoor J, Noyce AJ, Warner TT. Association between diabetes and subsequent Parkinson disease: a record-linkage cohort study. Neurology. 2018 Jul 10;91(2):e139-e142. **(Chapter 5)**

4. De Pablo-Fernández E, Tur C, Revesz T, Lees AJ, Holton JL, Warner TT. Association of autonomic dysfunction with disease progression and survival in Parkinson disease. JAMA Neurol. 2017 Aug 1;74(8):970-976. **(Chapter 6)**

5. De Pablo-Fernández E, Lees AJ, Holton JL, Warner TT. The prognosis and neuropathological correlation of clinical subtypes of Parkinson disease. JAMA Neurol. 2019 (in press). **(Chapter 7)**

Other publications during the degree period

1. **De Pablo-Fernández E**, Doherty KM, Holton JL, Revesz T, Djamshidian A, Limousin P, Bhatia KP, Warner TT, Lees AJ, Ling H. Concomitant fragile X-associated tremor ataxia syndrome and Parkinson's disease: a clinico-pathological report of two cases. J Neurol Neurosurg Psychiatry. 2015 Aug;86(8):934-6.

2. Gami P, Murray C, Schottlaender L, Bettencourt C, **De Pablo Fernández E**, Mudanohwo E, Mizielinska S, Polke JM, Holton JL, Isaacs AM, Houlden H, Revesz T, Lashley T. A 30-unit hexanucleotide repeat expansion in *C9orf72* induces pathological lesions with dipeptide-repeat proteins and RNA foci, but not TDP-43 inclusions and clinical disease. Acta Neuropathol. 2015 Oct;130(4):599-601.

3. Doherty KM, **De Pablo-Fernández E**,** Houlden H, Polke JM, Lees AJ, Warner TT, Holton JL. MSA-C or SCA 17? A clinico-pathological case update. Mov Disord. 2016 Oct;31(10):1582-1584.

** shared first author

4. **De Pablo-Fernández E**, Breen DP, Bouloux PM, Barker RA, Foltynie T, Warner TT. Neuroendocrine abnormalities in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2017 Feb;88(2):176-185.

5. Noyce AJ, Kia DA, Hemani G, Nicolas A, Price TR, **De Pablo-Fernández E**, Haycock PC, Lewis PA, Foltynie T, Davey Smith G; International Parkinson Disease Genomics Consortium, Schrag A, Lees AJ, Hardy J, Singleton A, Nalls MA, Pearce N, Lawlor DA, Wood NW. Estimating the causal influence of body mass index on risk of Parkinson disease: a Mendelian randomisation study. PLoS Med. 2017 Jun 13;14(6):e1002314.

6. **De Pablo-Fernández** E, Sierra-Hidalgo F, Benito-León J, Bermejo-Pareja F. Association between Parkinson's disease and diabetes: data from NEDICES study. Acta Neurol Scand. 2017 Dec;136(6):732-736.

7. **De Pablo-Fernández E**, Warner TT. Dystonia. Br Med Bull. 2017 Sep 1;123(1):91-102.

8. **De Pablo-Fernández E**, Cerdán Santacruz D, Warner TT, Holton JL. No evidence of iatrogenic human transmission in autopsy confirmed multiple system atrophy. Mov Disord. 2018 Jul;33(7):1183-1184.

9. **De Pablo-Fernández E**, Warner TT. Autonomic dysfunction in Parkinson's disease: the hidden game changer? Mov Disord. 2018 Jul;33(6):1028.

10. Batla A, **De Pablo-Fernández E****, Erro R, Reich M, Calandra-Buonaura G, Barbosa P, Balint B, Ling H, Islam S, Cortelli P, Volkmann J, Quinn N, Holton JL, Warner TT, Bhatia KP. Young onset multiple system atrophy: clinical and pathological features. Mov Disord. 2018 Jul;33(7):1099-1107.

** shared first author

11. Oliveira MCB, Ling H, Lees AJ, Holton JL, **De Pablo-Fernandez E****, Warner TT. Association of autonomic symptoms with disease progression and survival in progressive supranuclear palsy. J Neurol Neurosurg Psychiatry. 2018 (epub ahead of print).

**shared last author

Collaborative work

Chapter 2 and 3:

Immunostaining of brain samples was performed by Mr Robert Courtney and Ms Kate Strand, senior technicians at the Queen Square Brain Bank for Neurological Disorders. Analysis and interpretation of the histological findings was performed under the supervision of Prof Janice Holton, Professor in Neuropathology and Neuropathology Director at the Queen Square Brain Bank for Neurological Disorders.

Chapter 4:

Anorectal manometry studies were performed and interpreted by Dr Valentina Passananti and Dr Anton Emmanuel, both from the Gastrointestinal Physiology Unit at University College Hospital in London.

Chapter 5:

Statistical analysis was performed in conjunction with Dr Julia Pakpoor and Dr Raphael Goldacre, from the Oxford Record Linkage Group, Unit of Health-Care Epidemiology, Nuffield Department of Population Health, University of Oxford.

List of abbreviations

AgRP; agouti-related peptide

ARM; anorectal manomery

AutD; autonomic dysfunction

CART; cocaine- and amphetamine-regulated transcript

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

CI; confidence interval

GI; gastrointestinal

GLP1; glucagon-like peptide 1

H&E; haematoxylin and eosin

HES; Hospital Episode Statistics

HR; hazard ratio

ICD-10; International Classification of Diseases - Revision 10

INF; infundibular nucleus

MCH; melanin-concentrating hormone

MDS-UPDRS; Movement Disorders Society-Unified Parkinson's Disease Rating Scale

MSA; multiple system atrophy

MSH; melanocyte-stimulating hormone

MRI; magnetic resonance imaging

NIA; National institute on Aging

NMS; non-motor symptoms

NMSQ; non-motor symptom questionnaire

NPY; Neuropeptide Y

PD; Parkinson's disease

PPMI; Parkinson's disease Progression Markers Initiative

PPAR-γ; peroxisome proliferator-activated receptor γ

PSP; progressive supranuclear palsy

PVN; paraventricular nucleus

QSBB; Queen Square Brain Bank for neurological disorders

RBD; Rapid eye movement (REM) sleep behaviour disorder

SCN; suprachiasmatic nucleus

SNpc; substantia nigra pars compacta

SON; supraoptic nucleus

SNpc; substantia nigra pars compacta

T2DM; type 2 diabetes mellitus

TDP43; transactive response DNA binding protein 43

TH; tyrosine hydroxylase

TRH; thyrotropin-releasing hormone

UPDRS; Unified Parkinson's Disease Rating Scale

VIP; vasointestinal peptide

Table of contents

Abstract **7**

Impact statement **9**

Publications related to this thesis **11**

Other publications during the degree period **13**

Collaborative work **15**

List of abbreviations **17**

Table of contents **19**

List of tables **25**

List of figures **27**

Chapter 1. Non-motor features in Parkinson's disease. **29**

1.1 The clinical relevance. 32

1.2 Pathophysiological implications of non-motor features. 35

1.3 The evolving concept of Parkinson's disease and other unanswered questions 38

Section I: Pathophysiological mechanisms of non-motor features in Parkinson's disease **41**

Chapter 2. Hypothalamic dysfunction in Parkinson's disease: a clinicopathological study of the hypothalamus. **43**

2.1 Introduction 43

2.1.1 The human hypothalamus 43

2.1.2 The paraventricular, supraoptic and infundibular nuclei of the hypothalamus 44

2.1.3 Hypothalamic dysfunction in Parkinson's disease 47

2.2 Methods. 50

2.2.1 Study design and settings 50

2.2.2 Clinical assessment 50

2.2.3 Neuropathological assessment.....54

2.3 Results 57

2.4 Discussion.....61

Chapter 3. Circadian system in Parkinson's disease: A histological analysis of the suprachiasmatic nucleus and pineal gland..... 65

3.1 Introduction.....65

3.1.1 Circadian system in humans 65

3.1.2 The circadian system in degenerative parkinsonian disorders 67

3.2 Methods 70

3.2.1 Study design and participants.....70

3.2.2 Neuropathological assessment.....70

3.3 Results 73

3.4 Discussion.....76

Chapter 4. Constipation in Parkinson's disease: a clinical, colonic transit, high-resolution manometry and MRI defecography study. 81

4.1 Introduction.....81

4.1.1 Role of the GI system in Parkinson's disease pathogenesis 81

4.1.2 Constipation in Parkinson's disease.....83

4.1.3 Evaluation of constipation 84

4.2 Methods 88

4.2.1 Subjects and study design.....88

4.2.2 Clinical assessment of constipation 88

4.2.3 Colonic transit, high resolution anorectal manometry and MRI defecography 89

4.2.4 Statistical analysis 90

4.3 Results 92

4.3.1 Clinical assessment of constipation 94

4.3.2 Colonic transit, high resolution anorectal manometry and MRI defecography 97

4.3.3 Slow transit and anorectal dysfunction 97

4.4 Discussion 100

4.4.1 Clinical assessment 100

4.4.2 Colonic transit, high resolution anorectal manometry and MRI defecography 101

4.4.3 Conclusions 104

Section II: The role of non-motor features on the pathogenic process of Parkinson's disease 107

Chapter 5. Association between diabetes and subsequent Parkinson's disease: a record-linkage cohort study 109

5.1 Introduction 109

5.2 Methods 111

5.2.1 Study design, population and data 111

5.2.2 Statistical analysis 112

5.3 Results 114

5.4 Discussion 116

5.4.1 Epidemiological evidence 116

5.4.2 Pathogenic mechanisms 117

5.4.3 Clinical and potential therapeutic implications 118

5.4.4 Limitations and conclusions 119

Chapter 6. Autonomic dysfunction in Parkinson's disease – impact on disease progression and survival. 121

6.1 Introduction 121

6.1.1 The autonomic nervous system 121

6.1.2 Autonomic dysfunction in Parkinson's disease 124

6.2 Methods 128

6.2.1 Study design 128

6.2.2 Clinical assessment 128

6.2.3 Neuropathological assessment 129

6.2.4 Statistical assessment 130

6.3 Results 132

6.3.1 Demographics and clinical features 132

6.3.2 Association between AutD and other variables 137

6.3.3 Effect of autonomic dysfunction on disease progression 139

6.3.4 Effect of autonomic dysfunction on survival 139

6.3.5 Effect of individual autonomic symptoms on disease progression and survival 143

6.3.6 Other predictors of survival 143

6.4 Discussion 145

Chapter 7. The prognosis and neuropathological correlation of clinical subtypes in Parkinson's disease. 151

7.1 Introduction 151

7.2 Methods 154

7.2.1 Study design 154

7.2.2 Clinical assessment 154

7.2.3 Subtype definitions 156

7.2.4 Neuropathological assessment 157

7.2.5 Statistical analysis 158

7.3 Results 159

7.3.1 Disease progression and survival in Parkinson's subtypes.....163

7.3.2 Neuropathology in Parkinson's subtypes.....168

7.4 Discussion.....170

7.4.1 Parkinson's subtype classification. 170

7.4.2 Disease progression and prognosis. 171

7.4.3 Neuropathological correlation and pathophysiological implications of clinical subtypes.....172

Summary of findings and future work 181

Acknowledgements 189

Bibliography 191

Appendix 215

List of tables

Table 1.1. Non-motor symptoms and anatomical correlation.....33

Table 2.1. Main hypothalamic nuclei with their major neuropeptides and functions. 45

Table 2.2. Clinical characteristics of study participants. 53

Table 2.3. Demographic and histological data. 58

Table 3.1. Demographics and histological findings. 74

Table 4.1. Demographics, clinical data and GI investigations. 93

Table 4.2. Correlation of constipation severity. 95

Table 5.1. Hazard ratios and 95% confidence intervals in the exposed T2DM cohort (compared with reference cohort). 115

Table 6.1. Peripheral structures of the autonomic nervous system. 123

Table 6.2. Patient demographic and clinical features. 135

Table 6.3. Association between autonomic dysfunction and other variables. .138

Table 6.4. Risk for disease progression and survival 141

Table 6.5. Multivariable analysis of survival predictors. 144

Table 7.1. Demographic and clinical data by PD subtype. 162

Table 7.2. Disease progression and survival data by PD subtype. 164

Table 7.3. Cox proportional hazard regression models of PD subtypes and other significant variables for milestones and survival. 166

Table 7.4. Neuropathological findings by PD subtypes. 169

List of figures

Figure 2.1. Neuroanatomy of PVN, SON and INF hypothalamic nuclei. 55
Figure 2.2. Representative sections of INF, PVN and SON hypothalamic nuclei.56
Figure 2.3. Diagram representing Lewy pathology in PVN, INF and SON. 60
Figure 2.4. Diagram representing TH immunoreactivity in PVN, INF and SON...60
Figure 3.1. The circadian system. 66
Figure 3.2. Representative sections of the SCN and pineal gland. 72
Figure 3.3. Bar graph representing neuropathological findings in SCN and pineal by disease. 75
Figure 4.1. Scatter showing correlation of constipation severity. 96
Figure 4.2. Pathophysiological interpretation of GI investigations.....99
Figure 6.1. Flow chart and comparison between excluded vs included patients. 133
Figure 6.2. Box-and-whisker plots showing time from diagnosis to autonomic symptoms, disease milestones and death.....136
Figure 6.3. Box-and-whisker plots showing time from diagnosis to milestones by early vs late autonomic dysfunction.....140
Figure 6.4. Kaplan-Meier curves for survival (left) and risk of first milestone (right) by early vs late autonomic dysfunction.....140
Figure 6.5. Kaplan-Meier curves for disease progression by early vs late autonomic dysfunction.....142
Figure 7.1. Flow chart.....160
Figure 7.2. Box-and-whisker plots showing time from diagnosis to milestones and death by PD subtypes. 165
Figure 7.3. Kaplan-Meier curves of cumulative probability and risk of disease milestones.....167
Figure 7.4. Schematic representation of the clinical course illustrating symptom severity over time for PD subtypes. 175
Figure 7.5. Contributing factors of neuropathological heterogeneity in PD. ...178

Chapter 1. Non-motor features in Parkinson's disease.

Parkinson's disease (PD) is a common and complex neurodegenerative disorder with an exponentially increasing burden.(1) The first detailed description of the disease was provided by James Parkinson in his famous monograph *An essay on the shaking palsy* in 1817, which was further elaborated and refined by Jean-Martin Charcot who proposed the name of Parkinson's to this disease. PD is the most common neurodegenerative condition after Alzheimer's disease and, given its strong association with age, PD prevalence is rapidly increasing in the world's aging population. Epidemiological studies revealed a global prevalence of more than 6.1 million worldwide in 2016, comprising about 1% in population over 60 years of age in industrialised countries with a slight male predominance.(2)

PD is still defined clinically by the classical parkinsonian motor features with the combination of bradykinesia alongside either resting tremor or muscular rigidity, whilst postural instability tends to be a later feature. The diagnosis of the disease is clinical and the United Kingdom Parkinson's Disease Society Brain Bank criteria are the most widely accepted, requiring the presence of parkinsonian motor features with additional supportive findings in the absence of alternative causes of parkinsonism.(3) Despite significant efforts on the search of specific biomarkers for PD, no test allows confirmation of the diagnosis during life and post-mortem neuropathological examination remains the gold standard.

The essential neuropathological hallmark of PD is the combination of moderate to severe cell loss in the ventrolateral tier of the substantia nigra pars compacta (SNpc) associated with Lewy pathology in brainstem structures, although no consensus standardised neuropathological recommendations are available.(4) Both neuropathological abnormalities are consistently found in patients with idiopathic PD but they are not specific in isolation and can be found in a wide range of disorders. Gliosis and cell loss in the SNpc is present in other

degenerative parkinsonian disorders associated with abnormal protein deposition (including multiple system atrophy - MSA, progressive supranuclear palsy - PSP, corticobasal degeneration - CBD and some forms of frontotemporal lobe degeneration) but can also be found without other distinctive histological key features in some monogenic forms of PD, such as those associated with *parkin* or *LRRK2* mutations.(5) Lewy pathology refers to the constellation of neuronal aggregates including Lewy bodies (round eosinophilic inclusions with a halo found in the neuronal perikarya) and other inclusions in neuronal processes such as Lewy neurites. The main component of Lewy pathology is misfolded α-synuclein (6), and therefore immunostaining with antibodies against this protein has become the standard and most sensitive immunohistochemical technique for diagnostic purposes. Lewy pathology is commonly found in other brainstem structures outside SNpc such as the locus coeruleus, the dorsal motor nucleus of the vagus or nucleus basalis of Meynert, but also in many selectively vulnerable neuronal populations throughout the central nervous system. Staging systems have been proposed for the assessment of the distribution of Lewy pathology. As it has been hypothesised that Lewy pathology spreads in a stereotyped pattern among different susceptible anatomical regions, Braak and colleagues proposed a system with six different stages based on the caudo-rostral progression.(7) This staging system has gained attention as it may explain, at least in a great proportion of patients, the clinical progression of the disease, with α-synuclein deposition starting in structures of the enteric nervous system and the olfactory bulb responsible for the prodromal symptoms, progressing eventually to the motor impairment once the pathology spreads to the SNpc. Although the Braak staging system has become popular, its acceptance and general applicability is still a matter of significant debate.(8-10) The Dementia with Lewy Bodies Consortium recommended a system based not only on the distribution but also on the severity of Lewy pathology in multiple representative central nervous system areas using a semiquantitative scoring system differentiating three stages (brainstem, limbic and neocortical)(11) which is widely accepted in the neuropathological evaluation of Lewy body disorders.

From a pathophysiological perspective, involvement of the SNpc by the neuropathological process is the key element in the development of the classical motor symptoms of PD. Dopaminergic neurons of the SNpc project their terminals to the striatum in a somatotopic pattern and there is a well-established pathophysiological correlation between the SNpc neuronal loss and the classic motor features of the disease. Classically, PD has been viewed as a neurodegenerative disease with prominent involvement of the dopaminergic neurons in the SNpc resulting in a dopamine deficiency in the basal ganglia leading to the cardinal motor symptoms. It has been estimated that a loss of 30% of total SNpc dopaminergic neurons and about 50-60% of their terminals (with a much more profound reduction of putaminal dopamine) is required for the onset of motor symptoms.(12, 13)

1.1 The clinical relevance.

Although motor symptoms and signs continue to be the defining features of PD, it is now well recognised that a constellation of non-motor features are also an integral part of the disease. Some non-motor features were reported in James Parkinson's initial description of the condition but it is only in recent years that they have attracted the attention of clinicians and researchers. These include autonomic dysfunction (AutD), sleep disturbances, sensory symptoms and neuropsychiatric complications (**Table 1.1**).

Non-motor features	Anatomical correlation
Autonomic dysfunction	Hypothalamus, insula, sympathetic ganglia/parasympathetic nuclei, dorsal motor nucleus of the vagus
Orthostatic hypotension	Cardiac sympathetic nerves, adrenal gland
Urogenital dysfunction	Pelvic plexus
Constipation	Enteric nervous system
Neuropsychiatric	
Dementia	Neocortex, limbic system, nucleus basalis of Meynert, locus coeruleus,
Mood disorders	Locus coeruleus, raphe nuclei, limbic system, temporal cortex
Psychosis	Limbic system, visual cortex
Impulse control disorders	Basal forebrain (amygdala, accumbens)
Sensory	
Anosmia	Olfactory bulb, amygdala, perirhinal cortex
Pain	Thalamus, locus coeruleus, raphe nuclei, epidermal nerves
Sleep	
Insomnia and sleep fragmentation	Lateral hypothalamic area, laterodorsal pontine tegmentun nuclei, reticular formation
REM sleep behaviour disorder	Laterodorsal pontine tegmentun nuclei
Restless legs syndrome	Cingulate, striatonigral system, thalamus

Table 1.1. Non-motor symptoms and anatomical correlation.

In their various combinations, they may eventually dominate the clinical picture and present a therapeutic challenge for clinicians, particularly in advanced stages of the disease. Non-motor symptoms (NMS) have been long neglected in clinical practice and significant progress has been made to improve their recognition and quantification with the development of specific diagnostic tools and severity scales.(14) They are an integral feature of PD and not secondary to disruption of other physiological processes or side effects from medication, are frequent at any stage of the disease and contribute significantly to the quality of life, disability and morbidity burden.(15) This burden is aggravated by the poor response of non-motor features to dopaminergic replacement therapies commonly used in PD, suggesting that other neurotransmitters may be involved in the pathophysiology of these symptoms. Moreover, evidence from histological studies has shown that Lewy pathology is never restricted to the nigrostriatal system and α-synuclein is distributed in multiple neuroanatomical sites.(16) Although an exact clinico-pathological correlation is still to be established, it is well accepted that non-motor manifestations of PD are likely to arise from the additional involvement of other non-dopaminergic neurotransmitters in multiple structures of the central, peripheral and autonomic nervous system (**Table 1.1**). Therefore, their management should include therapeutic interventions and drugs targeting other areas apart from the dopaminergic nigrostriatal system, although the evidence to support their recommendation is weak in most cases (17) and further research is needed in this area to provide more effective treatments.

1.2 Pathophysiological implications of non-motor features.

Some of the NMS in PD such as constipation, rapid eye movement behaviour disorder (RBD), dysautonomia, anosmia or neuropsychiatric disturbances frequently precede the onset of the classical motor symptoms, sometimes by decades.(18) This prodromal phase of the disease, in which non-motor features and other early signs of neurodegeneration occur before full parkinsonism develops, has important pathophysiological implications. Several studies have demonstrated that α-synuclein deposition occurs early in the disease even at prodromal stages in other anatomical structures outside the SNpc such as the medulla oblongata, olfactory bulb, enteric and autonomic nervous system.(19) It is accepted that symptoms during the prodromal phase are due to the pathogenic process and neurodegeneration, and α-synuclein deposition in peripheral and central structures outside the SNpc is well underway before a clinical diagnosis can be reliably made. Diagnosis of prodromal PD would therefore be essential to provide a window of opportunity for future disease-modifying drugs and several non-motor features have been suggested as biomarkers for early diagnosis of the disease and identification of at risk populations.(20)

The staging system proposed by Braak fits with this idea that α-synuclein deposition starts in the peripheral nervous system, olfactory bulb and medulla during the prodromal phase before spreading to more rostral structures, reaching the SNpc when classical motor symptoms develop.(7) This staging system has gained acceptance, although whether this scheme is valid for every PD patient, and whether the pathological progression correlates with the timing of non-motor features in PD is still a matter of controversy.(8-10) The early development of anosmia and constipation associated with neuropathological changes in the olfactory bulb and enteric nervous system also led to the hypothesis that these structures could be the starting or triggering neuroanatomical site of the PD pathogenesis.(21) Although this hypothesis is speculative and under debate, it has attracted significant attention from

researchers on the early pathophysiology of PD and the spread of neuropathological changes and clinical correlations during the disease course.

The pathophysiology of the non-motor features in PD is complex and poorly understood but it seems likely that most of these symptoms are multifactorial with other external elements (including dopaminergic medication) contributing to their pathophysiology. Clinico-pathological correlations between non-motor symptoms and Lewy pathology affecting multiple neuroanatomical structures have been attempted in order to provide a better understanding of the underlying pathogenic abnormalities (**Table 1.1**). However, this association may not be as straightforward as with the classical motor features and further research is warranted.(16, 22)

PD is markedly heterogeneous with respect to the presence and severity of symptoms, disease course and response to treatments, and the wide range of non-motor features plays a significant role in this clinical variability.(23) In order to estimate prognosis and to provide a better understanding of disease progression, significant efforts have been made to identify disease features with prognostic value, and definition of PD subtypes with similar disease characteristics and course has become a research priority. Traditionally PD subtypes have been defined by motor features and other general demographics such as age at onset. However, growing evidence suggests that some non-motor features such as orthostatic hypotension, RBD or cognitive impairment might be potential key determinants in PD progression and prognosis, and new PD subtypes incorporating these symptoms have been recently proposed. (24-26) The mechanisms by which some NMS can modify the course of the disease are poorly understood, and whether they can influence the degenerative process of Lewy pathology or their effect is a consequence of increased morbidity remains to be elucidated.

Finally, as key symptomatic features specific to PD, it seems reasonable that non-motor symptoms should be considered in the clinical diagnosis of PD and

new criteria incorporating non-motor features have been recently proposed.(27) The utility of these diagnostic criteria has been recently validated in a large cohort of prospective patients in the United Kingdom with good results.(28)

1.3 The evolving concept of Parkinson's disease and other unanswered questions.

Advances over the last two decades in the understanding of the underlying pathophysiological mechanisms, the anatomical areas involved and the clinical heterogeneity of the disease regarding NMS have provided valuable insights on clinical progression and disease pathogenesis. These advances have challenged the traditional concept of PD as a predominantly motor disorder due to dopaminergic deficits in the nigrostriatal system. This concept has been gradually replaced by a more complex model encompassing a slowly neurodegenerative disorder that starts many years before the clinical diagnosis, involving multiple neuroanatomical areas of the central and peripheral nervous system, affecting dopaminergic and non-dopaminergic pathways, and that manifests with a wide range of motor and non-motor symptoms. Research on non-motor aspects has therefore led to significant advances in the understanding of PD pathogenesis and progression although important questions remain unanswered. These include better clinico-anatomical correlations and understanding of the mechanisms and neurotransmitters involved in the underlying pathogenesis, the potential influence that some non-motor features may have in the progression of PD or its associated neurodegenerative process and how this can be applied for prognostic estimations, PD subtyping and, eventually, individualised symptomatic and disease modifying treatments.

Through different projects using clinical, epidemiological, imaging, electrophysiological and neuropathological methods, this thesis aims to provide further evidence that improves the understanding of the bidirectional influence between some of the non-motor features and the pathogenic mechanisms of PD. The first part explores the underlying pathophysiological mechanisms and neuroanatomical correlations of some of the non-motor features associated with PD including hypothalamic dysfunction, circadian abnormalities and gastrointestinal dysfunction. As previously discussed, evidence suggests that

some non-motor features are not just clinical manifestations of the underlying neurodegeneration in PD, but can also influence the pathogenic process as predisposing factors, modifying the clinical phenotype or affecting the clinical course and prognosis. In this regard, the second part of the thesis investigates how abnormalities of glucose metabolism and autonomic dysfunction affect the pathophysiology of PD, and how relevant non-motor features can be used to determine clinical PD subtypes.

Section I: Pathophysiological mechanisms of non-motor features in Parkinson's disease

Chapter 2. Hypothalamic dysfunction in Parkinson's disease: a clinicopathological study of the hypothalamus.

2.1 Introduction

2.1.1 The human hypothalamus

The hypothalamus is a small but complex structure situated in the ventral part of the diencephalon and laterally to the walls of the third ventricle. The functional neuroanatomical organisation of the hypothalamus is very complex and its neurons and networks are involved in the integration and regulation of several important body functions.

The hypothalamus consists of several interconnected nuclei that are cytologically and neurochemically well characterised. From a physiological perspective, hypothalamic function can be divided into neuroendocrine regulation through the release of neurohormones and maintaining of body homeostasis using several neurotransmitters and neuromodulators. Some of the hypothalamic nuclei are critically involved in the regulation of the main neurendocrine axes including the hypothalamic-pituitary-adrenal, hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid. These neuroendocrine cells project to the neurohypophysis or pituitary gland where the released factors act as neurohormones. The hypothalamus receives the negative feedback from the circulating hormones which in turn modulate the neuroendocrine hypothalamic output signals.

Over the last few decades, the additional role of the hypothalamus in the regulation of additional body functions and body homeostasis has been better understood. A large number of neurochemically diverse neurons in the hypothalamus create a complex network within and outside the hypothalamus where their released factors function as neurotransmitters or neuromodulators.

These hypothalamic networks act as integrators and regulators of multiple body functions such as sleep, energy metabolism, fluid regulation or autonomic responses. It is also well accepted that some of these neurons possess complex bidirectional connections with the limbic system and other cortical areas, and the hypothalamus can influence higher functions such as memory, emotions, behaviour or mood.

2.1.2 The paraventricular, supraoptic and infundibular nuclei of the hypothalamus

The hypothalamus is formed by multiple cytologically and neurochemically diverse nuclei with a complex functional neuroanatomical organization. An overview of the main hypothalamic nuclei with their neuropeptides and functions are shown in **Table 2.1**.

Nuclei	Hypothalamic anatomical area	Neuropeptides	Functions
Lateral hypothalamus	Lateral	Orexin (hypocretin), CART, MCH	Sleep-wake, energy metabolism, emotion
Paraventricular	Periventricular	Vasopressin, oxytocin, CRH, TRH	Autonomic function, pituitary control (HPA, HPT axes), body fluid and blood pressure
Infundibular	Periventricular	CART, MSH, AgRP, NPY, GHRH	Energy metabolism, feeding and growth
Supraoptic	Lateral	Vasopressin, oxytocin	Body fluid and blood pressure, labour and lactation
Tuberomammillary	Mammillary	Histamine	Arousal, sleep-wake, thermoregulation
Suprachiasmatic	Anterior	VIP, vasopressin	Sleep-wake and circadian regulation

Table 2.1. Main hypothalamic nuclei with their major neuropeptides and functions.

CRH, corticotropin-releasing hormone; GHRH, growth hormone releasing hormone; HPA, hypothalamic-pituitary-adrenal axis; HPT, hypothalamic-pituitary-thyroid axis; MCH, melanin-concentrating hormone; TRH, thyrotropin-releasing hormone; VIP, vasointestinal peptide.

The paraventricular nucleus of the hypothalamus (PVN) plays a pivotal role in the integration of adaptive autonomic responses necessary for body homeostasis. (29) It contains parvocellular neurons projecting to the autonomic control centres in the lower brainstem nuclei and the neurohypophysis which regulates stress responses of the hypothalamic-pituitary-adrenocortical axis.(30) The projections of the magnocellular neurons of the PVN are joined by similar neurons in the supraoptic nucleus (SON) to the posterior pituitary gland releasing vasopressin and oxytocin to the bloodstream regulating a complex neuroendocrine pathway involved in body fluid homeostasis, plasma volume expansion and blood pressure regulation.(31) The infundibular nucleus (INF) acts as the central regulator of homeostatic feeding behaviour and energy metabolism receiving input from peripheral humoral signals and central hedonic information.(32) The INF integrates this information and its activity has two opposite functions: anorexigenic cells produce cocaine- and amphetamine-regulated transcript (CART) and melanocyte-stimulating hormone (MSH), while orexigenic activity is controlled by the secretion of agouti-related peptide (AgRP) and neuropeptide Y (NPY).(33)

2.1.3 Hypothalamic dysfunction in Parkinson's disease

The wide range of non-motor features in PD includes the disruption of several neuroendocrine functions in which the hypothalamus has a significant regulatory role. Autonomic function, body weight and blood pressure are all regulated, at least to a certain degree, by hypothalamic structures and they can all be disrupted in PD.(15) These findings suggest that hypothalamic dysfunction caused by the neurodegenerative process may be responsible for some of these NMS in PD.

AutD is common in patients with PD and includes cardiovascular (orthostatic hypotension, supine hypertension), gastrointestinal (gastroparesis, constipation) urogenital (overactive bladder, urinary retention, erectile dysfunction) and thermoregulatory symptoms.(34) AutD in patients with PD has been traditionally associated with involvement of peripheral autonomic structures by Lewy pathology (as opposed to MSA where there is predominant involvement of the central nervous system) although neuropathological abnormalities in central autonomic centres are commonly found in post-mortem studies.(35) Orthostatic hypotension, defined as a sustained fall of > 20 mmHg systolic or > 10 mmHg diastolic within 3 minutes of standing, is arguably one of the most disabling signs of AutD and has been associated with risk of falls, cognitive impairment and reduced survival.(36, 37) It has an estimated prevalence of 30% in patients with PD although only a third of them develop symptoms such as syncope, blurred vision or coat hanger pain.(38) Abnormalities of body weight are well recognised and an unintentional progressive loss in body weight has been consistently reported affecting 50% of PD patients with an overall reduction of 1.73kg/m^2. (39-41) Weight loss in PD has important clinical implications as it is shown to be associated with disease severity and to a more rapid disease progression.(42) The mechanisms underlying the body weight abnormalities in PD are likely to be multifactorial and not just secondary to an energy expenditure/intake imbalance.(43) Dysregulation of central mechanisms of feeding behaviour and body

metabolism have been suggested as patients undergoing deep brain stimulation of the subthalamic nucleus experience a rapid weight gain greatly exceeding the weight loss see in medically treated patients.(44)

Evidence from different areas of research supports the involvement of the hypothalamus in PD. Magnetic resonance imaging (MRI) studies have shown a reduction of hypothalamic grey matter volume.(45) Abnormalities involving both dopaminergic (46) and non-dopaminergic transmission (47) using positron tomography emission have also been reported. The presence of pathological changes in the hypothalamus of PD patients has been known since the initial anatomical studies in the late 1970s. Lewy bodies have been demonstrated in most of the hypothalamic nuclei including the SON and INF although the PVN could not be consistently identified with historical routine staining techniques.(48) Subsequent studies investigated specific hypothalamic neuronal populations in these nuclei in patients with PD describing cell loss and plasticity changes involving the magnocellular neurons of the SON (49) and oxytocin neurons of the PVN (50) but Lewy bodies were rarely present in the SON and could not be identified in the PVN. Given the crucial role of dopamine in PD, another study assessed neuropathological changes in the hypothalamic dopaminergic neurons of the PVN and INF nuclei but no cell loss was found (50, 51) despite evidence of dopaminergic dysfunction on a previous positron tomography emission study.(46) Although taken together these results from previous imaging and pathological studies showed evidence of hypothalamic involvement in PD, results are difficult to interpret, limited by the use of old immunohistochemical techniques and a correlation of histological abnormalities with disease progression and non-motor features has never been performed. As hypothalamic nuclei are neurochemicaly well defined, and some of them are involved in releasing hormones easily measurable in blood and plasma, a better understanding of the hypothalamic involvement in PD and its correlation with the presence of non-motor features could potentially help in the development of biomarkers and symptomatic treatments in the future.

In this study, we studied the presence of α-synuclein pathology and tyrosine hydroxylase (TH) immunoreactivity, as a dopaminergic marker, in three hypothalamic nuclei with potential clinical implications in PD symptomatology (PVN, INF and SON) at different stages of the disease and correlated the histological findings with the clinical severity of non-motor features.

2.2 Methods.

2.2.1 Study design and settings

This is a case-control clinicopathological study of participants selected from the archives of the Queen Square Brain Bank for neurological disorders (QSBB). General information on the QSBB donation program can be found in *Appendix 1*. Patients with neuropathological confirmation of the diagnosis of PD were matched with subjects with incidental Lewy body disease (people without evidence of neurological symptoms in life but presence of Lewy pathology on post-mortem examination) and healthy controls without clinical or neuropathological evidence of a neurological disease. The donation programme is approved by a London Multi-Centre Research Ethics Committee and the tissue is stored under a license from the Human Tissue Authority. Written consent was obtained from all participants for the use of clinical information and brain tissue for research purposes.

Statistical significance was set at $p<0.05$ and results were not adjusted for multiple comparisons given the exploratory character of the study. Kruskal-Wallis test was used for global comparisons and Mann-Whitney U test for pairwise comparisons between groups. Stata 12 (StataCorp, TX) software package was used for statistical analysis.

2.2.2 Clinical assessment

Clinical records including primary care medical notes and the correspondence between medical specialists and general practitioners were systematically reviewed. All PD patients were regularly reviewed by hospital specialists (neurologists or geriatricians) throughout the course of their illness. All other participants were registered with general practitioners in the United Kingdom.

Additionally, prospective brain donors were required to fill in QSBB registration and annual assessment forms containing relevant information about their medical condition.

Information on weight changes, AutD and orthostatic hypotension was documented and the severity of the symptoms was graded using a semiquantitative score and classified as absent, mild, moderate or severe based on the clinical impression of the treating physician. These symptoms were clinically defined for this research study as follows. Weight loss was defined as an unexplained sustained (> 2 years) average reduction in body weight at any time during the disease course greater than 1.5 kg/year based on longitudinal studies reporting an average weight loss of about 0.45 kg/year in PD patients,(40, 52): the amount of weight loss was subsequently graded as mild (>1.5-2 kg/year), moderate (>2-2.5 kg/year) or severe (>2.5 kg/year). Symptomatic orthostatic hypotension was defined as >20mm systolic or >10mm diastolic blood pressure drop upon standing associated with postural symptoms. AutD was defined by either abnormal cardiovascular autonomic function testing or documentation of any two of the following symptoms persistent for longer than 6 months and not attributable to a non-neurological cause:(53) (i) urinary urgency, increased daytime frequency and nocturia without hesitancy as defined by the International Continence Society,(54) (ii) constipation (< 3 defecations per week), having to strain to pass stools or regular use of laxatives, (iii) symptoms of upper gastrointestinal dysfunction including nausea, bloating and early satiety, (iv) symptomatic or documented orthostatic hypotension, (v) sweating abnormalities or (vi) erectile dysfunction in males.

PD patients were subdivided in two groups based on the presence and severity of non-motor features: those with moderate-severe weight loss, autonomic failure and symptomatic orthostatic hypotension (PD+NMS group) were matched by age and disease duration with patients with a pathological diagnosis of PD and absent or mild weight loss, autonomic failure or

symptomatic orthostatic hypotension (PD-NMS group). Groups were compared to age-matched asymptomatic subjects with incidental Lewy body disease (incidental group) and healthy control individuals (control group)(**Table 2.2**).

Case number	Weight loss	Autonomic dysfunction	Orthostatic hypotension
PD+NMS			
1	Moderate	Urinary symptoms-severe Constipation-severe Upper GI symptoms-severe Erectile dysfunction	Severe
2	Moderate	Urinary symptoms-severe Constipation-severe	Severe
3	Severe	Urinary symptoms-severe Constipation-severe	Severe
4	Severe	Constipation-severe Upper GI symptoms-severe Erectile dysfunction Abnormal AFT	Severe
5	Severe	Urinary symptoms-severe Constipation-severe	Severe
6	Severe	Urinary symptoms-severe Upper GI symptoms-severe Erectile dysfunction Excessive sweating Abnormal AFT	Severe
7	Moderate	Urinary symptoms-severe Constipation-moderate Erectile dysfunction	Moderate
8	Moderate	Urinary symptoms-severe Constipation-severe	Moderate
PD-NMS			
9	Absent	Constipation-mild Urinary symptoms-mild	Absent
10	Absent	Absent	Absent
11	Absent	Constipation-mild Urinary symptoms-mild	Mild
12	Absent	Absent	Absent
13	Absent	Absent	Absent
14	Absent	Constipation-mild Urinary symptoms-mild	Absent
15	Absent	Constipation-mild Urinary symptoms-mild	Absent
Incidental Lewy body disease			
16 - 22	Absent	Absent	Absent
Healthy controls			
23 - 29	Absent	Absent	Absent

Table 2.2. Clinical characteristics of study participants.

*AFT, autonomic function test; GI, gastrointestinal.

2.2.3 Neuropathological assessment

Formalin-fixed brain tissue blocks were sampled from representative areas of the central nervous system following standard QSBB protocols. The neuropathological diagnosis of PD was defined as the presence of moderate to severe cell loss in the ventrolateral tier of the SNpc associated with Lewy pathology in key vulnerable neuronal populations of the brainstem.(3, 55) Formalin-fixed tissue blocks including the hypothalamus were sampled and 8µm thick sections were stained with haematoxylin and eosin (H&E) complemented with appropriate immunohistochemistry (**Figure 2.1**). TH catalyses the conversion of tyrosine to levodopa and it is the rate limiting enzyme in the cathecholamine synthesis pathway. TH expression is a well-recognised marker of dopaminergic neurons and immunohistochemistry to this enzyme (1:5000, T2928, Sigma-Aldrich, St Louis, MO, USA) was used as a dopaminergic marker. TH immunoreactivity was graded in the three hypothalamic nuclei and also in the putamen and caudate nuclei using a semiquantitative scoring (intense, moderate, mild, absent). Additional immunohistochemistry against specific hypothalamic cell populations was used to help in the identification of hypothalamic nuclei, including oxytocin (1:2000, ab2078, abcam, Cambridge, MA, USA) for PVN and SON, CART (1:200, sc-366086, Santa Cruz, CA, USA) for PVN and INF and AgRP (1:1000, Phoenix Pharmaceuticals H-003-53, Belmont, CA, USA) for INF (**Figure 2.2**).

Lewy pathology was assessed for each case in each of the hypothalamic nuclei using a semiquantitative grading system (absent, mild, moderate, severe, very severe) identical to the consensus on synuclein pathology staging system.(11) For each case, distribution of α-synuclein pathology was also evaluated according to the staging system proposed by Braak (stage 0-6).(7) Assessment of the severity of histological changes was performed blinded to the symptomatology and clinical diagnoses.

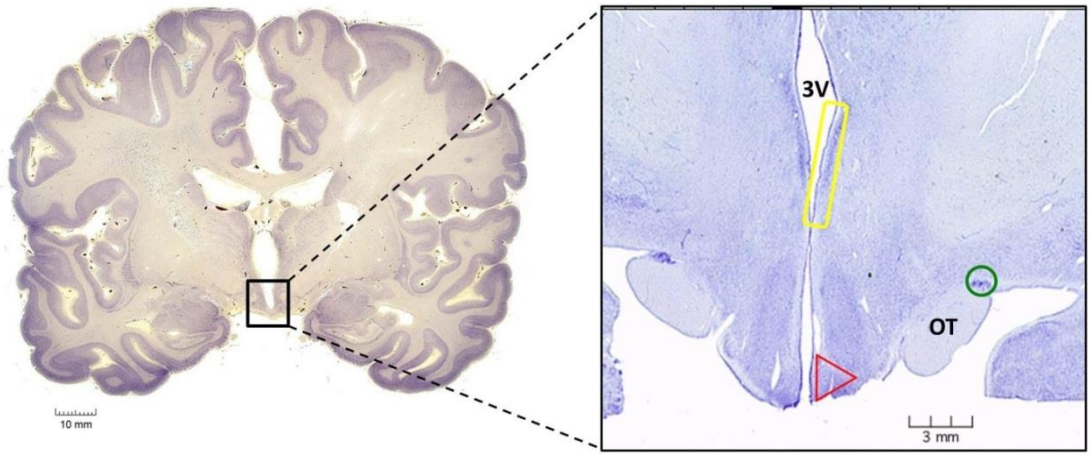


Figure 2.1. Neuroanatomy of PVN, SON and INF hypothalamic nuclei.

Representative coronal section of the brain (left) and hypothalamus (right) showing the neuroanatomy of the paraventricular (yellow rectangle), supraoptic (green circle) and infundibular (red triangle) hypothalamic nuclei. Photomicrographs are stained with Nissl cresyl violet method (original images obtained with permission from the Michigan State University Brain Biodiversity Bank).

3V, third ventricle; OT, optic tract.

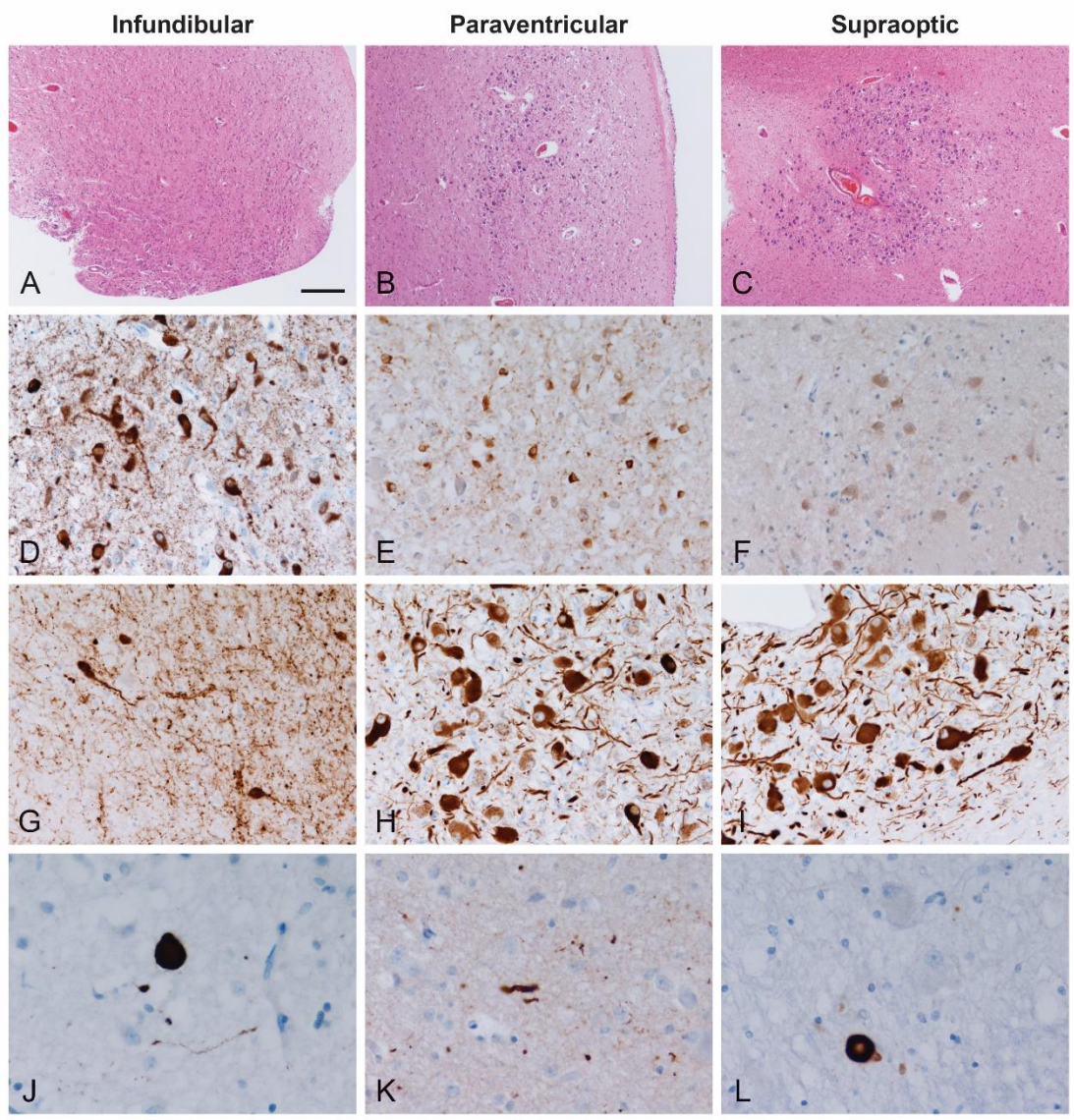


Figure 2.2. Representative sections of INF, PVN and SON hypothalamic nuclei.

Images of H&E staining (A-C) complemented with immunohistochemistry for specific hypothalamic cell populations: AgRP (D), CART (E) and oxytocin (F). TH immunoreactivity was used as dopaminergic marker (G-I) and α-synuclein immunoreactivity (J-L) for assessment of Lewy pathology. Bar in a represents 260µm in A-C, 50µm in D-I and 25µm in J-L.

2.3 Results

Cases were retrospectively selected and due to limited hypothalamic tissue availability a total of eight PVN, five INF and five SON nuclei were examined in the PD+NMS group; seven PVN, seven INF and seven SON in the PD-NMS group; seven PVN, five INF and six SON in the incidental group; six PVN, five INF and five SON in the healthy controls. Comparisons of the main demographics and histological analysis are presented in **Table 2.3**.

	Controls	Incidental	PD-NMS	PD+NMS
Female:male	5:2	2:5	1:6	0:8
Age in years (mean ± SD)	81.51 ± 8.27 (*P*=0.82)	84.59 ± 4.89 (*P*=0.16)	77.49 ± 3.40 (*P*=0.25)	80.55 ± 4.29
Braak stage (n)		*P*=0.005	*P*=0.56	
Stage 2		1	0	0
Stage 3		2	0	0
Stage 4		3	0	1
Stage 5		0	1	1
Stage 6		1	6	6
Disease duration in years (mean ± SD)	NA	NA	9.99 ± 5.26 (*P*=0.49)	11.67 ± 4.44
PVN α-synuclein	*P*<0.001	*P*=<0.001	*P*=0.20	
Absent	6	6	0	0
Mild	0	1	4	7
Moderate	0	0	3	1
PVN TH	*P*=0.94	*P*=0.63	*P*=0.33	
Mild	0	0	1	1
Moderate	5	4	6	5
Intense	1	3	0	2
INF α-synuclein	*P*<0.001	*P*=0.02	*P*=0.92	
Absent	5	3	0	0
Mild	0	2	3	2
Moderate	0	0	4	3
INF TH	*P*=0.13	*P*=0.51	*P*=0.58	
Absent	0	3	0	0
Mild	0	2	3	2
Moderate	5	0	4	3
SON α-synuclein	*P*=0.003	*P*=0.008	*P*=1	
Absent	5	5	0	0
Mild	0	1	7	5
SON TH	*P*=0.51	*P*=0.83	*P*=0.33	
Moderate	4	4	6	3
Intense	1	2	1	2

Table 2.3. Demographic and histological data.

P values of Mann-Whitney test using PD+NMS group as a reference.

No α-synuclein immunoreactivity was found in the group of healthy individuals. Lewy pathology was present in the three hypothalamic nuclei examined in all PD cases, with Braak stages 4-6, and also in 14.3% of PVN, 40% of INF and 16.7% of SON in the incidental group with Braak stages ranging 2-6 (**Table 2.3**). In all three hypothalamic nuclei Lewy pathology was more severe in the PD+NMS group in comparison to incidental Lewy body disease (PVN: $P = 0.001$, INF: $P = 0.02$, SON: $P = 0.008$) but there was no difference between PD+NMS and PD-NMS groups (PVN: $P = 0.20$, INF: $P = 0.92$, SON: $P = 1$) (**Figure 2.3**).

As expected, TH immunoreactivity in the striatum was significantly reduced in the PD+NMS group in comparison with healthy controls and the incidental group, although there were no differences in the TH staining between PD+NMS and PD-NMS groups (**Figure 2.4**). TH immunoreactivity in the assessed hypothalamic nuclei did not show any difference between the PD+NMS and the PD-NMS, incidental group and healthy controls.

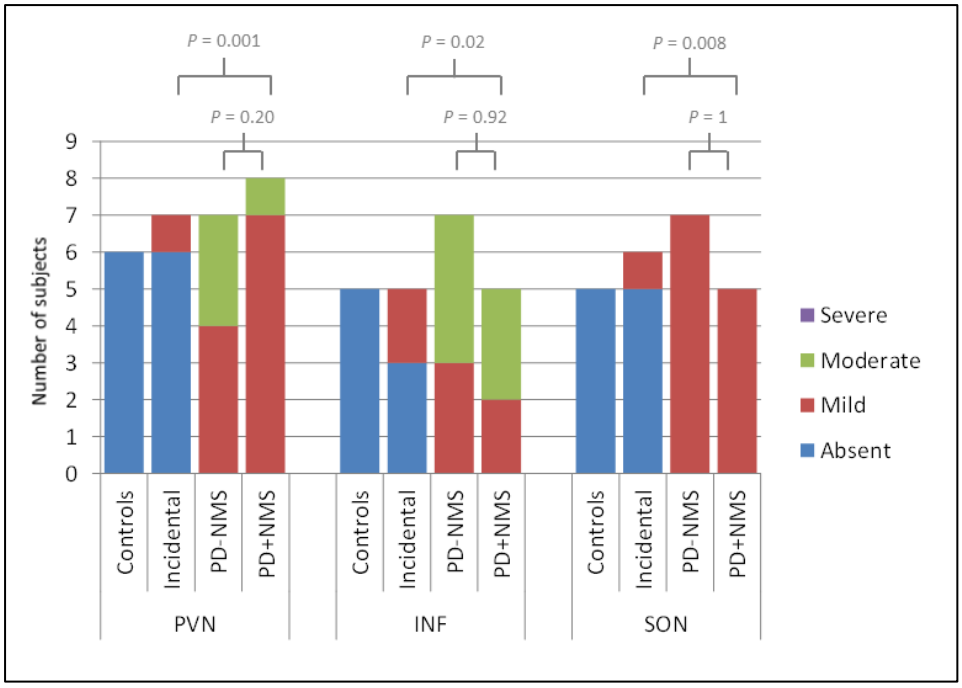


Figure 2.3. Diagram representing Lewy pathology in PVN, INF and SON.

Comparisons between each group and PD+NMS group as reference (*P* values of Mann-Whitney test applied).

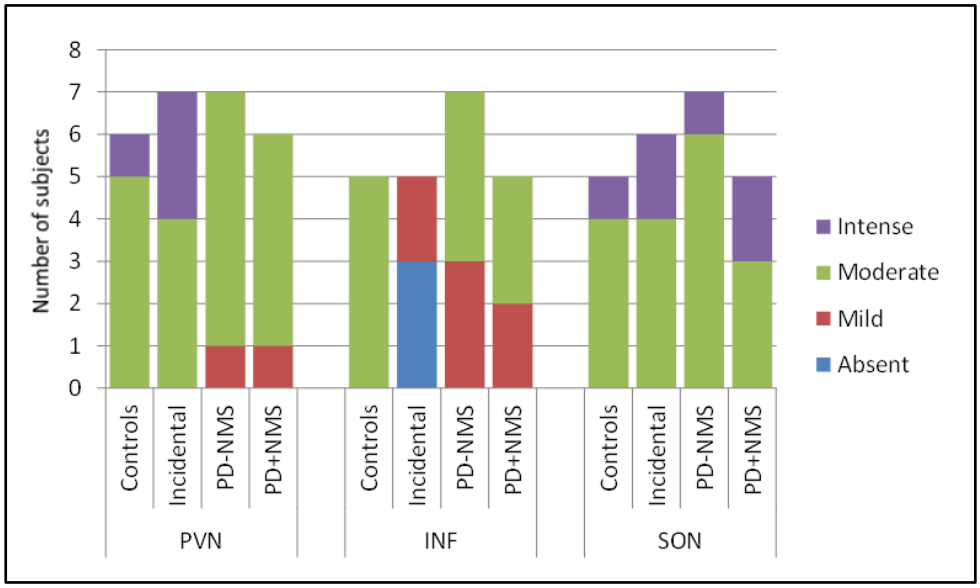


Figure 2.4. Diagram representing TH immunoreactivity in PVN, INF and SON.

2.4 Discussion

Our study demonstrated Lewy pathology in the PVN, INF and SON of the hypothalamus in all of the PD patients which included cases ranging from Braak stage 4-6. Importantly, we also demonstrated that Lewy pathology in these nuclei may precede motor symptoms as, in the incidental group, Lewy pathology was observed as early as Braak stage 2. The significance of incidental Lewy body disease is still under debate but evidence from histological studies suggests that it is a pathologic condition representing preclinical PD rather than age-related α-synuclein pathology.(56-58) Therefore our study also showed that the severity of α-synuclein deposition increased with disease progression, as pathological changes were more severe in patients with advanced PD (PD+NMS and PD-NMS) in comparison to subjects with incidental Lewy body disease.

The presence of Lewy bodies in the hypothalamic nuclei has been previously investigated with conflicting results which might be explained by the heterogeneity in the histological methods used for assessment.(48-50) The more frequent hypothalamic involvement by PD pathology in our cases cannot be explained by a selection bias towards more severe individuals as Lewy pathology could be found in early preclinical stages. Previous studies might have underestimated the true amount of hypothalamic pathology and the use in our study of more sensitive anti-α-synuclein immunohistochemistry, as opposed to anti-ubiquitin, might have increased the detection of Lewy pathology. Although our results contrast with those of the Braak staging system which describes involvement of the tuberomamillary and ventromedial nuclei in stages ≥ 4 (7), it should be noted that Lewy pathology was never greater than moderate in the SON, PVN and INF in our case series. We hypothesize that the involvement of these hypothalamic nuclei at an early stage (Braak stage 2) might be explained by the extensive hypothalamic connections with the lower brainstem. (29, 33) This early hypothalamic involvement may also have clinical implications as hypothalamic dysfunction could contribute to some of the NMS in pre-motor stages of the disease.

Our study is the first to provide a clinical correlation of the histological changes with the severity of NMS. The PVN, INF and SON were assessed in this study because of their physiological role and potential clinical implications in AutD, weight loss and blood pressure dysregulation respectively, which are well known symptoms of PD.(15) However, our study did not show any differences in severity of α-synuclein deposition in any of the hypothalamic nuclei assessed between patients with and without severe NMS. Previous studies failed to show a correlation between the presence of Lewy bodies and neuronal loss questioning the pathophysiological significance of Lewy body pathology in hypothalamic dysfunction. (49, 50, 59) In addition to neuropathological studies, the presence of hypothalamic dysfunction in PD is also supported by *in vivo* studies with functional imaging describing involvement of dopamine and other monaminergic systems.(46, 47) Recent MRI studies using various sequences have tried to correlate hypothalamic involvement with some of the non-motor features in PD although this technique is not able yet to analyse specific hypothalamic nuclei. Volumetric MRI has demonstrated a reduction of hypothalamic grey matter correlating with disease severity and melatonin levels, (45) while resting-state functional MRI has shown a reduction in hypothalamic-thalamic-striatal functional connectivity in patients with severe autonomic symptoms.(60) It is likely that the non-motor features evaluated in this study have a multifactorial origin and their severity is likely to be a combination of dysfunction of regulatory mechanisms with anatomical involvement in multiple areas of the central and peripheral nervous system. Because of this multifactorial origin making any firm conclusions regarding the clinical correlation of the histological changes of the hypothalamus in patients with PD may be challenging. The assessment of the hypothalamic dopaminergic systems using TH immunoreactivity in our patients did not show any differences between the groups, in agreement with previous pathological studies using TH-(50) and melanin-immunohistochemistry.(51) Therefore, taken together these findings suggest that it is likely that functional deficits involving non-dopaminergic systems rather than Lewy pathology may be responsible for

hypothalamic dysfunction which may contribute to some of the non-motor features in PD.(61)

Potential limitations of this study include the relatively small number of subjects and lack of quantitative assessment of specific hypothalamic neuronal populations which was not possible due to the retrospective nature of the study.

In conclusion we showed that Lewy pathology occurs at early stages (even pre-clinical phase) and progressively affects the PVN, INF and SON in PD. Lewy pathology in these hypothalamic nuclei does not correlate with the severity of certain NMS in our patients and the dopaminergic hypothalamic system was not impaired. Further functional studies assessing specific neurotransmitter levels and prospective histological studies to assess specific neuronal populations together with clinical correlations are warranted to further elucidate the role of hypothalamic dysfunction and its influence on NMS in PD.

Chapter 3. Circadian system in Parkinson's disease: A histological analysis of the suprachiasmatic nucleus and pineal gland.

3.1 Introduction

3.1.1 Circadian system in humans

Circadian rhythms are the biological cycles affecting physiological function and behaviour. In humans, circadian rhythms are generated with a periodicity of (nearly) 24 hours and they influence all body functions, the sleep-wake cycle being the most apparent. The suprachiasmatic nucleus (SCN) is a paired small structure in the anterior hypothalamus that acts as a central biological clock.(62) The SCN comprises a core region of vasointestinal polypeptide (VIP) synthesizing neurons that play a critical role in circadian regulation, and a shell region of vasopressin neurons which have complementary functions.(63) The SCN dictates a strong circadian output while remains sensitive to any changes in exogenous factors (seasonal changes, shift work, time-zone travel) or misalignments between endogenous and exogenous circadian rhythms. The circadian activity of the SCN is the result of the rhythmic pattern of expression of a group of core genes known collectively as clock genes (*PER, CLOCK, BMAL1, CRY*) at a molecular level. Their expression is regulated by a complex set of interlocking feedback loops at transcription, translation and post-translation level and their pattern of expression has been proposed as a peripheral biomarker of circadian activity.(64) The SCN synchronizes all body functions in central and peripheral structures using neural and humoral signals. (62) Melatonin is the main humoral circadian efferent of the SCN and it is also the most important endogenous entraining agent for the SCN. Melatonin is synthesized by the pinealocytes of the pineal gland during darkness and this is regulated by the SCN activity through a multisynaptic pathway via the superior cervical ganglion (**Figure 3.1**).(65)

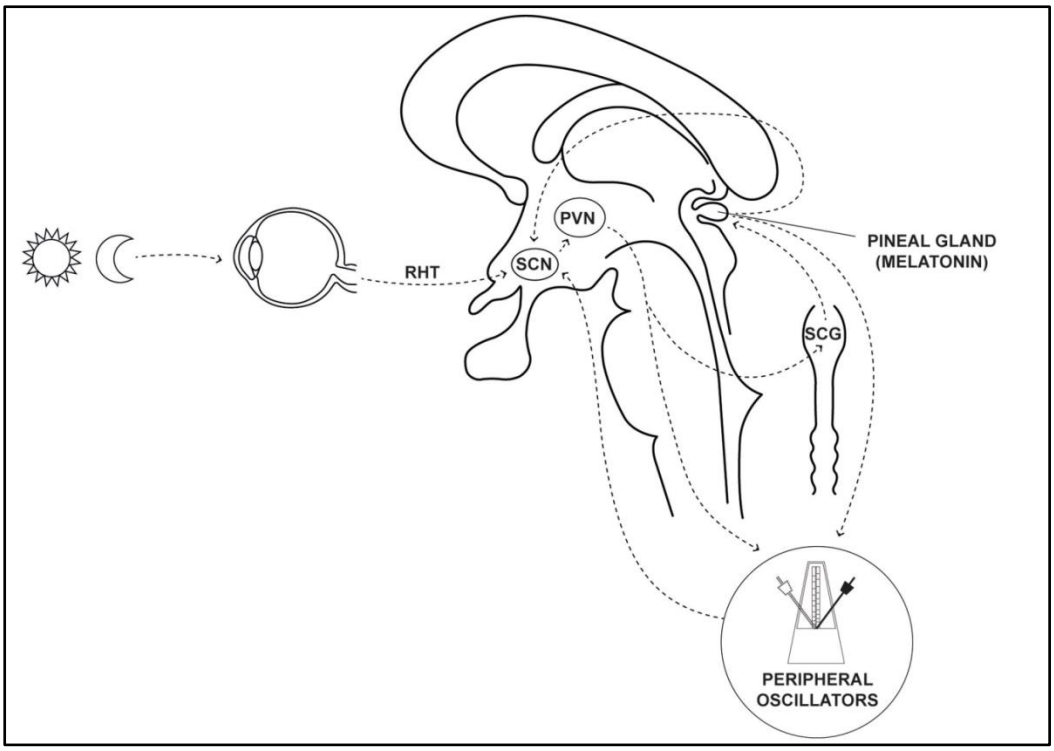


Figure 3.1. The circadian system.

PVN, paraventricular nucleus; RHT, retino-hypothalamic tract; SCG, superior cervical ganglion; SCN, suprachiasmatic nucleus.

There is no pineal storage of melatonin and circulating concentrations show a strong circadian pattern which is considered a good biological marker of circadian activity.(66) The SCN receives information from exogenous and endogenous sources in order to maintain the entrainment of circadian activity with environmental rhythms. The main external information comes from the light through the retino-hypothalamic tract to the phase sensitive VIP neurons of the SCN. The SCN also receives endogenous input from multiple peripheral oscillators throughout the body and feedback from circulating melatonin.

Good synchronisation of circadian rhythms is essential for optimal physical and mental health and symptoms of acute circadian misalignment can be experienced after shift work or time-zone air flights.(67, 68) Chronic circadian dysfunction can cause more deleterious effects on human health and increasing evidence is an association of their disruption with metabolic disturbances,(69) increased cancer risk,(70) immune disorders,(71) renal dysfunction,(72) cardiovascular disease,(73) impaired cognition (74) and mental health disorders.(75) Not surprisingly, circadian dysfunction has also been reported in neurodegenerative disorders such as Alzheimer's disease, Huntington's and PD contributing to their varied symptomatology. Moreover, recent evidence suggests that circadian dysfunction could also act as a causative or contributing factor to the neurodegenerative process in these disorders.(76)

3.1.2 The circadian system in degenerative parkinsonian disorders

Circadian function in PD has attracted research interest in the last several years and growing evidence from clinical studies demonstrated the potential contribution of circadian dysfunction to PD symptomatology. Disruption of the sleep-wake cycle is the most obvious symptom and recent studies with careful design and strict protocols to control exogenous factors have shown disruption of sleep architecture and excessive daytime sleepiness associated with reduced

circulating melatonin. (77, 78) In addition, actigraphic studies have demonstrated disruption of the physiological motor pattern, with PD patients displaying increased activity at bedtime and reduced activity levels during the day which correlates with disease stage.(79, 80) PD patients exhibit worsening of their motor symptoms with diminished motor response to levodopa therapy in the evening unexplained by pharmacokinetic factors (81, 82) which may reflect underlying circadian dysregulation of the dopaminergic systems.(83) Fluctuation in retinal dopamine levels has been proposed as responsible for the diurnal variation in visual contrast sensitivity seen in patients with PD.(84) Circadian dysfunction has been implicated in variations of autonomic physiological functions including heart rate,(79, 85) blood pressure profile(86, 87) and body temperature regulation.(88)

Although evidence is more limited in other neurodegenerative parkinsonian disorders, observational studies have evaluated the possibility of circadian dysfunction contributing to abnormal fluctuations of body functions in patients with MSA and PSP. AutD including blood pressure dysregulation is a well-known symptom in MSA and loss of vasopressin neurons in the SCN (89) and abnormalities of cortisol and vasopressin secretion (90, 91) have both been implicated as potential indicators of underlying circadian dysfunction contributing to symptom fluctuation. Moreover, abnormalities of body thermoregulation have been described in MSA and PSP patients (92, 93) and disruption of the pattern of rest-activity (94) and blood pressure profile (95) have been shown in PSP.

It is worth noting that disruption of physiological rhythms in neurodegenerative parkinsonian disorders cannot always or exclusively be attributed to circadian dysfunction and a primary involvement of those physiological functions in association with other multiple factors may contribute to the symptomatology. As an example, primary disruption of sleep architecture, nocturia, nocturnal motor symptoms and dopaminergic medications, in addition to circadian dysfunction, are well recognised factors contributing to sleep-wake cycle

abnormalities in PD.(96) The evidence supporting circadian dysfunction in PD is more robust and several studies have additionally demonstrated abnormalities in multiple biological markers of circadian activity including expression of clock genes (77, 97-99) and secretion of melatonin (77, 78, 100) and cortisol. (77, 101, 102) However, the extent of these circadian abnormalities and their clinical relevance remain uncertain. Although collectively these results indicate a direct involvement of the circadian system in PD, the neuroanatomical site of disruption of circadian abnormalities remains unknown and the underlying pathophysiological mechanisms are still not fully understood. Studies on markers of circadian activity in other parkinsonian conditions have never been performed and it is unclear whether circadian dysfunction is an intrinsic element of the neurodegenerative process in these disorders or an indirect consequence of dysfunction of other physiological networks (e.g. autonomic nervous system).

The aim of this study is to provide a detailed histopathological analysis of the key structures of the circadian system including the SCN of the hypothalamus and the pineal gland in patients with PD in order to provide a better understanding of the underlying pathophysiology of circadian dysfunction. Results in PD patients will be compared with a group of MSA and PSP patients to elucidate whether a direct neuropathological involvement of the circadian system is shared among these neurodegenerative disorders (as opposed to disruption of biological rhythms as a consequence of dysfunction of other physiological systems) or circadian involvement is a specific feature of PD (and some of the other parkinsonian conditions). Additionally, the detailed neuropathological evaluation will provide information on what specific anatomical structures within the circadian system are affected by the neurodegenerative process and are likely to be the dysfunctional target of future research.

3.2 Methods

3.2.1 Study design and participants

This is a retrospective neuropathological case-control study. Cases with a diagnosis of PD confirmed at autopsy were selected from the archives of the QSBB and the Parkinson's UK Brain Bank, both in London. A group of patients with pathologically confirmed diagnosis of PSP and MSA, and healthy individuals without clinical or pathological evidence of a neurological condition were selected from the QSBB and included as a disease / healthy control group. The donation programmes of both brain tissue resources have Research Ethics Committee approval and written consent was obtained for all donations. General information on the QSBB donation program can be found in *Appendix 1*.

Kruskal-Wallis test was used for global comparisons and Mann-Whitney test for pairwise comparisons between groups. Statistical significance was set at $p<0.05$ and results were not adjusted for multiple comparisons given the exploratory character of the study. Stata 12 (StataCorp, TX) software package was used for statistical analysis.

3.2.2 Neuropathological assessment

Post-mortem formalin-fixed hypothalamic and pineal tissue was sampled and 8µm thick sections were stained using QSBB standard protocols. As the SCN cannot be confidently identified with routine staining, VIP immunohistochemistry (1:80, ab8556, abcam, Cambridge, MA, USA) was used to identify specific VIP-expressing neurons which form the core region of the SCN and have a major role in circadian regulation.(103) Due to limited tissue availability a total of 13 SCNs and 17 pineal glands in the PD group, 5 SCNs and

19 pineal glands from PSP cases, 5 SCNs and 6 pineal samples from MSA patients, and 5 SCNs and 7 pineal glands from controls were examined.

Immunohistochemistry for α-synuclein (1:50, Vector, Burlingame, CA, USA) and phosphorylated tau (AT8; 1:600, Bioscience life sciences, Nottingham, UK) was performed. Lewy pathology was assessed using a semi-quantitative grading system identical to the consensus on assessment of Lewy body disorders (11)(absent, mild, moderate, severe, very severe) in the SCN and pineal gland of PD cases (**Figure 3.2**). Based on the severity and distribution of Lewy pathology a Braak stage (0-6)(7) and a Lewy pathology subtype (brainstem, limbic, neocortical) was assigned for each PD case.(104) Neuropathological diagnosis of MSA was made according to relevant diagnostic criteria (105) and glial cytoplasmic inclusions (the pathological hallmark of this condition) were graded using a similar semiquantitative scale in SCN and pineal tissue of MSA cases. The diagnosis of PSP was established based on the accumulation of tau-immunoreactive inclusions (including neurofibrillary tangles, neuropil threads, tufted astrocytes and coiled bodies) and neuronal loss in typical distribution (106) and severity of histological findings were graded using a semiquantitative approach.

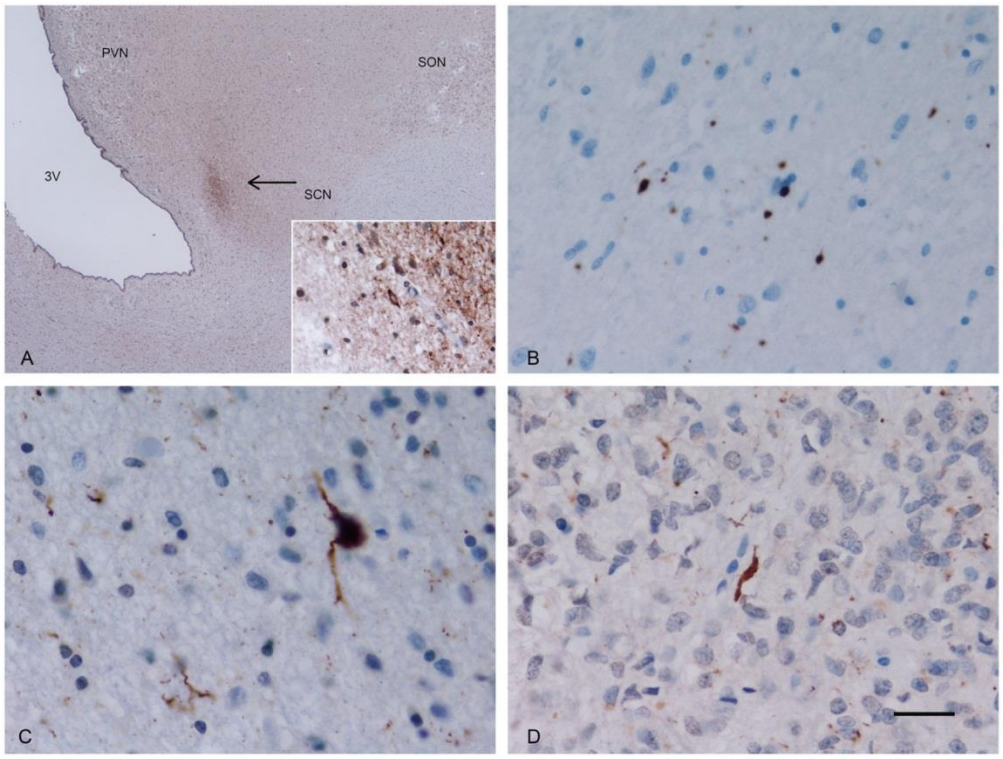


Figure 3.2. Representative sections of the SCN and pineal gland.

Immunohistochemistry for VIP was used for identification of the SCN (A; arrow); inset shows expression in neuronal cell bodies and processes. Immunohistochemical staining of the SCN for α-synuclein (B) showing Lewy pathology in a patient with PD, and for phosphorylated tau (C) showing PSP-tau related pathology in a patient with PSP. Immunohistochemical staining of pineal tissue for α-synuclein (D) showing Lewy pathology in a PD patient. Scale bar in D represents 520 µm in A, and 25 µm in inset of A, B-D.

3V, third ventricle; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus.

3.3 Results

A total of 28 PD, 21 PSP and 11 MSA patients were included, in addition to 12 healthy controls. Demographics and clinical characteristics are shown in **Table 3.1**. Groups did not differ by gender (P = 0.321). MSA (P < 0.001) and PSP patients (P < 0.001) were younger than controls but the latter did not differ by age from patients with PD (P = 0.098). As expected, disease duration was shorter in MSA (P < 0.001) and PSP cases (P < 0.001) in comparison to PD patients.

In PD cases, Lewy pathology was demonstrated in 9 cases (69.2%) in the SCN with mild or moderate severity but in none of the controls (P = 0.014). By contrast, mild α-synuclein deposition was only found in 2 pineal glands in PD cases (11.8%). These findings did not reach statistical significance when compared to healthy controls (P = 0.354) (**Table 3.1** and **Figure 3.3**). To assess whether SCN α-synuclein deposition was associated with the extent and severity of Lewy pathology in the central nervous system, an analysis by Braak stage (Braak stage 5 vs Braak stage 6; P = 0.245) and Lewy pathology type (Limbic vs neocortical; P = 0.536) was performed without any differences being identified between groups.

In PSP cases the SCN showed PSP-tau pathology of mild or moderate severity in all cases (P = 0.005 compared to controls) but no tau pathology was found in pineal tissue (**Figure 3.2**). By contrast MSA cases did not show any α-synuclein deposition in either the SCN or the pineal gland (**Table 3.1** and **Figure 3.3**).

	Control (n = 12)	**PD (n = 28)**	**MSA (n = 11)**	**PSP (n = 21)**
Sex (male:female)	6:6	22:6	7:4	13:8
P value		0.074	0.519	0.512
Age diagnosis (y)	NA	64.6 ± 12.6	63.3 ± 6.4	66.9 ± 6.8
Age death (y)	82.7 ±	79.4 ± 6.4	70.0 ± 7.1	74.4 ± 6.9
***P* value**	5.4	*P* = 0.098	*P* < 0.001	*P* < 0.001
Disease duration (y)	NA	14.7 ± 8.7	6.8 ± 3.4	7.5 ± 3.2
***P* value (PD as reference)**			*P* < 0.001	*P* = 0.002
SCN (n)	5	13	5	5
SCN pathology; n (%)	Absent 5	Absent 4 (30.8%)	Absent 5	Mild 2 (40%)
***P* value**	(100%)	Mild 7 (53.8%)	(100%)	Moderate 3 (60%)
		Moderate 2 (15.4%)	*P* = 1	*P* = 0.005
		P = 0.014		
Pineal (n)	7	17	6	19
Pineal pathology; n (%)	Absent 7	Absent 15 (88.2%)	Absent 6	Absent 19 (100%)
***P* value**	(100%)	Mild 2 (11.8)	(100%)	*P* = 1
		P = 0.354	*P* = 1	
Braak stage; n (%)	NA	Braak 5: 3 (12%)	NA	NA
***P* value**		Braak 6: 22 (88%)		
(Braak 5 vs Braak 6)		P = 0.245		
Lewy body subtype	NA	Limbic: 5 (21.7%)	NA	NA
***P* value**		Neocortical: 18		
(limbic vs neocortical)		(78.3%)		
		P = 0.536		

Table 3.1. Demographics and histological findings.

P values of Mann-Whitney test applied for comparisons with control group as reference unless stated otherwise.

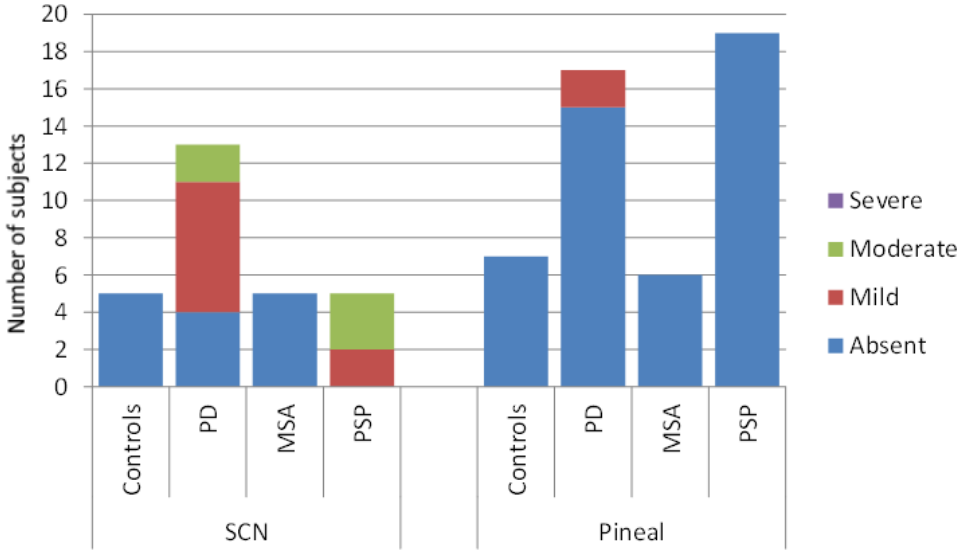


Figure 3.3. Bar graph representing neuropathological findings in SCN and pineal by disease.

3.4 Discussion

This is the first study to provide a histological analysis of the key regulatory structures of the circadian system in PD and other parkinsonian disorders and has demonstrated a different pattern of involvement in PD, PSP and MSA with important clinical and pathophysiological implications.

First, our findings showed that neuropathological involvement of the circadian system is not common to all neurodegenerative diseases, as there was no evidence of abnormal α-synuclein deposition in patients with MSA. Therefore, our results suggest that specific neuronal populations of the circadian structures (core VIP-expressing neurons of the SNC) exhibit certain vulnerability to specific neurodegenerative diseases (Alzheimer's and Huntington's disease,(76, 107) in addition to PD and PSP) rather than the circadian system being just a fragile network susceptible to any neurodegenerative process. Further research is needed to elucidate the shared molecular mechanisms by which these neurodegenerative diseases cause circadian degeneration although mechanisms such as oxidative stress, abnormalities of autophagy and disruption of metabolic pathways have been proposed.(107)

Second, our histological analysis identified a clear distinct pattern of neuropathological involvement of the circadian system in PD and also PSP, suggesting that the neuroanatomical site of circadian disruption is located in the SCN (rather than the pineal gland). Circadian dysfunction in PD and PSP is likely to be caused by direct neuropathological involvement of the central circadian pacemaker (SCN) as it was found to be exclusively affected in PSP and more consistently involved in PD. Studies on melatonin secretion in PD support this pattern of involvement as, when interpreted together, in addition to reduced circulating melatonin, there is also abnormalities in the circadian rhythmicity of melatonin secretion.(77, 78, 100, 108, 109) We explored any potential relationship between SCN α-synuclein deposition and the neurodegenerative process in PD but we did not find any association with

histological progression in the central nervous system (measured by 2 different accepted staging systems). Although as expected in a brain bank series our study only included PD cases in advanced stages of the disease, this suggests that histological involvement of the SCN is independent of the stereotyped progression of the Lewy pathology which is consistent with previous clinical studies showing that circadian dysfunction can be seen even in newly diagnosed PD patients.(77) We could only find α-synuclein deposition in pineal tissue in 2 PD patients (11.8%), although this was of mild severity, showing Lewy neurites but without Lewy bodies, and these findings did not reach statistical significance when compared to controls. The pineal gland was unaffected by tau pathology in all PSP cases.

To our knowledge, this is the first systematic neuropathological assessment of the circadian system in PD and other neurodegenerative parkinsonian disorders which has identified the VIP-neurons of the SCN as the key neuroanatomical structure likely to be dysfunctional in PD and PSP. The SCN has never been previously assessed in PD or PSP, and pineal gland tissue had only been analysed before in 3 patients with PD,(110) with no Lewy bodies found although, the Lewy pathology may have been underestimated as sensitive α-synuclein immunohistochemistry was not available at that time. The potential role of the SCN in circadian dysfunction in PD was suggested by a previous study showing reduced hypothalamic grey matter on MRI which correlated with reduced melatonin output,(45) although dedicated imaging of the SCN or pineal gland were not technically possible.

Clinical studies have shown disruption of some of the biological functions in patients with PSP including rest-activity cycle, blood pressure pattern and body temperature regulation.(93-95) However, our study provides for the first time direct evidence of a primary involvement of the circadian system in PSP, as no studies had included biological markers of circadian activity. In contrast with PSP, the SCN and pineal gland seem to be spared from α-synuclein deposition in MSA. Previous clinical studies have shown evidence for fluctuations of

symptoms and body functions suggestive of circadian rhythm disruption in patients with MSA (89, 90, 92) but there are no studies directly assessing markers of circadian activity. Our results do not support the primary involvement of the circadian system by glial or neuronal α-synuclein containing inclusions in MSA and it seems plausible that disruption of biological function rhythms in these patients may be secondary to degeneration of other systems such as autonomic and neuroendocrine networks. Previous pathological studies have shown loss of vasopressin neurons in the SCN of patients with MSA (89) although vasopressin neurons may be more involved in autonomic function control while circadian rhythm regulation is exerted by VIP-expressing neurons.(63, 103)

Whether an intrinsic part of the neurodegenerative process or a secondary consequence of dysfunction of other systems, circadian disruption has been linked to regulation of oxidative stress, protein degradation, autophagy, mitochondrial dysfunction, DNA repair and brain metabolism.(107, 111) These mechanisms are impaired in neurodegeneration and, therefore, it has been proposed that circadian disruption does not only contribute to some of the non-motor symptoms associated with these diseases but also could influence the pathological process in neurodegenerative conditions.(76, 107, 111) This is important, as restoring normal circadian activity by therapeutic interventions could potentially represent a novel target for symptomatic and disease-modifying interventions.(112) In this regard, a few studies have explored the potential symptomatic effect of restoring circadian rhythmicity by administration of melatonin or exposure to bright light therapy in patients with PD. Data on the use of melatonin for the treatment of sleep abnormalities in PD have been inconclusive(17, 113) based on two randomised studies which reported minimal benefits of uncertain clinical significance and subjective improvement of quality of sleep at different doses.(114, 115) Promising results have been reported regarding the use of bright light therapy in sleep, motor function and mood in PD (116) although it has only been assessed in two randomised placebo-controlled trials with different light therapy regimes and

assessment protocols, making it difficult to draw firm conclusions. The most recent one showed significant improvement of excessive daytime sleepiness, several measures of sleep quality and architecture, rest-activity cycles on actimetry among PD patients in the treatment group, and interestingly, an improvement of PD severity in parts I, II and III of the Unified Parkinson's Disease Rating Scale (UPDRS) in patients in both groups (placebo group received dim-red light therapy).(117) A previous randomised trial had shown also benefits in mood disturbances, parts I, II and IV of the UPDRS in comparison to the placebo group, but failed to improve motor UPDRS or sleep.(118)

Our study had few limitations. Assessment of circadian rhythms requires a strict control of external modifying factors and no accurate clinical information on circadian activity was available in our patients preventing any clinical correlation of our histological findings. Because of limited tissue availability we were unable to perform quantitative assessment of specific neuronal populations, particularly of the VIP neurons of the core of the SCN, in order to evaluate neuronal loss.

In conclusion, we have shown a direct neuropathological involvement of the circadian system in patients with PD and PSP, while no histological involvement was found in MSA patients. The pattern of neuropathological involvement was similar in PD and PSP, suggesting that circadian dysfunction is likely to be secondary to the involvement of the VIP neurons of the SCN. In contrast, abnormalities of biological rhythms in MSA are unlikely to be related to a primary involvement of the circadian system, and may be secondary to degeneration of other functional networks.

Chapter 4. Constipation in Parkinson's disease: a clinical, colonic transit, high-resolution manometry and MRI defecography study.

4.1 Introduction

The gastrointestinal (GI) system in PD has attracted much attention in the last few decades in PD research. A much more precise and detailed account of the extent of GI dysfunction and the associated clinical implications in PD have now emerged although the importance of the GI is not only restricted to the clinical aspects. Accumulating evidence has emerged from many different studies and areas of research implicating the early changes in the function of the GI system in the pathogenesis of PD and it has been suggested that the enteric nervous system might be a crucial neuroanatomical structure in the spreading of α - synuclein deposition and the neurodegenerative process of the disease.(21)

4.1.1 Role of the GI system in Parkinson's disease pathogenesis

GI symptoms can manifest during the prodromal phase and several studies have shown that constipation is associated with an increased risk of development of PD even in those with duration of symptoms for more than 10 years.(18, 119, 120) This early clinical involvement is also supported by the presence of early pathological changes in peripheral anatomical structures of the enteric nervous system, namely the mucosal nerve fibres and the submucosal and myenteric plexuses. α-Synuclein accumulation has been demonstrated at virtually all the levels throughout the GI system including submandibular gland, oesophagus, stomach, appendix and colon, showing a rostro-caudal gradient with greatest involvement in the submandibular gland and lower oesophagus.(121-123) These early promising results led to further

studies investigating immunohistochemical detection of α-synuclein as a potential biomarker. However, heterogeneity of the results and the inability of the current techniques to distinguish between physiological and pathological synuclein are currently unable to provide enough specificity as a diagnostic biomarker, although further research is ongoing to overcome these challenges.(124)

The early clinical and pathological involvement of the enteric nervous system in PD supports the crucial role of the GI system in the pathogenesis of PD and the hypothesis that PD may start in the gut has attracted much research attention in recent years. In this gut-brain hypothesis, two different anatomical structures, the epithelial intestinal mucosa and the vagus nerve, are fundamental in the process of allowing a potential external pathogen to spread the neurodegenerative process from the enteric nervous system to the brain. The enteric nervous system innervation is supplied by the vagal nerve and the projecting neurons in the dorsal motor nucleus of the vagus are among the first central structures to show pathological involvement in PD.(7) Pathological changes in PD are considered to progress among vulnerable anatomically connected structures rostrally from the nuclei in the lower brainstem to cortical areas in a predictable manner. The pathological stages of the disease proposed by Braak are based on this stereotyped progression after a comprehensive neuropathological assessment of Lewy pathology of a large group of PD patients at different stages of the disease.(7) Based on the early involvement of the enteric nervous system and the caudo-rostral progression of histopathological changes, it has been proposed that the GI system could be the site of onset of the pathogenesis of PD which subsequently would spread through vagal connections to the brainstem and other central structures.(125) Recent studies have shown that people with complete truncal vagotomy had a reduced risk of subsequent PD supporting the idea of the vagus nerve as potential route for α-synuclein spreading.(126) The GI mucosa provides the greatest surface in the human body for interaction with exogenous substances and, in this scenario, it would also provide the gateway where a potential

neurotropic pathogen could induce α-synuclein aggregation. Dysfunction of the epithelial barrier with increased permeability, changes in the composition of the microbiota and increased levels of inflammation in the gut lumen have all been demonstrated in patients with PD and considered to contribute to the initiation of the PD pathogenic cascade.(127, 128) Although an appealing hypothesis that is tentatively supported by preclinical data, this theory is not widely accepted, there is a whole body of evidence against and it is the matter of an intense debate in the research community.(129, 130)

4.1.2 Constipation in Parkinson's disease

In addition to the potential role in PD pathogenesis, GI dysfunction is associated with a wide range of symptoms including sialorrhoea, dysphagia, impairment of gastric motility and constipation, that contribute significantly to the clinical burden of non-motor features associated with PD. They are also likely to play part in the pathophysiological changes that underlie the development of motor fluctuations, as impairment of gastric motility and its consequences (small intestinal bacterial overgrowth, *Helicobacter pylori* infection) can interfere with levodopa absorption.(131)

Constipation is one of the most common symptoms in PD with an estimated prevalence of approximately 50%, although results vary widely among studies due to application of different criteria for definition.(132) Because of the variability in patient symptomatology and multiple definitions used, the widely accepted ROME III criteria for functional constipation were developed by gastroenterologists in order to standardise diagnosis.(133) To date, these criteria have not been validated in PD research and they have not been widely used to evaluate constipation in patients with PD. Prevalence of constipation seems to increase with disease progression and severity of the disease although, as discussed earlier, constipation might be one of the earliest

prodromal manifestations of PD presenting many years before the onset of motor symptoms.(131)

4.1.3 Evaluation of constipation

From a pathophysiological perspective, constipation can be divided in slow transit constipation and defecatory (anorectal) dysfunction. Each of them is associated with different symptoms and underlying mechanisms requiring specific investigations and management. Slow transit constipation is the most widely recognised feature leading to a reduction in bowel movements and it is presumably due to the impaired colonic motility secondary to the pathological involvement of the enteric nervous system.(131, 134) Defecatory dysfunction (also known as outlet obstruction constipation) resulting in incomplete evacuation, pain and excessive straining, is a consequence of the impairment of the multiple complex coordinated mechanisms involved in defecation.(135)

The first step in the assessment of constipation should be a systematic evaluation of the symptoms and their severity. Generic questionnaires to assess NMS in PD do not evaluate constipation in detail (14) and, until a specific questionnaire for constipation in PD is designed, it seems reasonable that whatever assessment tool is used it should evaluate both slow transit and defecatory type of symptoms.(133) When patients do not respond to stool regulation, additional investigations are useful in the evaluation of the mechanisms underlying the symptoms of constipation and response to treatment.

Colonic transit time can be easily estimated measuring the movements of radiopaque markers through the gut. Although different protocols are available, markers are ingested, subsequently identified on an abdominal X-ray and the

transit time is estimated based on the numbers of remaining markers in three colonic regions, namely right, left and rectosigmoid colon.(136)

Normal defecation involves coordinated complex reflex and voluntary functions which are triggered by the distension of the rectal wall. If socially appropriate, the voluntary contraction of the abdominal wall muscles increases colonic pressure, the relaxation of the puborectalis muscle widens the anorectal angle and the relaxation of anal sphincter allows the evacuation of the faeces. Assessment of defecatory function is crucial for understanding the mechanistic pathophysiology in order to provide effective symptomatic management of constipation and it is generally agreed that no single test can provide a full assessment of anorectal function. A comprehensive battery of tests measuring anorectal sensorimotor function, evacuation and structure should be included and results should be interpreted in conjunction given the common multifactorial origin of constipation and the overlap between pathological and physiological parameters.(137, 138)

Anorectal manometry (ARM) is the best established test for the assessment of defecatory function in clinical settings and provides a comprehensive evaluation of anorectal sensorimotor function comprising a battery of tests at rest and during defecatory manoeuvres which include anal sphincter function, rectal sensation, rectoanal reflex activity and pressure measurements during attempted defecation.(139) With recent technical advances, high-resolution ARM is increasingly replacing conventional ARM as it provides a more accurate assessment, allows integrated measurement of pressures and performance of balloon expulsion test.

Defecography is a simple test involving the insertion of a stool substitute into the rectum followed by imaging at rest and during rectal evacuation that, in addition to an assessment of pelvic floor structure, allows evaluation of real-time dynamic defecatory function (pelvic floor descent, puborectalis relaxation and measurement of anorectal angle) and evacuation of faeces. MRI is

superseding traditional videofluoroscopic techniques as it avoids ionising radiation and allows excellent soft tissue differentiation.

The assessment of constipation in patients with PD has previously been evaluated in several studies and both, slow transit and anorectal dysfunction have been reported.(132, 135) However, its pathophysiology remains poorly understood and this carries important clinical implications as symptomatic management of slow transit and defecatory dysfunction differ significantly. Although previous studies provided significant advances in the characterization of constipation in PD, several limitations regarding the design of the studies and heterogeneity in the results prevent any firm conclusions. Firstly, most of the previous studies report results of investigations for the assessment of constipation in isolation, without other additional complementary tests to allow a systematic evaluation of the symptoms.(140-143) It is well accepted that these investigations are of little clinical utility when individually performed because of the difficulties of interpreting the results given the overlap between health and disease, and the lack of correlation between a single physiological abnormality and symptoms.(137-139, 144) Isolated tests of defecatory function can also erroneously attribute the origin of the symptoms to a single pathophysiological abnormality, failing to diagnose and address the commonly multifactorial cause of the problem. Secondly, most of the studies assessing constipation in PD were performed a few decades ago with conventional historical diagnostic techniques, and, with a few exceptions reporting results using high resolution ARM,(142, 145) more recent studies have not incorporated technological advances in the evaluation of the symptoms such as MRI defecography.

In this study, we report the results of a systematic assessment of patients with PD and subjective constipation including validated clinical questionnaires, colonic transit, high-resolution ARM and MRI defecography in order to provide a correlation of the results with the severity and extent of the symptoms, and

understand the pathophysiological mechanisms driving constipation in patients with PD.

4.2 Methods

4.2.1 Subjects and study design

Participants with a diagnosis of PD based on the United Kingdom Parkinson's Disease Society Brain Bank diagnostic criteria (3) and subjective complaints of constipation were prospectively recruited from the Movement Disorders clinic at the National Hospital for Neurology and Neurosurgery, Queen Square between June 2016 and February 2018. Patients with the following features were excluded from the study: signs or symptoms consistent with secondary or atypical parkinsonian disorders, pregnancy, major colonic or pelvic floor surgery, significant organic colonic disease (inflammatory bowel disease, severe diverticulosis, etc.), significant systemic connective tissue disorders (scleroderma), significant active psychiatric disorder or chronic regular opioid use (> 1 take per day). All patients provided informed written consent and the study was approved by the assigned Research Ethics Committee.

Patients were assessed while on their regular medications including treatment with anticholinergics and laxatives. Clinical and demographic data were recorded. Severity of PD related symptoms was assessed using the Hoehn and Yahr scale (HY) for disease stage, the part III of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) for motor symptoms and the non-motor symptom questionnaire (NMSQ).

4.2.2 Clinical assessment of constipation

No specific questionnaire has been validated to specifically assess constipation in patients with PD, although the score for questions related to these symptoms from the NMSQ (questions 5-7) were recorded separately. To specifically evaluate the extent and severity of the symptoms of constipation in detail

including slow transit and defecatory dysfunction symptoms, patients were asked to complete self-administered questionnaires namely the Patient Assessment of Constipation Symptoms questionnaire (PACSYM) and the Neurogenic Bowel Dysfunction score (NBD). PACSYM evaluates in detail symptoms of constipation comprising 12 questions divided into three domains (abdominal, rectal and stools) using a five-point scale from absent to very severe with a total score of 60 points and is validated and widely used in patients with chronic idiopathic constipation.(133, 146) (**Error! Reference source not found.**) NBD score is a symptom-based questionnaire comprising a total of 10 items with an overall score ranging from 0-47 points (higher scores indicating more severe symptoms) developed and validated to assess colorectal dysfunction in patients with spinal cord injury and a modified version has been previously used in PD patients.(147, 148) (**Error! Reference source not found.**)

4.2.3 Colonic transit, high resolution anorectal manometry and MRI defecography

A subgroup of patients (15 out of 42) underwent additional investigations to evaluate colorectal function including colonic transit time, high-resolution ARM and MRI defecography. Results of the investigations were interpreted blinded to the severity of constipation.

Colonic transit was evaluated using radiopaque markers according to the standard technique which has been previously described.(149) In brief, three sets of radiologically distinct markers were taken at 24 hour intervals and an abdominal *X*-ray taken 120 hours after ingestion of the first set. Retention of more than the normal range (< 3 markers of day 1, < 5 of day 2 and < 11 of day 3) for any one of the three sets of markers was regarded as reflecting slow whole gut transit.

ARM was undertaken using a custom 16 channel 4.4mm diameter water perfused high resolution manometry catheter. The intra-anal array consisted of 13 of the 16 channels, starting at 5mm from the anal verge and at 5mm intervals extending to 6.5cm from the anal verge. The proximal channels were positioned posteriorly to optimally measure the action of puborectalis muscle. There were two further rectal channels either side of the balloon, with the final channel 15cm distal to the anal verge measuring atmospheric pressure. Once the catheter was correctly positioned it was secured in place using a clamp. Normal values for resting and incremental squeeze pressures were based on previously published data.(150) Evacuatory measurements were made during attempted balloon expulsion with the catheter clamped in situ.

MRI defecography was performed using closed 1.5 Tesla magnet with the patient supine.(151) Patients are given a glycerine suppository 30 minutes prior to the procedure to empty the rectum of faecal content. Patients then lay on the MRI table in the left lateral position and 120ml of standard ultrasound gel was introduced into the rectum via bladder syringe. After a planning sequence, sagittal, axial and coronal T2 weighted images are performed through the pelvis during maximum strain and maximum contraction. The mid sagittal plane through the rectum was selected and a video recording was taken through this plane during evacuation of the rectal jelly. MRI evidence of failure of relaxation or descent of puborectalis during attempted evacuation on at least two attempts was defined as “MRI dyssynergia”; evidence of inability to void the contrast gel was defined as “MRI evacuation failure”.

4.2.4 Statistical analysis

Demographic and clinical features are reported as number (percentage) for categorical variables and median ± standard deviation (SD) for continuous variables. Comparisons between groups were performed using chi square test

for categorical variables, and t test for continuous variables as appropriate. Association between variables was evaluated using Pearson correlation coefficient when appropriate. Statistical significance was set at $p<0.05$ and Stata 12 (StataCorp, TX) software package was used for statistical analysis.

4.3 Results

A total of 42 patients with PD (69.1% male; age 67.7 ± 7.9 years; disease duration 10.5 ± 6.1 years) and subjective complaints of constipation were included in the study. Main demographic and clinical features are shown in **Table 4.1**.

	Total n = 42	CTr n = 15	ARM n = 20	MRI defecography n = 9
Age	67.7 ± 7.9	66.8 ± 6.6	66.8 ± 7.9	68.8 ± 7.0
Gender (male)	29 (69.1)	12 (80.0)	16 (80.0)	7 (77.8)
Disease duration (y)	10.5 ± 6.1	8.9 ± 5.3	9.3 ± 5.6	7.9 ± 4.0
Ldopa response				
Mild	1 (2.4)	1 (6.7)	1 (5.0)	1 (11.1)
Moderate	4 (9.5)	1 (6.7)	1 (5.0)	1 (11.1)
Good	37 (88.1)	13 (86.7)	18 (90.0)	8 (77.8)
Ldopa equivalent dose (mg)	770.38 ± 429.88	629.13 ± 388.01	633.85 ± 378.39	619.8 ± 290.5
MDS-UPDRS III	26.8 ± 18.2	21.27 ± 14.22	24.8 ± 15.97	24.2 ± 16.6
HY stage				
1	4 (9.5)	2 (13.3)	3 (15.0)	1 (11.1)
2	29 (69.1)	11 (73.3)	13 (65.0)	6 (66.7)
3	5 (11.9)	1 (6.7)	2 (10.0)	1 (11.1)
4	2 (4.8)	0 (0.0)	1 (5.0)	0 (0.0)
5	2 (4.8)	1 (6.7)	1 (5.0)	1 (11.1)
NMSQ	12.74 ± 5.00	12 ± 4.77	11.80 ± 4.34	13.2 ± 5.5
Constipation definition	33 (78.6)	12 (80.0)	16 (80.0)	7 (77.8)
PACSYM score	12.2 ± 8.0	14.1 ± 9.1	14.8 ± 9.7	17.1 ± 9.9
NBD score	6.6 ± 4.8	5.8 ± 4.6	6.7 ± 5.1	7.7 ± 5.0
Laxatives	20 (47.6)	8 (53.3)	12 (60.0)	6 (66.7)
Levodopa	38 (90.5)	13 (6.7)	17 (85.0)	9 (100.0)
Dopamine agonists	17 (40.5)	7 (46.7)	7 (35.0)	2 (22.2)
COMT	16 (38.1)	4 (26.7)	6 (30.0)	2 (22.2)
MAO-B inhibitors	19 (45.2)	6 (40.0)	8 (40.0)	3 (33.3)
Anticholinergics	9 (21.4)	2 (13.33)	9 (45.0)	2 (22.2)
Amantadine	18 (42.9)	7 (46.7)	5 (25.0)	5 (55.6)
Abnormal CTr		10 (66.7)		
ROM distribution		Right 2 (14.3) Left 7 (50.0) SR 5 (35.7)		
Abnormal MRI				6 (66.7)
MRI evacuation				6 (66.7)
MRI dyssynergia				5 (55.6)
ARM			14 (70.0)	
Sensation			13 (65.0)	
Expulsion			1 (5.0)	
ARM sphincter			6 (30.0)	
RAI reflex			1 (5.0)	

Table 4.1. Demographics, clinical data and GI investigations.

Data shown in number (%) or mean ± SD.
CTr colonic transit; RAI, rectoanal inhibitory; ROM, radiopaque marker; SR, sigmoid-rectum.

4.3.1 Clinical assessment of constipation

Despite subjective complaints of constipation, only 33 (78.6%) reported a frequency of < 3 bowel movements per week or straining, which is one of the most commonly used definitions of constipation used in patients with PD, (132) and only 20 (47.6%) were on regular treatment with laxatives.

The strongest correlation of severity of constipation measured by PACSYM and NBD questionnaires was found with severity of non-motor symptoms (Pearson correlation; $r = 0.506$; $P < 0.001$ and $r = 0.387$; $P = 0.011$ respectively) but scores were also correlated with motor impairment measured by MDS-UPDRS part III, HY stage of the disease only for NBD score, and treatment with laxatives (all P values < 0.05; see **Table 4.2** and **Figure 4.1**). No correlation was found between constipation severity and disease duration, treatment with anticholinergics or other medications used for the symptomatic treatment of PD (all $P > 0.05$; see **Table 4.2**).

	PACSYM score	NBD score	NMSQ constipation
Age*	-0.176; 0.266	0.107; 0.500	-0.181; 0.252
Gender**	0.149	0.040	0.627
Disease duration*	0.203; 0.198	0.190; 0.229	0.120; 0.450
HY stage***	0.272; 0.081	0.367; 0.017	0.030; 0.850
MDS-UPDRS III*	0.317; 0.041	0.393; 0.010	0.061; 0.700
NMSQ*	0.506; 0.001	0.387; 0.011	0.366; 0.017
Laxatives**	0.003	0.002	0.857
Anticholinergics**	0.094	0.082	0.743
Colonic transit**	0.211	0.248	0.836
ARM**	0.981	0.941	0.865
MRI defecography**	0.016	0.010	0.105

Table 4.2. Correlation of constipation severity.

*r Pearson correlation coefficient; P value
** t test P value
*** Spearman rank correlation coefficient; P value

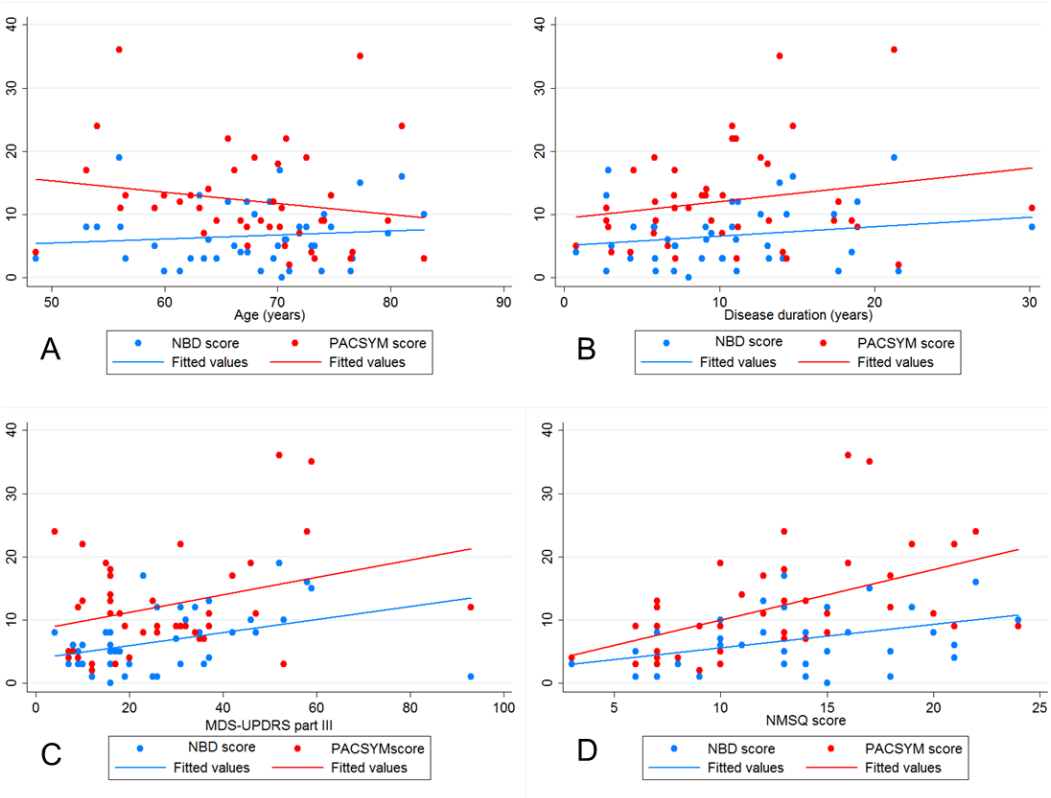


Figure 4.1. Scatter showing correlation of constipation severity.

Constipation severity (NBD score in blue; PACSYM score in red) correlation with age (A), disease duration (B), MDS-UPDRS part III score (C) and NMSQ score (D). See **Table 4.2** for r correlation coefficient and P values.

4.3.2 Colonic transit, high resolution anorectal manometry and MRI defecography

A subgroup of patients underwent further investigations including colonic transit (n = 15), high-resolution ARM and balloon expulsion test (n = 20), and MRI defecography (n = 9). These selected patients did not differ significantly regarding age, gender, disease duration, severity of motor and non-motor symptoms (all P values > 0.05) although constipation severity measured by PACSYM (but not by NBD) was slightly higher on those patients having ARM and MRI defecography.

Table 4.1 shows a summary of the results of the GI investigations. ARM was abnormal in 17 (70.0%) patients, with reduced rectal sensation (65.0%) and attenuated external anal sphincter function (30.0%) as the abnormal parameters most commonly reported, while balloon expulsion test and rectoanal reflex activity was normal in almost all patients. These abnormalities on high-resolution ARM did not correlate with more severe constipation on self-administered questionnaires. Colonic transit was delayed in 10 (66.7%) patients and the distribution of the radiopaque markers predominated in the left hemicolon followed by sigmoid-rectum. Functional disorders on MRI defecography were identified in two thirds of patients, particularly defecatory dyssynergia (lack of relaxation and failure of descent of puborectalis muscle). Abnormalities on MRI defecography was the only investigation correlated with severity of constipation measured by PACSYM (t test; P = 0.016) and NBD questionnaires (t test; P =0.010) (**Table 4.2**).

4.3.3 Slow transit and anorectal dysfunction

A total of 15 patients underwent assessment of both colonic transit and anorectal function (high resolution ARM and/or MRI defecography) with a

subgroup of 9 patients having all three investigations. The pathophysiological interpretation of the results of these investigations is shown in **Figure 4.2**. Based on these findings, a mixed pattern involving slow transit and anorectal dysfunction is the most common underlying pathophysiology of constipation in our patients (60.0%) and overall, anorectal dysfunction is more prevalent than slow transit in PD. The analysis of the subgroup with all three investigations showed similar results.

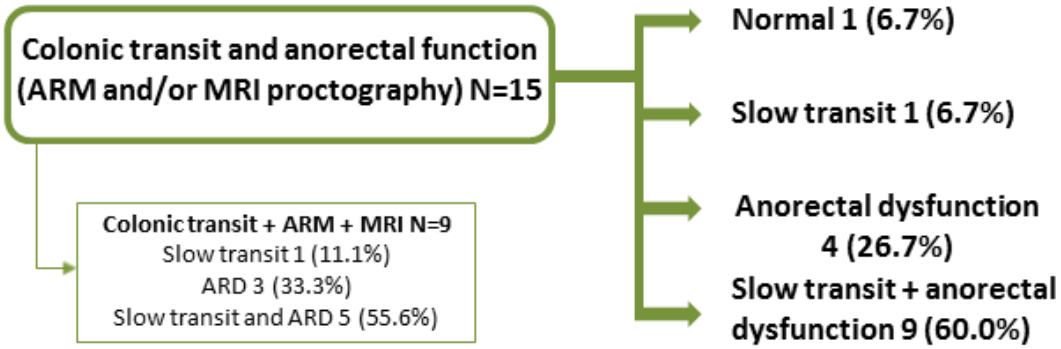


Figure 4.2. Pathophysiological interpretation of GI investigations.

4.4 Discussion

In this prospective study we performed a systematic assessment of constipation (including evaluation of slow transit and defecatory dysfunction), combining colonic transit and high-resolution manometric studies, and reporting for the first time results of MRI defecography in a group of PD patients. Our findings demonstrate the pathophysiological and clinical heterogeneity of this symptom in PD, showing that multiple complex overlapping mechanisms are involved with poor correlation between subjective symptoms, current definitions used in clinical practice and objective measurements of different physiological tests.

4.4.1 Clinical assessment

Definition of subjective symptoms of constipation is challenging and currently there are no accepted criteria to define these symptoms or validated questionnaires to evaluate their severity specifically in patients with PD. In this study only patients with subjective complaints of constipation were included but solely 78.6% of them fulfil the criteria of < 3 bowel movements per week or the need to strain to pass a stool, a definition commonly used in PD studies.(132) Similar figures are also present when these subjective symptoms are compared with the results of physiological tests, with only about two thirds of patients showing abnormal findings. Severity of constipation showed again poor correlation with a comprehensive range of investigations for the evaluation of this disorder. These discrepancies reflect the lack of well-accepted and accurate clinical tools to make the diagnosis, assess the severity and the impact of constipation specifically in patients with PD. Other studies have shown similar conflicting results between subjective complaints and objective abnormalities although the latter were more commonly present than subjective symptoms.(141, 152) However differences in methodologies and the lack of a comprehensive battery of investigations make any comparisons between

studies challenging. Development of specific assessment scales for constipation in PD should consider its symptom-based character, incorporating the patient's perception, and should also include questions to address slow transit and defecatory dysfunction.

Severity of constipation in our study varied widely among individuals although PACSYM scores in PD patients were lower compared to pooled results from a meta-analysis of patients with functional constipation.(153) Severity of constipation correlated with severity of the disease including motor and non-motor symptoms, in agreement with previous studies,(148, 154, 155) although there was no correlation with disease duration indicating that constipation is an early symptom that can predate the diagnosis of PD for many years. Supporting this idea, constipation and manometric abnormalities have been reported in early drug-naïve patients (141, 156) without significant differences when compared to advanced stages of the disease.(142, 143) No correlations were found between severity of constipation and any of the medications used for the symptomatic treatment of PD, including treatment with anticholinergic drugs.

4.4.2 Colonic transit, high resolution anorectal manometry and MRI defecography

Our study included a comprehensive battery of assessments incorporating recent developments in diagnostic techniques such as high-resolution ARM and MRI defecography. Our findings did not show a good correlation among different investigations reflecting the multifactorial complex origin of constipation in PD and the need of a comprehensive assessment to elucidate the overlapping contributing factors. Both slow transit and defecatory dysfunction contributed to constipation and overlapped in the majority of our PD patients. When analysed together, defecatory dysfunction (high-resolution ARM and MRI defecography abnormalities) seem more prevalent than isolated

slow transit constipation. Only a very few studies have evaluated colonic transit and anorectal dysfunction simultaneously and agree with a predominant role for anorectal dysfunction although either manometric parameters without additional defecography were used (145, 157) or studies were conducted decades ago using historical diagnostic techniques.(158) The understanding of pathophysiological mechanisms of constipation has important implications in clinical practice as, whilst slow transit is managed with diet modifications and laxatives, defecatory dysfunction is likely to be underdiagnosed and biofeedback therapy seems to be an effective therapy (137) with some patients responding to apomorphine (159, 160) and botulinum toxin injections.(161)

Delayed colonic transit was found in 66% of our patients and has been previously reported in multiple studies in approximately 80% of patients with PD when compared to controls. (152, 157, 162, 163) Although methodological differences make comparisons between studies difficult, the average transit time is estimated to be twice as long in PD patients,(134, 158) but not as high as in patients with idiopathic functional constipation (153). Similar to our results, in general there is poor clinical correlation of these abnormalities with constipation symptoms. (132) It has been speculated that the pathophysiological mechanisms behind delayed colonic transit is impaired colonic motility secondary to α-synuclein deposition in the enteric nervous system, although the clinical significance of these depositions has not been well established and distinction between physiological and pathological α-synuclein deposits is still an intense matter of research.(124, 164)

High-resolution ARM was pathological in 70% of our patients with abnormalities in tests of rectal sensation more commonly reported although abnormal sphincter function, rectal reflex activity and balloon expulsion were also found in some patients. Rectal hyposensitivity is a common finding in patients with chronic constipation and it is likely secondary to impaired rectal afferent input. Reduced rectal sensation causes diminished perception of rectal wall distension leading to loss or attenuation of the desire to defecate leading to faecal

impaction.(165, 166) Previous manometric assessment in patients with PD have shown abnormalities with anorectal dysfunction in a great proportion of patients even at early stages of the disease, although the pattern of dysfunction is very heterogeneous including rectal hyposensitivity and several motor abnormalities of sphincter pressure or contractility (dyssynergia)(140, 141, 143, 157, 158, 161, 167) although only two of them incorporated high-resolution manometry.(142, 145) Taken together, in addition to methodological differences and incorporation of more sensitive high-resolution equipment that may contribute to variability of results, these findings indicate that pathophysiology of defecatory dysfunction in PD is complex with multiple heterogeneous overlapping mechanisms. Nevertheless, these results should be interpreted carefully taking into context the clinical picture and other results from additional tests of defecatory function (defecography, sphincter electromyography) as no single test is able to provide a full assessment of anorectal function.(137, 138, 144)

We also reported, for the first time in PD patients, findings on the assessment of defecography using MRI as an additional test evaluating anorectal function. The prevalence of abnormal findings on MRI defecography was similar to colonic transit and high-resolution ARM with two thirds of patients showing evidence of anorectal dysfunction. All patients with abnormal results showed incomplete/delayed evacuation and findings consistent with anorectal dyssynergia (lack of relaxation of puborectalis muscle and pelvic floor descent) were found in 55%. Although no previous studies have been performed in PD patients, our results seem to be consistent with previous studies on patients with functional constipation showing that MRI defecography is able to detect evacuation difficulties not only due to dyssynergic defecation, and therefore a useful investigation in the assessment of constipation in PD.(168) Despite similar rates of abnormal results, correlation between high-resolution ARM and MRI defecography was very poor in our study. While manometric findings reported a predominant pattern of rectal hyposensitivity, the main abnormalities on defecography were related to poor coordination and

relaxation of motor anorectal function (dyssynergia) which was not detected by the manometry or balloon expulsion test. Although our results need to be replicated in other groups with greater number of patients, they suggest that MRI defecography is a sensitive technique to detect defecatory dysfunction and a useful complementary diagnostic test to ARM in the assessment of constipation.

The main strengths of our study are the presence of detailed clinical information on PD and constipation, the comprehensive range of investigations included to assess the multiple pathophysiological aspects of constipation in a systematic and structured manner and the inclusion of modern techniques such as high-resolution ARM or the report for the first time of MRI defecography findings in patients with PD. Our study also had some limitations. Although normal reference values and findings on patients with functional chronic constipation are available in the literature for some of these investigations, including colonic transit time (136, 153, 169) and high-resolution ARM, (150) we did not include a control group to compare our results. Findings on MRI defecography should be interpreted with caution given the relatively small sample size and the lack of previous studies using this technique on PD patients. Finally we did not discontinue any of the regular medications of the participants in order to evaluate the symptoms in real-life clinical settings, including dopaminergic medication and anticholinergics which may potentially have effects on colonic motility, although our analysis did not reveal any correlations between any PD medications and severity of the symptoms.

4.4.3 Conclusions

In summary, our study provided a comprehensive assessment with a battery of investigations that allowed a detailed pathophysiological evaluation of constipation in patients with PD. Results showed a discrepancy between patient

perception of the symptoms and the definitions and severity questionnaires commonly used in clinical practice which reflects the lack of specific diagnostic tools for the assessment of this symptom in PD. Development of specific clinical assessments should take into account the patient subjective perception of the symptoms and evaluate slow transit and defecatory symptoms. Our results showed a heterogeneous pattern of pathophysiological abnormalities suggesting multiple overlapping mechanisms contributing to the symptomatology involving both slow colonic motility and abnormalities of anorectal function including rectal hyposensitivity and defecatory dyssynergia. Given the multifactorial aetiology of the symptoms, the poor correlation between different investigations, the overlap between healthy and pathological parameters and the inability of any single test to provide a full assessment of constipation, a battery of tests including at least one investigation assessing colonic transit and two evaluating anorectal function should be included for a comprehensive pathophysiological evaluation in those with refractory constipation. Results of this systematic assessment should guide the multidisciplinary management of the symptoms with dietary modifications, laxatives and biofeedback therapy according to the underlying pathophysiological abnormalities.

Section II: The role of non-motor features on the pathogenic process of Parkinson's disease

Chapter 5. Association between diabetes and subsequent Parkinson's disease: a record-linkage cohort study

5.1 Introduction

Although historically insulin was regarded as a peripheral hormone regulating glucose metabolism, the role of this hormone in the central nervous system has started to be elucidated in the last few decades. It is now evident that insulin signalling in the brain, locally synthesized by neurons and the majority peripherally produced by pancreatic cells, has a neuromodulatory role promoting neuronal homeostasis and survival. This is in addition to regulation of other body functions such as weight, reproduction and cognition.(170) Growing evidence shows that disruption of the insulin signalling and its downstream pathways through the insulin receptors are down regulated in normal aging, and pathologically reduced in neurodegenerative conditions.(171) Evidence suggests that diabetes and some neurodegenerative disorders are synergistic conditions linked by dysregulated pathophysiological pathways, rather than parallel coincidental aging processes. Reduction of insulin receptors in the SNpc, neuroprotective effects of insulin on dopaminergic neurons and regulation of dopaminergic function by insulin signalling have been demonstrated in PD. (172) The relationship between diabetes and PD is complex and it is still a matter of debate whether insulin resistance in the brain is a cause or consequence of neurodegeneration or, more likely, there is a bidirectional interaction between the two. The underlying mechanisms of this complex interaction are likely to share disrupted pathways and abnormalities of brain insulin signalling interfere with apoptosis, neuroinflammation, mitochondrial dysfunction, autophagy, protein synthesis and synaptic plasticity, which are known to contribute to PD pathogenesis. These similarities have led some authors to propose that both type 2 diabetes (T2DM) and PD are chronic diseases of impaired glucose metabolism and insulin resistance sharing common molecular pathogenic pathways. (173-175)

Abnormalities of insulin signalling in PD patients would theoretically put them at risk of insulin resistance and deficient glucose metabolism. Several studies have reported a higher prevalence of impaired glucose tolerance test, with historical figures as high as 50-80%,(176) although more recent data provide a prevalence of impaired glucose metabolism around 20% in PD patients.(177)

Observational clinical studies have also provided evidence suggesting that concomitant T2DM may modify the PD phenotype and disease progression. In a case-control study, patients with PD and preceding T2DM had more severe disease, higher scores on the UPDRS scale for severity of motor symptoms, required higher doses of levodopa for symptomatic control.(178) The presence of T2DM has been associated with a faster progression of the disease(179) including earlier motor complications, (180) cognitive impairment,(179, 181-183) postural instability and gait difficulties.(184, 185)

Finally, several epidemiological studies have explored an association between diabetes mellitus and future risk of PD, which is of crucial importance given the high prevalence of T2DM in the general population in Western countries. Despite the converging biological and clinical data, evidence from epidemiological studies remains equivocal (186, 187) although meta-analyses of cohort studies have reported an increased pooled relative risk of developing PD after diabetes mellitus.(188, 189) The aim of this study is to provide further evidence using national record-linkage to evaluate whether pre-existent type 2 diabetes mellitus (T2DM) was associated with subsequent PD in a large nationwide hospital cohort.

5.2 Methods

5.2.1 Study design, population and data

A retrospective cohort study was conducted analysing in conjunction data from English national Hospital Episode Statistics (HES) and mortality data. HES records incorporate every episode of day-case (admission without overnight stay) or inpatient care (at least 1 overnight stay) in all National Health Service hospitals in England from January 1999 to December 2011. HES data were obtained from National Health System Digital (formerly the Health and Social Care Information Centre). Mortality data were obtained from the Office for National Statistics. The linked dataset with time-sequenced record of hospital admissions and death for each individual used in this study derived from HES and mortality data was constructed by the Oxford Record Linkage Group. The construction, maintenance and analysis of the dataset for research was approved by the Central and South Bristol Research Ethics Committee (04/Q2006/176).

A cohort of individuals with T2DM (exposed cohort) was constructed by identifying for each individual, the earliest known episode of day-case or inpatient admission in which T2DM was coded using the International Classification of Diseases Revision 10 (ICD-10) code E11, whether the main reason for admission or in any diagnostic position, within the study period. A reference cohort was comprised of all individuals without a coded diagnosis of T2DM admitted for a range of minor medical conditions and surgical procedures including any of the following: otitis, varicose veins, haemorrhoids, upper respiratory tract infections, nasal polyps, tonsillectomy, teeth disorders, inguinal hernia, nail diseases, sebaceous cyst, internal derangement of knee, bunions, contraceptive management, dislocations/sprains/strains, bruising, gall bladder disease, appendectomy, hip replacement, knee replacement. Individuals were excluded if they had record of PD (ICD-10 code G20) dated either before or at the same time as the earliest known T2DM record or, in the

unexposed cohort, their admission for their reference condition. Exposed and reference cohorts were then searched for any subsequent hospital admission with a coded diagnosis of PD (ICD-10 code G20).

Individuals with a code for ischaemic cerebral infarction (ICD-10 code I63), vascular parkinsonism (ICD-10 code G21.4), drug-induced secondary parkinsonism (ICD-10 code G21.1) or normal pressure hydrocephalus (ICD-10 code G91.2) recorded at any time were excluded from both cohorts.

5.2.2 Statistical analysis

Date of entry was the date of the first recorded episode of day-case or inpatient hospital admission coded for T2DM (exposed cohort) or any reference condition. Date of first hospital admission coded for PD in any diagnostic position, or death, or date of end of data collection (whichever occurred first) constituted the date of exit of the cohort.

Multivariable Cox proportional hazard regression models were used to estimate the risk of a subsequent diagnosis of PD. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated and adjusted for age, sex, calendar year of cohort entry, region of residence and patients' quintile of Index of Multiple Deprivation score (a measure of area-level deprivation).

Subgroup analyses were subsequently conducted to assess whether the HRs differed by age group, by length of follow-up, or by the presence of T2DM-related complications. Complicated T2DM was defined by the presence of a hospital episode coded for diabetic neuropathy (ICD-10 code G63.2), diabetic nephropathy (N08.3) or diabetic retinopathy (H36.0)

The following sensitivity analyses were performed: i) to assess the potential possibility of surveillance bias and reverse causality, those with an interval

between the earliest record of T2DM and PD <1 year were excluded, ii) to evaluate potential outcome misclassification patients with a diagnosis of schizophrenia or other psychotic disorders (ICD 10 code F20-29), other forms of secondary parkinsonism (ICD-10 codes G21 and G22), degenerative diseases of the basal ganglia (ICD-10 code G23), essential tremor (ICD-10 code G25.0), drug-induced and other forms of tremor (ICD-10 codes G25.1 and G25.2) were excluded, iii) to mitigate surveillance bias results were also controlled by total number of admissions per individual.

5.3 Results

A total of 2,017,115 individuals entered the T2DM cohort and 6,173,208 people entered the reference cohort. Results showed an overall higher rate of subsequent PD in the T2DM cohort with an adjusted HR of 1.32 (95% CI 1.29-1.35). Subgroup analysis revealed a substantially higher relative rate in younger individuals and those with complicated T2DM (**Table 5.1**). All sensitivity analyses as described earlier did not materially affect the point estimate and a significant association between T2DM and subsequent PD remained.

	PD observed	HR	95% CI	*P* value
T2DM cohort (N = 2,017,115)	14,252	1.32	1.29-1.35	<0.001
Age group				
25-44 y (n = 130,728)	58	3.81	2.84-5.11	<0.001
45-64 y (n = 650,387)	1,711	1.71	1.61-1.81	<0.001
65-74 y (n = 571,291)	5,112	1.40	1.35-1.45	<0.001
>75 y (n = 664,709)	7,371	1.18	1.14-1.21	<0.001
Sex				
Men (n = 1,068,269)	8,713	1.27	1.23-1.30	<0.001
Women (n = 948,846)	5,539	1.42	1.37-1.47	<0.001
T2DM-PD coded admission time interval (y)				
< 1	3,030	1.44	1.37-1.52	<0.001
> 1	11,222	1.29	1.26-1.33	<0.001
1-4	6,958	1.30	1.26-1.34	<0.001
5-9	3,737	1.28	1.23-1.33	<0.001
>10	527	1.32	1.19-1.46	<0.001
T2DM related complications**				
Complicated T2DM (n = 180,593)	1,824	1.49	1.42-1.56	<0.001
Uncomplicated T2DM (n = 1,836,522)	12,428	1.30	1.27-1.33	<0.001
Controlling by total number of admissions per individual				
		1.31	1.28-1.34	<0.001

Table 5.1. Hazard ratios and 95% confidence intervals in the exposed T2DM cohort (compared with reference cohort).

5.4 Discussion

Our results support an increased risk of PD in patients with previous T2DM. The magnitude of association was higher in individuals with T2DM who were younger or had T2DM related complications. The main strengths of this study are the large size of HES database with stratification of relative risks by age group, sex and T2DM complications, the cohort design, the ability to exclude patients with cerebrovascular disease, drug-induced and vascular parkinsonian disorders, adjustment for multiple potential confounding factors and sensitivity analyses. HES dataset includes all National Health Service hospitals in England and as health care is free to access in this country, it is considered to be representative of the entire population and results are likely generalizable.

5.4.1 Epidemiological evidence

Epidemiological evidence about the risk of subsequent PD in patients with T2DM is equivocal, with conflicting results among cohort and case-control studies.(186-189) Previous cohort studies have shown a positive (190, 191) or no association (192) between pre-existing T2DM and PD. The magnitude of association in our study is similar to the pooled estimate from recent meta-analyses: Cereda and colleagues included five studies with a total population of 681,000 (HR 1.26; 95% CI 1.03-1.55), (188) and a recent revision included seven population-based cohort studies with a total population of 1,761,632 (RR 1.38; 95 CI 1.18-1.62).(189) However, the population size of our study is far greater than the accumulated population included in both meta-analyses, with tight confidence intervals around the point estimate. There was significant heterogeneity in the studies included in the previous meta-analyses (I^2 = 60.2% and I^2 = 71.2% respectively) which would have affected precision and they are likely to be due to differences in study design (hospital-based as in our study, health/professional registries,(190) or population-based(191, 192)), study

population (results differ between Europe(191) and mainland USA(192)), ascertainment of PD and T2DM (self-reported, drug/medical registries or physician-confirmed diagnosis) and adjustment for confounding factors.

The magnitude of risk in our study was greater in younger individuals where genetic factors may relatively exert more of an effect and more than 400 genes, previously identified through genome-wide association studies, have been closely linked to both conditions using integrative network analysis.(193) On the other hand, the association in elderly patients may be the consequence of disrupted insulin signalling secondary to additional lifestyle and environmental factors causing cumulative pathogenic brain changes. This is supported by the higher risk among those with complicated T2DM in our cohort, and those with long disease duration T2DM (>10 years) reported in previous studies.(190, 194)

5.4.2 Pathogenic mechanisms

As discussed earlier, whether due to genetic predisposition, environmental factors or both, disrupted brain insulin signalling could lead to shared dysregulated cellular pathways including neuroinflammation (microglia activation, production of pro-inflammatory cytokines), mitochondrial dysfunction and increased oxidative stress. Little is known on how the effects of these dysregulated pathways ultimately promote neurodegeneration and contribute to the development and/or progression of PD. A few studies have attempted to elucidate these mechanisms using various methodologies although conflicting results prevent any conclusions. Despite preclinical evidence suggesting a reciprocal role between insulin and striatal dopaminergic degeneration in animal models of PD,(171) clinical studies using dopaminergic functional imaging have shown conflicting results with reduced (179, 183) but also similar (184) deficits in the striatum among PD patients with and without T2DM. Some of these dysregulated pathways are shared with other

neurodegenerative diseases and growing evidence from different areas of research shows an association between T2DM and Alzheimer's disease.(195) Study of cerebrospinal fluid biomarkers in PD patients showed greater total tau levels in those with T2DM, (179) with similar increased values in patients with Alzheimer's disease,(196) suggesting the possibility that tau pathology may contribute to the cognitive phenotype in patients with PD and concomitant T2DM. Further evidence of the impact of concomitant T2DM on PD comes from neuroimaging studies which have consistently reported brain atrophy and cortical volume loss affecting several areas including total grey matter,(197, 198) frontal (197) and temporal lobe,(183, 198) although again, these results were not replicated in other study.(179)

5.4.3 Clinical and potential therapeutic implications

Given the common molecular pathways shared between T2DM and PD and based on promising preclinical results on animal models, it has been proposed that restoration of brain insulin signalling could have neuroprotective effects and anti-diabetic drugs are currently being repurposed as potential PD treatments.(171) One of the therapeutic targets that has brought more attention is the peroxisome proliferator-activated receptor γ (PPAR-γ) given its pivotal role in mitochondrial function and gluconeogenesis. This hypothesis is further supported by observational epidemiological studies showing that treatment with thiazolidinediones (a class of PPAR-γ agonists) showed a reduction in the risk of PD in several cohort studies in the United Kingdom, Norway and Taiwan.(199-201) These promising results prompted a large, multicentre, double-blind, placebo-controlled trial including 210 patients randomly assigned to 45 mg/day pioglitazone, 15 mg/day pioglitazone or placebo to evaluate potential neuroprotective effects on PD patients. Results failed to show a significant benefit on symptoms (measured using total UPDRS) and the authors concluded that pioglitazone was unlikely to modify clinical

progression in PD at the doses studied.(202) More promising are the preliminary clinical results for exenatide, a glucagon-like peptide 1 (GLP1) receptor agonist licensed for the treatment of diabetes that is being evaluated for neuroprotective effects on PD.(203) A recent double-blind trial involving 62 patients with PD randomly assigned to placebo or exenatide 2 mg weekly injections showed positive and persistent effects on motor symptoms measured by the section 3 of the MDS-UPDRS.(204) Whether effect is secondary to long-lasting symptomatic benefit or neuroprotection remains unclear although a larger trial is being organised to further explore this potential therapeutic avenue.

5.4.4 Limitations and conclusions

Limitations of the study include the lack of clinical information for PD ascertainment beyond routinely collected data. However, in England, PD diagnosis is based upon recommendations by national clinical guidelines and it is common clinical practice for patients with suspected diagnosis to be referred untreated to a movement disorder specialist for diagnosis confirmation.(205) In addition, individuals with cerebrovascular disease, secondary parkinsonian disorders and other movement disorders that could potentially be misdiagnosed as PD were excluded to reduce potential diagnostic misclassification. However extensive strict sensitivity analyses were applied with no qualitative modification of the results of the association between T2DM and PD. The study used routinely collected data and we were unable to adjust for other potential confounders such as anti-diabetic medication or smoking. As this is a hospital-based study, potential selection bias cannot be ruled out (although this is mitigated by using a hospital-based reference cohort), and individuals included in the T2DM cohort may represent the more severe spectrum of disease. Moreover, the study uses prevalent T2DM cases based on first recorded hospital diagnosis and is not a follow-up from first point of onset.

This national record-linkage cohort study shows an increased risk of PD in patients with T2DM, particularly in those with complicated diabetes and younger patients. Our results support the link between these two conditions which may be the result of genetic predisposition and / or disrupted shared pathogenic pathways with potential clinical and therapeutic implications.

Chapter 6. Autonomic dysfunction in Parkinson's disease – impact on disease progression and survival.

6.1 Introduction

6.1.1 The autonomic nervous system

The autonomic nervous system innervates all the tissues and organs of the body and maintains, in close collaboration with the endocrine system, body homeostasis and the appropriate balance of autonomic body functions. In the central nervous system, it comprises a network of interconnected areas at different levels including the autonomic spinal neurons, brainstem nuclei (nucleus of the solitary tract, ventrolateral medulla, parabrachial nucleus and periaqueductal grey matter) and basal forebrain (hypothalamus and limbic system). The central autonomic nervous system integrates visceral and somatosensory information and generates the appropriate autonomic, endocrine and motor responses mediated by preganglionic neurons.(30) Autonomic output is organised in a two-neuron efferent pathway including a preganglionic and postganglionic neuron with the former acting as the link between the central and peripheral autonomic nervous system (**Table 6.1**). From a physiological perspective the peripheral autonomic nervous system is divided into the sympathetic, parasympathetic and enteric nervous systems. The preganglionic sympathetic neurons are located in the intermediolateral cell column at the T1-L2 levels of the spinal cord and activate the neurons in the paravertebral and prevertebral sympathetic ganglia. The parasympathetic preganglionic neurons lie in the brainstem nuclei or the sacral segments (S2-S4) of the spinal cord and their output is mediated by the vagal and pelvic nerves to the postganglionic parasympathetic neurons located just outside or inside the target organs. All the preganglionic neurons use acetylcholine to activate the nicotinic receptors of the ganglion cells while several neurotransmitters are involved in postganglionic activation depending on the division of the

autonomic nervous system and the target organs. The parasympathetic ganglionic neurons use acetylcholine as the main neurotransmitter stimulating muscarinic receptors mediating local reflexes such as pupillary reactions, gastrointestinal motility and secretion (in conjunction with the enteric nervous system), micturition and defecation, lacrimal and salivary secretion and beat-to-beat control of the heart rate. Acetylcholine is also the main ganglionic neurotransmitter for sympathetic neurons innervating the sweating glands. Most sympathetic ganglion neurons use norepinephrine as the main neurotransmitter to innervate blood vessels and visceral organs which are involved in blood pressure and flood regulation, body temperature and responses to stress and external stimuli. The enteric nervous system consists of two ganglionated plexuses located in the submucosa (submucosal or Meissner's plexus) and the muscle layer (myenteric or Auerbach's plexus) of the gut. The enteric nervous system controls gastrointestinal motility and secretion and, although their activity is largely independent, they also receive modulatory input from the sympathetic system via the celiac and mesenteric ganglia, and parasympathetic signals from the dorsal motor nucleus of the vagus.

	Preganglionic (Ach nicotinic)	Postganglionic	Function
Sympathetic	T1/T3	Superior cervical	Pupillary dilatation (NE) Vasoconstriction (NE) Facial sweating (Ach)* Melatonin secretion (NE)
	T1/T8	Stellate and upper thoracic	Bronchodilatation (NE) Cardiac stimulation (NE) Vasoconstriction and piloerection (NE) Sweating (Ach)*
	T5/T12	Celiac, mesenteric, adrenal medulla	Vasoconstriction (NE) Reduction of GI motility (NE) Epinephrine, glucagon and renin secretion
	T10/L2	Lumbar paravertebral	Vasoconstriction and piloerection (NE) Sweating (Ach)*
	T2/L2	Inferior mesenteric and hypogastric	Urine storage Ejaculation Rectal control
Parasympathetic	Edinger-Westphal	Ciliary	Pupillary contraction and accommodation (Ach)*
	Superior salivatory	Pterygopalatine and submaxillary	Lacrimation, mucosal secretion and salivary secretion (Ach)*
	Inferior salivatory	Otic	Salivatory secretion (Ach)* Vasodilatation (Ach)*
	Dorsal motor nucleus vagus	Pulmonary, myenteric and submucosal plexus	Bronchoconstriction (Ach)* Increase GI motility (Ach, SP)* GI secretion (Ach, VIP)
	Nucleus ambiguus	Cardiac plexus	Cardiac inhibition

Table 6.1. Peripheral structures of the autonomic nervous system.

*Muscarinic receptors
Ach, acetylcholine; NE, norepinephrine; SP, substantia P; VIP, vasointestinal polypeptide.

6.1.2 Autonomic dysfunction in Parkinson's disease

AutD is an important feature of synucleinopathies including PD. Symptoms secondary to AutD in PD are common and can happen at early stages of the disease even before the diagnosis.(18) They include a wide range of manifestations and reflect the multisystem character of PD. Unlike MSA, AutD in PD is considered to be secondary to an earlier and more extensive involvement of the peripheral autonomic structures, although there is also evidence of impairment of key autonomic regulatory central nuclei.(35, 206) The autonomic nervous system can be affected at any level, including the sympathetic, parasympathetic and, as discussed earlier, the enteric nervous system, resulting in a constellation of symptoms involving multiple body functions. In most cases multiple factors may contribute to the symptomatology (particularly medications), although a primary neuropathological involvement of autonomic structures has been demonstrated.(22, 35, 121, 206, 207)

Several abnormalities of cardiovascular dysfunction have been described in PD; neurogenic orthostatic hypotension is the most disabling, although postprandial hypotension, supine hypertension and non-dipping have also been reported.(36) Orthostatic hypotension is one of the most common signs of AutD being present in 30% of patients with PD although it is symptomatic only in a proportion of these.(38) Manifestations of orthostatic hypotension include lightheadedness, blurred vision, coat hanger pain and cognitive complaints that are classically triggered when standing. The underlying pathophysiology involves the impairment of baroreflex sympathetic vasoconstriction as opposed to MSA, where orthostatic hypotension is mainly mediated by impairment of central autonomic networks.

Symptoms of gastrointestinal dysfunction in PD have been previously discussed at length in this thesis and a degree of AutD is likely to contribute to their pathophysiology. A reduction in salivary secretion (drooling is secondary to

motor impairment of swallowing mechanisms) has been reported in several studies, (208, 209) likely caused by direct involvement of the salivary gland, the salivatory nucleus and submandibular ganglion.(207) Abnormal α-synuclein accumulation in the enteric nervous system is believed to contribute to problems with gastrointestinal motility at different levels leading to symptoms such as impaired gastric emptying, slow colonic transit and anorectal dysfunction. Gastroparesis may alter levodopa pharmacokinetics by delaying its absorption contributing to the development of motor fluctuations.(131)

Urinary symptoms can be classified in voiding (incomplete bladder emptying, post-void residual, reduced flow and urinary retention) or storage symptoms (urinary urgency, increased urinary frequency, nocturia and incontinence) the latter being more common in patients with PD with findings of detrusor overactivity on urodynamic studies.(210) The pathophysiological mechanisms are not fully understood although dysfunction of the control of the micturion centre as a consequence of disruption of the frontal-basal ganglia circuit may be responsible.(211) Urinary symptoms are usually associated to sexual dysfunction with symptoms including erectile dysfunction and difficulties in ejaculation in men, and reduced vaginal lubrication, painful intercourse and incontinence during sexual activity in women.(212)

Sweating is an important thermoregulatory activity and anhidrosis can be a manifestation of AutD in PD. Depending on its distribution and severity it may be asymptomatic or manifest as reduced heat tolerance or reactive hyperhidrosis in preserved body areas.

The wide range of autonomic symptoms contributes significantly to PD clinical heterogeneity. In clinical practice, prediction of the disease course, complications and prognosis is crucial to provide adequate treatment, support and counselling. In this regard, several studies have attempted to identify prognostic factors for PD. Older age at diagnosis and the postural-instability and gait-difficulty (PIGD) subtype have been consistently identified as predictors of

faster progression to disability. (213, 214) Increasing age and dementia have been the two predictors most commonly associated with shorter survival.(215)

Impairment of the autonomic nervous system is not exclusive of PD and it is a notable feature of other synucleinopathies. (34) In MSA, AutD is one of the key defining features included in the clinical diagnostic criteria (216) and, in addition to the contribution to the clinical burden, development of AutD has been consistently associated with shorter survival in large cohort studies in different populations (217-219) including clinico-pathological studies with post-mortem confirmation of the diagnosis.(53, 220, 221) Symptoms of AutD are also common in dementia with Lewy bodies and it has been suggested that dysautonomia may be associated with reduced survival although evidence is less robust.(222)

Despite its importance on other synucleinopathies and the increasing awareness of autonomic symptoms as part of the clinical spectrum in patients with PD, studies assessing the prognostic impact of AutD on this condition are scarce. Most of them evaluate autonomic symptoms in isolation, particularly orthostatic hypotension (37) or cardiovascular dysfunction using autonomic function tests,(223) and they do not provide a systematic assessment of the different domains of the autonomic system. Other studies are limited by short follow up periods which makes the assessment of the prognostic value of AutD difficult in a clinically meaningful way.(24, 223) More importantly, none of these studies provided post-mortem confirmation of the diagnosis which is of crucial importance given the low diagnostic accuracy rate in patients presenting with parkinsonism and AutD.(224-226)

Our hypothesis is that development of AutD can also have a similar negative prognostic impact in patients with PD. The aim of this study is to investigate whether the time from diagnosis to development of AutD has a prognostic impact on disease progression and survival in a large series of PD patients with

well-documented clinical progression throughout the disease course and post-mortem confirmation of the diagnosis.

6.2 Methods

6.2.1 Study design

This is a retrospective review of the clinical records of 100 consecutive patients with a pathological diagnosis of PD from the archives of the QSBB in London, United Kingdom, between January 2006 and June 2016. Patients with the following criteria were excluded: (i) those diagnosed with dementia prior to or within one year of onset of motor symptoms,(104) (ii) monogenic forms of PD (e.g. LRRK2, parkin, alpha-synuclein), (iii) coexistent neuropathological diagnosis deemed to affect PD progression (e.g., Alzheimer disease-related neurofibrillary tangle pathology Braak and Braak stage ≥ V, pathological changes consistent with other concomitant neurodegenerative conditions), (iv) comorbidities known to affect the autonomic nervous system (e.g., diabetic ganglionopathies / neuropathies), (v) patients with insufficient information documenting autonomic symptoms and disease progression.

6.2.2 Clinical assessment

All patients were regularly assessed in life by hospital specialists (neurologists or geriatricians) in the UK throughout the course of their illness. A systematic review of the medical records was performed recording clinical features and disease progression data. Symptoms were recorded as absent if not reported. Date of onset of each feature was documented and the time from diagnosis was calculated.

AutD was defined by either cardiovascular autonomic failure on autonomic function testing or documentation of any two of the following symptoms/signs persistent for longer than 6 months:(53) (i) urinary urgency, increased daytime frequency and nocturia without hesitancy as defined by the International

Continence Society,(54) (ii) constipation (< 3 defecations per week), having to strain to pass stools or regular use of laxatives, (iii) symptoms of upper gastrointestinal dysfunction including nausea, bloating and early satiety, (iv) symptomatic or documented orthostatic hypotension defined by >20mmHg drop in systolic blood pressure or >10mmHg in diastolic pressure on standing, (v) sweating abnormalities or (vi) erectile dysfunction in males.

Six milestones with significant functional impact and implications in care management reflecting the involvement of different functional domains in PD were selected to define disease progression:(53) (i) frequent falls defined by >2 episodes per year, (ii) cognitive impairment severe enough to impact significantly on tasks of daily living, (iii) unintelligible speech or the offering of communication aids, (iv) severe dysphagia or the offering of percutaneous endoscopic gastrostomy (PEG), (v) dependence on wheelchair for mobility, and (vi) placement in residential or nursing home care. These milestones were selected because of their clinical relevance and they are well documented in clinical records.

Other demographic data and clinical features were also recorded, including PD motor subtype (tremor-predominant, akinetic-rigid or PIGD), response to initial levodopa treatment (nil-mild, moderate, good, excellent)(53), maximum levodopa equivalent dose(227) and clinical diagnosis at time of death. Times from diagnosis to development of: (i) AutD, (ii) each autonomic symptom, (iii) first and (iv) each disease milestones and (v) death were calculated.

6.2.3 Neuropathological assessment

Formalin-fixed brain tissue samples were obtained from representative areas of the central nervous system and examined using routine stains supplemented with immunohistochemistry for Aβ peptide, tau protein (AT8 antibody), TDP-43,

ubiquitin and α-synuclein following QSBB standard protocols. Systematic histological analysis of neuronal loss in the substantia nigra and immunohistochemistry sections for inclusions known to be associated with neurodegenerative diseases was performed blinded to the clinical data on autonomic dysfunction. Lewy pathology (Lewy bodies and Lewy neurites) was assessed using a semiquantitative grading system (absent, mild, moderate, severe, very severe) in representative sections of the brainstem, basal forebrain and neocortical areas following consensus criteria and, based on the pattern of regional involvement, a Lewy body pathology type (brainstem, limbic, diffuse neocortical) was assigned for each case.(104)

6.2.4 Statistical assessment

Summary statistics are described as frequency and percentage for categorical data and mean and standard deviation (SD) for continuous variables. Linear regression was used to perform a preliminary analysis of potential associations between main explanatory variables. Time from diagnosis to development of AutD was considered as dependent variable and other relevant explanatory variables (e.g. age, gender, levodopa response, PD motor subtype, Lewy pathology type) were included as predictors in the model.

To assess the effect of time from PD diagnosis to AutD on the risk of developing a disease milestone or the risk of death (survival), patients were divided into two subgroups (e.g. early vs late AutD) using the median value, and Kaplan-Meier curves were plotted. Multivariable Cox proportional hazards models were then used to estimate the association between the main explanatory variable and the risk of developing a disease milestone or the risk of death (survival), which were considered in turn as dependent variables. Other explanatory variables included age, gender, PD motor subtype and response to levodopa. Adjusted HRs and 95% CIs were estimated. Visual inspection of

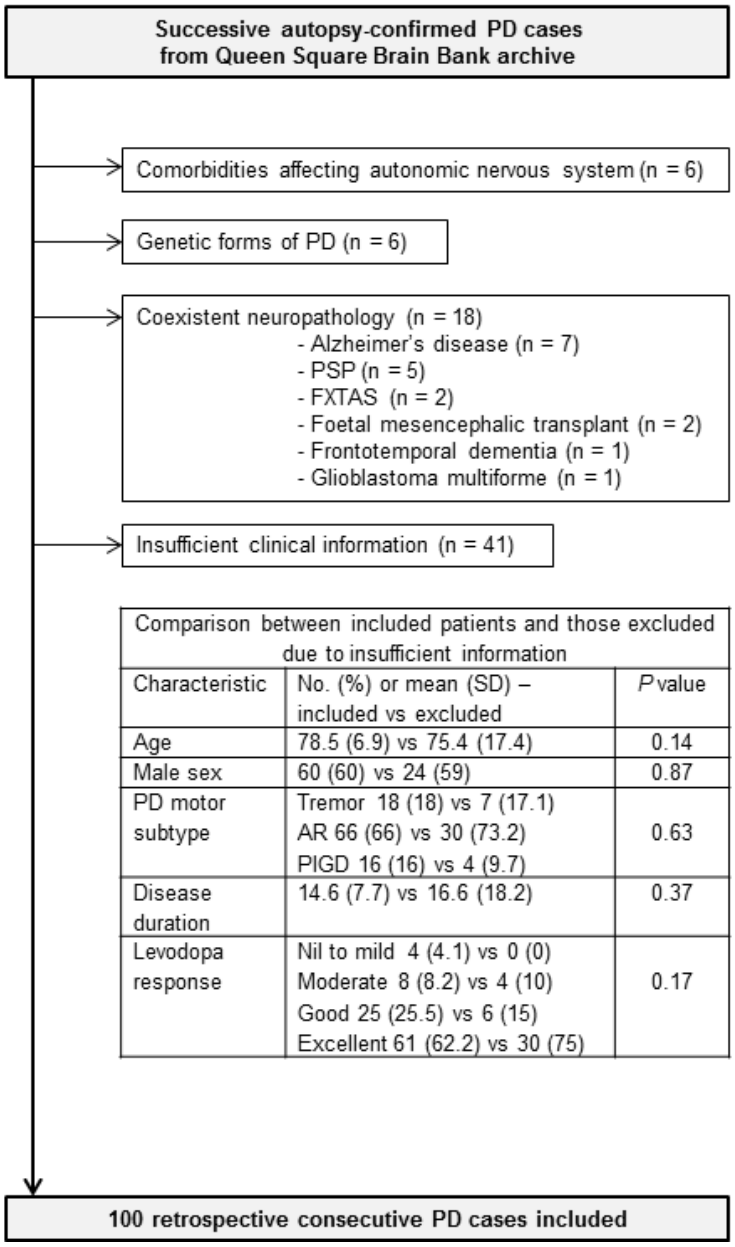
Kaplan-Meier curves and plots of scaled Schoenfeld residuals against time were used to assess the proportional hazards assumption. Censoring was considered uninformative.

Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the statistical software Stata 12 (StataCorp, TX).

6.3 Results

6.3.1 Demographics and clinical features

After exclusion of 71 patients, a total of 100 consecutive patients (60.0% male; age at diagnosis 63.9 ± 10.3 years; disease duration 14.6 ± 7.7 years) were included in the study. Details of excluded patients and flow chart of the study are shown in **Figure 6.1**. Of note, patients excluded with insufficient clinical information did not differ from the patients of the study in age, gender, disease duration, levodopa response or PD motor subtypes (all comparisons shown in **Figure 6.1**).



Comparison between included patients and those excluded due to insufficient information

Characteristic	No. (%) or mean (SD) – included vs excluded	*P* value
Age	78.5 (6.9) vs 75.4 (17.4)	0.14
Male sex	60 (60) vs 24 (59)	0.87
PD motor subtype	Tremor 18 (18) vs 7 (17.1) AR 66 (66) vs 30 (73.2) PIGD 16 (16) vs 4 (9.7)	0.63
Disease duration	14.6 (7.7) vs 16.6 (18.2)	0.37
Levodopa response	Nil to mild 4 (4.1) vs 0 (0) Moderate 8 (8.2) vs 4 (10) Good 25 (25.5) vs 6 (15) Excellent 61 (62.2) vs 30 (75)	0.17

Figure 6.1. Flow chart and comparison between excluded vs included patients.

AR, akinetic-rigid; FXTAS, fragile-X tremor ataxia syndrome; PIGD, postural instability and gait difficulty; PD, Parkinson's disease; PSP, progressive supranuclear palsy

Demographic and clinical features of patients included in the study are summarised in **Table 6.2**. Eighty three patients were clinically diagnosed with PD during life while 12 were misdiagnosed with MSA and 5 with progressive supranuclear palsy (PSP). At least one disease milestone was reached by 96% of patients with a mean time from diagnosis of 9.9 ± 6.2 years, with regular falls (83%), use of wheelchair (61%), care placement (52%) and cognitive impairment (50%) the most frequent. AutD developed in 85% of patients with a mean time from diagnosis of 6.7 ± 7.7 years (**Figure 6.2**). Thirteen patients developed AutD before the diagnosis of PD. The most common AutD disturbances were urinary symptoms (84%), constipation (83%) and orthostatic hypotension (60%). Histological analysis revealed that diffuse neocortical (65%) was the most common Lewy pathology type.

Feature	Total patients = 100 Percentage* or mean ± SD	Time from diagnosis (y); mean ± SD
Gender	F 40; M 60	
Age at diagnosis (y)	63.9 ± 10.3	
Age at death (y)	78.5 ± 6.9	
Disease duration (y)	14.6 ± 7.7	
Clinical diagnosis	PD 83; MSA 12; PSP 5	
PD motor subtype	Tremor 18; AR 66; PIGD 16	
Levodopa response (total n = 98)*	Nil 4 (4%); moderate 8 (8%); good 25 (26%); excellent 61 (62%)	
Maximum LED (mg)	917 ± 446	
Cause of death	Infection 45; deterioration 36; cardiovascular 4; other 15	
Lewy pathology type (total = 99)*	Brainstem 2 (2%); Limbic 33 (33%); Neocortical 64 (65%)	
First milestone	96	9.9 ± 6.2
Regular falls	83	10.2 ± 6.4
Wheelchair	61	11.6 ± 7.3
Speech	12	14.1 ± 7.0
Severe dysphagia	13	14.0 ± 8.1
Cognitive	50	10.8 ± 7.0
Care placement	52	12.0 ± 7.3
AutD	85	6.7 ± 7.7
OH	60	7.6 ± 8.8
Upper GI	16	3.7 ± 7.3
Constipation	83	7.0 ± 7.4
Urinary	84	5.0 ± 8.3
Erectile dysfunction (total males = 60)*	23 (38%)	3.0 ± 7.7
Sweating	12	5.5 ± 6.7

Table 6.2. Patient demographic and clinical features.

* number of patients and percentage are the same (total n = 100) unless stated otherwise, where data are shown as n (%)
AR, akinetic-rigid; F, female; LED, levodopa equivalent dose; M, male; MSA, multiple system atrophy; OH, orthostatic hypotension; PIGD, postural-instability and gait-difficulty; PSP, progressive supranuclear palsy; upper GI, upper tract gastrointestinal symptoms; y, years.

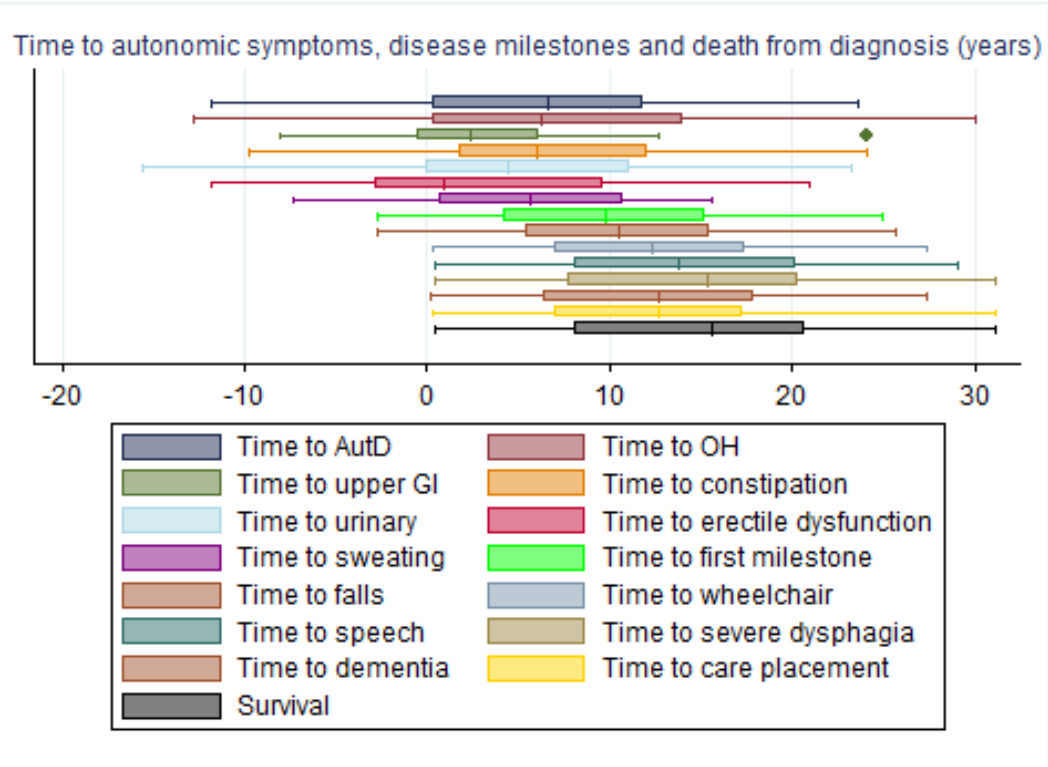


Figure 6.2. Box-and-whisker plots showing time from diagnosis to autonomic symptoms, disease milestones and death.

Box represents 25th and 75th percentiles with the line showing the median value. Whiskers represent adjacent values and outliers are represented by dots.

6.3.2 Association between AutD and other variables

Assessment of the relationship between explanatory variables (**Table 6.3**) showed that patients with earlier AutD were older (Regression coefficient -0.40; *P* <0.001; 95% CI -0.54 to -0.27), predominantly male (Regression coefficient -7.13; *P* <0.001; 95% CI -10.06 to -4.20), with a poorer levodopa response (Regression coefficient 12.19; P = 0.001; 95% CI 4.87 to 19.52), lower maximum levodopa equivalent dose (Regression coefficient 5.56; *P* = 0.002; 95% CI 2.14 to 8.98), and had PIGD motor subtype (Regression coefficient -10.08; *P* <0.001; 95% CI -15.16 to -5.00).

A clinicopathological correlation with the distribution and severity of α-synuclein pathology was assessed but development of AutD and Lewy pathology subtype did not show any significant association.

Explanatory variables	Regression coefficient	P value	95% CI
Age at diagnosis	-0.40	<0.001	-0.54 to -0.27
Male gender (vs female)	-7.13	<0.001	-10.06 to -4.20
PIGD PD subtype (vs tremor predominant)	-10.08	<0.001	-15.16 to -5.00
Excellent levodopa response (vs nil-mild)	12.19	0.001	4.87 to 19.52
Maximum LED (g)	5.56	0.002	2.14 to 8.98
Lewy pathology type limbic (vs brainstem)	2.27	0.70	-9.42 to 13.96
Lewy pathology type neocortical (vs brainstem)	2.96	0.61	-8.57 to 14.48

Table 6.3. Association between autonomic dysfunction and other variables.

AR, akinetic rigid; LED, levodopa equivalent dose; PIGD, postural-instability and gait-difficulty.

6.3.3 Effect of autonomic dysfunction on disease progression

Earlier development of AutD was significantly associated with an increased risk of reaching a disease milestone (**Table 6.4**, **Figure 6.3**, **Figure 6.4**, **Figure 6.5**). For every year delay in the development of AutD there was a reduction of risk of reaching the first milestone of 14% (HR 0.86; 95% CI 0.83 to 0.89; $P < 0.001$). The risk of reaching each individual milestone was also estimated. Earlier development of AutD was associated with a higher risk of falls (HR 0.88; 95% CI 0.84 to 0.92; $P < 0.001$), wheelchair dependence (HR 0.93; 95% CI 0.89 to 0.97; $P = 0.002$), cognitive impairment (HR 0.90; 95% CI 0.85 to 0.95; $P < 0.001$), and care placement (HR 0.91; 95% CI 0.87 to 0.96; $P = 0.001$). Results did not show an association between AutD with an increased risk of severe dysphagia or speech impairment although only a small number of patients developed these milestones.

6.3.4 Effect of autonomic dysfunction on survival

The impact of time from diagnosis to AutD on survival is summarised in **Table 6.4**, **Figure 6.3**, **Figure 6.4**, **Figure 6.5**. Survival analysis was adjusted by gender, age at diagnosis, PD motor subtype and response to levodopa as they have been previously reported as survival predictors in the literature (213) and they also showed a statistically significant association with shorter survival in the univariable analysis model in our study (see below *Other predictors of survival*). Shorter time to development of AutD was associated with shorter survival and for every year delay in the development of AutD there was a reduction of risk of death of 8% (HR 0.92; 95% CI 0.88 to 0.96; $P < 0.001$).

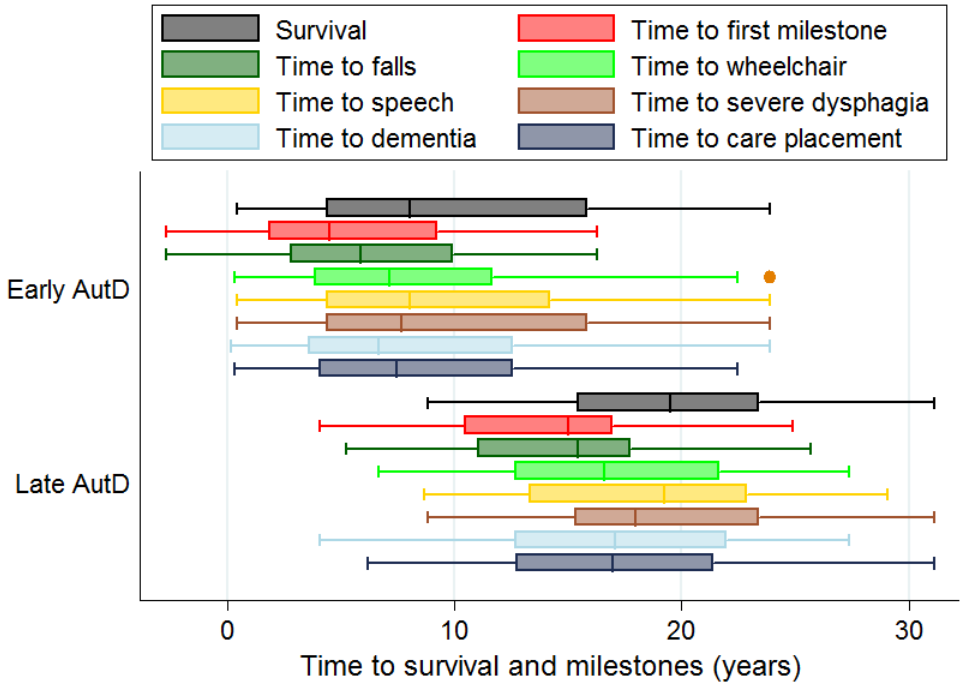


Figure 6.3. Box-and-whisker plots showing time from diagnosis to milestones by early vs late autonomic dysfunction.

Box represents 25th and 75th percentiles with the line showing the median value. Whiskers represent adjacent values and outliers are represented by dots.

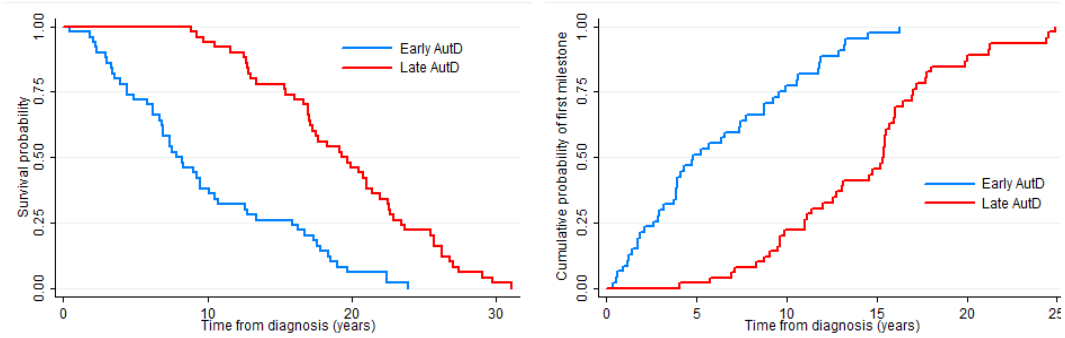


Figure 6.4. Kaplan-Meier curves for survival probability (left) and risk of first milestone (right) by early vs late autonomic dysfunction.

OUTCOME VARIABLE	Time to autonomic dysfunction* HR (95% CI) *P* value
First milestone	0.86 (0.83 to 0.89); *P* < 0.001
Regular falls	0.88 (0.84 to 0.92); *P* < 0.001
Wheelchair	0.93 (0.89 to 0.97); *P* = 0.002
Speech	0.93 (0.84 to 1.03); *P* = 0.18
Severe dysphagia	0.97 (0.88 to 1.07); *P* > 0.57
Cognitive impairment	0.90 (0.85 to 0.95); *P* < 0.001
Care placement	0.91 (0.87 to 0.96); *P* = 0.001
Survival	0.91 (0.88 to 0.95); *P* < 0.001
	Time to autonomic symptoms* HR (95% CI) *P* value
Orthostatic hypotension	First milestone: 0.94 (0.91 to 0.97) *P* < 0.001 Survival: 0.92 (0.89 to 0.95) *P* < 0.001
Upper gastrointestinal symptoms	First milestone: 0.93 (0.90 to 0.96) *P* < 0.001 Survival: 0.93 (0.91 to 0.96) *P* < 0.001
Constipation	First milestone: 0.94 (0.90 to 0.97) *P* = 0.001 Survival: 0.95 (0.9 to 0.98) *P* = 0.005
Urinary symptoms	First milestone: 0.92 (0.89 to 0.95) *P* < 0.001 Survival: 0.94 (0.91 to 0.97) *P* < 0.001
Erectile dysfunction	First milestone: 0.92 (0.89 to 0.96) *P* < 0.001 Survival: 0.86 (0.82 to 0.89) *P* < 0.001
Sweating abnormalities	First milestone: 0.88 (0.84 to 0.92) *P* < 0.001 Survival: 0.89 (0.86 to 0.93) *P* < 0.001

Table 6.4. Risk for disease progression and survival

*Cox proportional hazard models of autonomic dysfunction (top) and individual autonomic symptoms (bottom) for disease progression and survival. The different forms of the main explanatory variables were considered in turn in different Cox regression models (not in the same model).

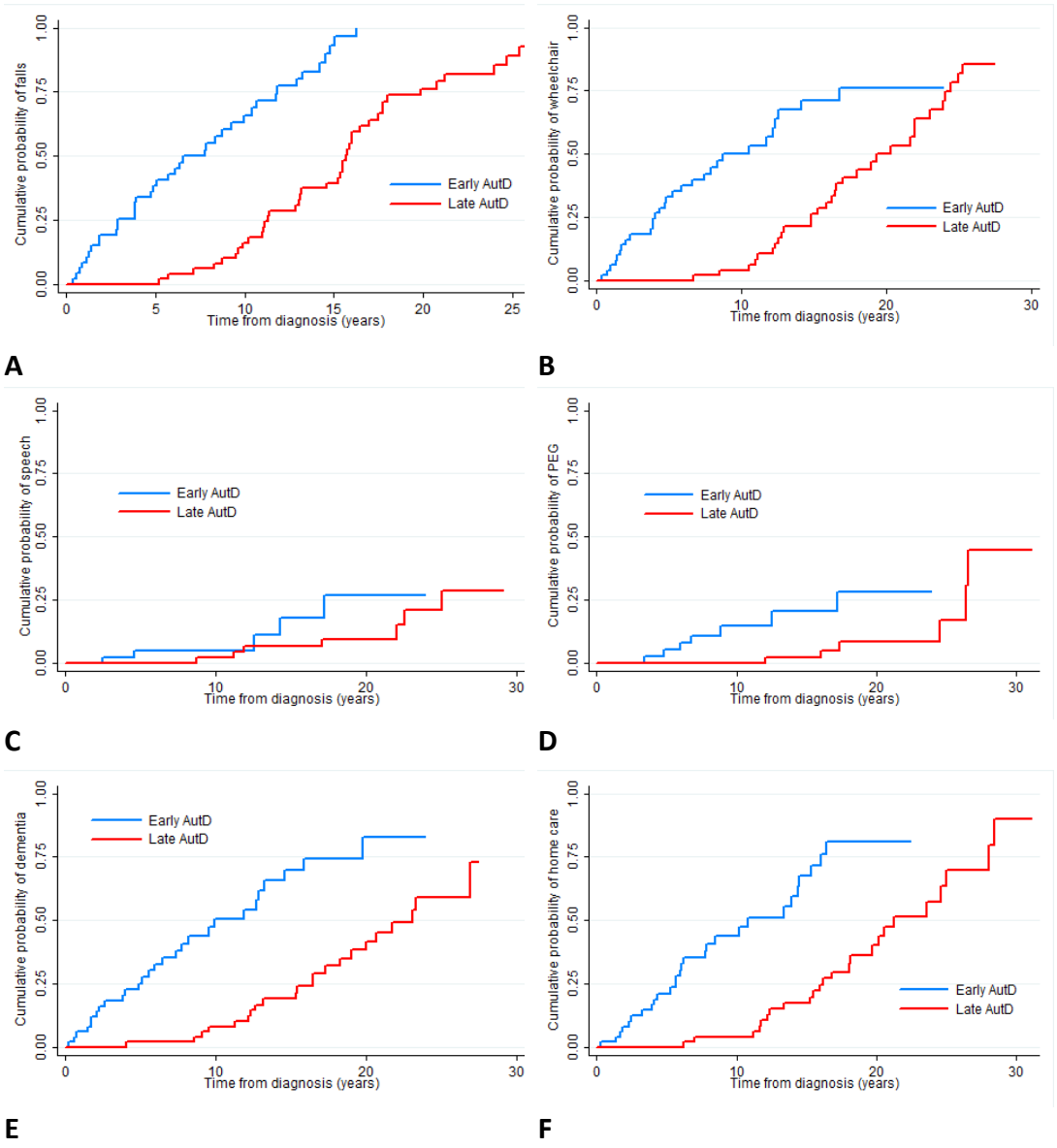


Figure 6.5. Kaplan-Meier curves for disease progression by early vs late autonomic dysfunction.

Risk of outcome: falls (A), wheelchair dependence (B), unintelligible speech (C), severe dysphagia (D), dementia (E) and home care placement (F).

6.3.5 Effect of individual autonomic symptoms on disease progression and survival

The influence of each individual autonomic symptom/sign (orthostatic hypotension, urinary symptoms, constipation, upper gastrointestinal symptoms, sweating abnormalities and erectile dysfunction in males) on survival and disease progression was also estimated.

Earlier development of each individual autonomic symptom/sign was associated with a significant higher risk of reaching a disease milestone and shorter survival (**Table 6.4**).

6.3.6 Other predictors of survival

Univariate models were used to study the influence of other clinical factors and demographic data on survival. In addition to earlier AutD development, factors associated with shorter survival included older age at diagnosis, male gender, PIGD motor subtype and poor levodopa response. Only three of these variables (age at diagnosis, poor levodopa response and earlier development of AutD) retained statistical significance and were maintained as independent predictors in the adjusted multivariate analysis (**Table 6.5**).

Variable	Crude HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
Age at diagnosis (y)	1.14 (1.11 to 1.18)	< 0.001	1.12 (1.08 to 1.16)	< 0.001
Time to AutD (y)	0.88 (0.85 to 0.91)	< 0.001	0.92 (0.88 to 0.96)	< 0.001
Levodopa response good (vs nil-mild)	0.11 (0.03 to 0.37)	< 0.001	0.22 (0.07 to 0.74)	0.014
Levodopa response excellent (vs nil-mild)	0.05 (0.02 to 0.16)	< 0.001	0.19 (0.05 to 0.64)	0.008
Male gender	1.72 (1.14 to 2.61)	0.01	1.62 (0.97 to 2.71)	0.063
PIGD subtype (vs tremor predominant)	5.54 (2.69 to 11.40)	< 0.001	1.91 (0.76 to 4.79)	0.166

Table 6.5. Multivariable analysis of survival predictors.

6.4 Discussion

This is the first study to evaluate systematically the impact of AutD throughout the disease course in a large group of autopsy-confirmed cases with PD. The main finding is that earlier development of AutD or individual autonomic features (including orthostatic hypotension, urinary symptoms, upper gastrointestinal symptoms, constipation, sweating abnormalities and erectile dysfunction in males) are independent predictors of more rapid disease progression, disability and shorter survival in PD patients.

The main strength of the study is the neuropathological confirmation of the diagnosis available for all cases. Due to the clinical heterogeneity of PD and relative lack of diagnostic tests, it is essential to include patients with a high level of diagnostic certainty that can only be achieved with post mortem examination. Autonomic symptoms can predate the diagnosis of PD by several years (228) and in some patients autonomic failure can dominate the clinical picture throughout the course of the disease (229) and lead to misdiagnosis of multiple system atrophy (224, 225) as was the case in 12 patients in our study. Data regarding accuracy of clinical diagnosis in our series of patients is similar to other previous clinico-pathological studies with a recent meta-analysis reporting a pooled diagnostic accuracy of 80.6%, with the highest of 86% being achieved by movement disorders experts, (230) and MSA being the condition most commonly misdiagnosed.(231) Our data highlights the fact that a significant proportion of patients with PD with AutD are misdiagnosed as multiple system atrophy in life meaning that it is likely that many population studies have excluded this subgroup from the analysis. Therefore, caution is needed in the interpretation of clinical studies, particularly those involving patients at early stages of the disease or those where the symptomatology overlaps with other conditions included in the differential diagnosis such as AutD.

Our study provided a systematic evaluation of autonomic symptoms including different autonomic domains and also provided detailed and regular clinical information throughout the whole disease course. Only a few studies have assessed the impact of the severity of autonomic symptoms on PD although their conclusions are limited by short follow up periods or assessment of individual autonomic symptoms in isolation. A retrospective cohort study failed to show any association between severity of AutD on autonomic cardiovascular tests and mortality rates at 7 years. (223) AutD measured using a specific validated questionnaire (SCOPA-AUT) did not show an increased risk of mortality after less than 5 years of follow up (232) although another study using the same questionnaire showed an association with deterioration of quality of life and performance of daily activities.(233) Compared to our results, follow up periods may not have been long enough to detect any potential significant effects although it is unclear whether the time of development of AutD (rather than its severity) seems to be the key determinant on survival. Other studies have assessed the influence of the presence (rather than the severity) of specific symptoms of AutD on PD progression and most of the studies have focused on orthostatic hypotension. A recent prospective cohort study of 176 patients with clinical diagnosis of PD and anosmia followed up throughout their disease course showed that the presence of orthostatic hypotension at the initial assessment was associated with significant shorter survival, although other symptoms and signs of AutD were not evaluated.(37) In addition to orthostatic hypotension, Stubendorff and colleagues (222) also evaluated the presence of constipation and urinary incontinence as markers of AutD in 30 patients with dementia with Lewy bodies and PD with dementia. Orthostatic hypotension was the only symptom associated with shorter survival after 36 months in this group, although the prognosis was worse in those patients with additional constipation or urinary incontinence. In addition to the effects on survival, there is a growing body of evidence that links orthostatic hypotension with development of dementia (234, 235) and even transient cognitive performance during orthostatism.(236) The underlying mechanisms are not fully understood and intense research is attempting to elucidate whether there

is just an association of symptoms or there is a causative link between them. It is noteworthy that the studies showing a negative prognostic value of autonomic symptoms in PD only focused on the presence or absence of the symptoms at initial assessments rather than their severity. In our study, analysis of individual autonomic symptoms also showed similar results to the analysis of global AutD, with a faster progression to first milestone and shorter survival for those developing each autonomic symptom. These findings suggests that time of development of AutD rather than the severity of the symptoms is the factor impacting on disease progression, suggesting a faster and more diffuse neurodegenerative process.

Recent studies have attempted to define new clinical subtypes of PD incorporating non-motor symptoms (including AutD) using different methodologies.(237, 238) These studies have shown that autonomic symptoms are more common in patients without tremor predominant subtypes of PD and also that AutD might associate with other features such as postural instability,(214, 239-241) RBD (25) and cognitive impairment (241, 242) suggesting a more extensive neurodegenerative process in these patients. A few of these studies have assessed the prognosis of the different PD clinical subtypes though in general they include a relatively small number of patients and short follow up periods (see chapter 7 for extended discussion). Those subtypes with prominent autonomic symptoms have shown worse disease progression (25) and shorter survival.(243) As the analysis was carried out in the context of other motor and non-motor symptoms, no conclusion regarding the prognostic value of these autonomic symptoms in isolation can be made. Our study is in agreement with the view that some symptoms might cluster together as earlier AutD was associated with PIGD motor subtypes which had also a higher risk of developing cognitive disability.

Analysis of the neuropathological data in our patients did not show any significant association of AutD with the distribution and severity of α-synuclein pathology in the central nervous system. These findings go against the

hypothesis that a more aggressive neurodegenerative process in these patients may spread faster to different brain areas and be responsible for the association of several non-motor symptoms (AutD, RBD, cognitive impairment) and the worse prognosis. In contrast to MSA, autonomic nervous system degeneration in PD is considered to be caused mainly by involvement of postganglionic neurons in the peripheral autonomic nervous system which may explain the lack of significant neuropathological findings in central structures.(34) However, there is histological evidence of involvement of key autonomic regulatory areas in the central nervous system in PD (some of them not included in the analysis of common PD staging systems) that may play a role in AutD. (34, 244) A more targeted histological assessment of central regulatory autonomic areas may reveal potential histological differences and help to elucidate the role of central involvement in AutD in PD. Whether a consequence of peripheral or central (or both) involvement of autonomic structures, earlier AutD was a significant prognostic factor for disease progression and survival independent of the association with other non-motor features with prognostic implications as shown in the multivariable analysis (**Table 6.5**). Based on this, we propose that AutD should be considered a non-motor marker of disease for the characterization of different PD subtypes.

Although not the main objective of this study, our data also showed that older age at diagnosis, male gender, PD subtype and response to levodopa were predictors of survival in univariable analysis. In multivariable analysis, however, only age at diagnosis and levodopa response remained significant. These features have been reported as survival predictors in PD in previous studies.(215) Our findings also showed that AutD is a common, early and sometimes severe clinical feature in PD but these data are likely to be a conservative estimate and they may not represent the figures in the general PD population.

The retrospective nature of the study and the assessment by different professionals without methodological homogeneity may potentially account for

some limitations in the accuracy of the recording of autonomic symptoms. The study used strict criteria and included a large group of PD patients with detailed and regular clinical information from hospital specialists (neurologists or geriatricians) and the family doctor that allowed confident documentation of the disease process to mitigate this potential bias. Disease milestones were well documented and they have been shown to describe accurately disease progression in other parkinsonian conditions.(53) On the other hand, brain bank cases may be biased towards more severe or atypical clinical cases.

In summary, this study of a large number of pathologically confirmed PD cases shows that an earlier development of autonomic symptoms or AutD is associated with a shortened survival and worse progression of the disease. As the presence of autonomic symptoms in the majority of our patients was assessed in clinical settings, these findings have important implications in clinical practice. Assessment of these symptoms should be part of the routine clinical assessment of PD patients, to identify those individuals who may require more detailed evaluation and follow up. The role of more aggressive treatment of autonomic symptoms or AutD on progression and survival, however, remains unclear. Further studies with pathologically proven PD cases and objective assessment of AutD severity are warranted to corroborate these findings.

Chapter 7. The prognosis and neuropathological correlation of clinical subtypes in Parkinson's disease.

7.1 Introduction

As discussed previously, the clinical presentation of PD, with its wide range of motor and non-motor features, varies considerably from patient to patient. Moreover, the way in which those symptoms respond to different symptomatic therapies and how the disease progresses over time can be remarkably heterogeneous.(23, 245) This variability has prompted many attempts to explore clinically relevant features to classify patients into subgroups with a similar clinical phenotype. Successful subtyping of patients at the time of diagnosis would have important practical implications for clinicians and patients, allowing prediction of the range of clinical manifestations likely to appear and allowing an accurate estimation of the prognosis in order to provide more individualised treatment plans and counselling. The underlying mechanisms behind this clinical variability are poorly understood, although the assumption of different Parkinson's clinical subtypes would have important pathophysiological implications. Some authors argue that the presence of different PD clinical subtypes really constitutes different disease subtypes with differing pathogenic processes and aetiologies while others support the idea of a common pathophysiological mechanism with individual factors influencing rate of progression.(237, 238) Whether multiple or one single pathogenic process, PD subtyping may help to better understand the pathophysiology of neurodegeneration in patients with PD and its implementation in clinical research may be a useful tool to develop specific treatments.

PD subtyping has attracted significant attention and multiple systems have been proposed exploring different ways of classification. The initial PD subtypes were based on the empirical observation of classical motor features based on a priori hypothesis. The most commonly proposed empirical groups are 'tremor-

dominant' and 'PIGD' subtypes which are usually defined using the motor scores of the UPDRS. (246) The prognostic significance of these clinical subtypes has been demonstrated in numerous studies (23, 245) and they are easy to apply in clinical practice, although they have been criticised for their lack of consistency over the disease course.

Empirical observations have been gradually replaced by data-driven approaches where the association between clinical variables is not influenced by a priori hypotheses. These cluster analyses are methodologically more robust as they do not imply any assumptions although results are highly dependent on the clinical variables and number of clusters selected which explains the considerable discrepancy between different studies. Most initial cluster analyses focused on demographics and motor symptoms, and 'old age-at-onset and rapid disease progression' and a 'young age-at-onset and slow disease progression' are the subtypes most commonly proposed.(247) As our understanding of PD advanced, some of the NMS of the disease with prognostic implications have been incorporated into PD subtype studies and cognitive impairment, RBD or depression have been shown to be key determinants in the definition of new PD subtypes in most recent cluster studies. (24-26, 241, 248) In addition to the inherent bias of variable and cluster selection, these new clinical subtypes have other limitations. Classification of PD patients purely based on data association may not reflect the underlying pathophysiological processes driving such clinical heterogeneity. Some of these studies analysed a wide range of motor and non-motor features some of which are not routinely measured in clinical practice, and some subtype classifications are based on complex scoring systems using lengthy questionnaires, therefore limiting its applicability in clinical settings.

Despite these important research efforts, to date, no classification system has proved superior over others and there is no consensus on a specific subtyping classification to use. Most cluster studies have no longitudinal follow up and in the only two studies with prospective assessments, this is limited to < 3

years,(24, 249) therefore further evidence is needed to assess the long-term validity of the proposed clinical subtypes on disease progression. PD subtypes need to be validated in different populations and, ideally, correlated with neuropathological findings or other biomarkers to support their neurobiological basis. However, most of the new cluster studies lack validation in other cohorts and, with few exceptions,(24, 26) supporting data on biomarkers. One of these studies used a comprehensive list of data on motor and non-motor clinical manifestations at time of diagnosis, with additional neuroimaging and cerebrospinal fluid biomarker data from the Parkinson's disease Progression Markers Initiative study (PPMI).(250) Four clinical features (motor severity, cognitive impairment, dysautonomia and RBD) were identified as the key determinants for subtyping based on cluster analysis and three distinct subgroups proposed: 'mild motor-predominant', 'diffuse malignant' and 'intermediate'. (24) The prognostic value of this subtype classification (or any other subtype classification system) has not been proven in the long-term (as patients were followed up for < 3 years), and has not been validated in other populations apart from the PPMI cohort.

Previous work performed at the QSBB correlated a subtype classification based on motor features and age at onset (251) with neuropathological findings. (252) By using clinical records incorporating non-motor features and the detailed pathological data from the QSBB on a separate large group of pathology-confirmed PD cases, longitudinal life-time data and neuropathological correlation among the newly proposed clinical subtypes are presented. Moreover, possible pathophysiological mechanisms responsible for the clinical and neuropathological heterogeneity are proposed.

7.2 Methods

7.2.1 Study design

This is a retrospective cohort study including consecutive patients with a neuropathologically-confirmed diagnosis of PD (defined as moderate to severe neuronal loss in the SNpc associated with Lewy pathology). Cases were identified from the archives of the QSBB in London, United Kingdom, between January 2009 and December 2017. Cases were excluded from the study if: (I) a diagnosis of dementia before or within 1 year of the onset of motor symptoms was made (11) (II) neuropathological changes consistent with diagnosis of an additional neurodegenerative condition were present on post-mortem examination, (III) had a monogenic form of PD,(5) or (IV) detailed and regular clinical records documenting the entire disease course were not available. The brain donor program was approved by a London Multi-Centre Research Ethics Committee, tissue was stored for research under a license from the Human Tissue Authority, and written informed consent was obtained from all donors.

7.2.2 Clinical assessment

Clinical records were systematically reviewed. All patients were assessed by hospital specialists (neurologists or geriatricians) in the United Kingdom regularly throughout their disease course. Only cases with detailed and regular documentation of clinical assessments were included in the study. Data on demographics, clinical features and disease progression were collected. The presence and severity of the cardinal motor signs of PD at the time of diagnosis, including tremor, bradykinesia, rigidity and postural instability, were recorded. The following non-motor features and their severity at the time of diagnosis were also documented: (I) autonomic dysfunction defined by the presence of any two of the following symptoms or signs persistent for longer than 6 months

(urinary symptoms, constipation, symptoms of upper gastrointestinal dysfunction, orthostatic hypotension, sweating abnormalities or erectile dysfunction in males) as previously defined, (II) clinically diagnosed RBD defined as presence of repeated episodes of sleep related vocalisation and/or complex motor behaviour, (III) cognitive dysfunction (graded according to the degree of impairment of tasks of daily living from mild cognitive impairment to severe dementia) and (IV) symptoms of major depressive disorder. Grading of symptom severity was performed blinded to disease course and neuropathological data, based on the clinical relevance of the symptoms on the premise that this would be well documented in the clinical notes. Using a 4-point semiquantitative scale based on the clinical impression by the treating physician, symptoms and signs were graded as follows:

- 0 – absent
- 1 – mild symptom severity / mild distress to patient / no therapeutic intervention required
- 2 – moderate symptom severity / moderate distress to patient / good symptomatic control with therapeutic intervention
- 3 – severe intensity / severe distress to patient / no symptomatic control despite therapeutic intervention

To assess disease progression, the occurrence and time of onset from diagnosis to specific disease milestones were recorded: (I) presence of regular falls or (II) dependence on wheelchair for mobility as milestones of motor disability, (III) dementia, defined as cognitive impairment severe enough to significantly affect tasks of daily living and (IV) placement in residential or nursing home care as a measure of global disability. Time from diagnosis to death (survival) was also recorded.

7.2.3 Subtype definitions

Definitions proposed by Fereshtehnejad *et al* were used to assign individuals in our study to a specific PD subtype.(24) As their classification system is based on a 4-item composite of multiple clinical questionnaires and scales which may not be available in clinical practice, the suggested algorithm was adapted to our retrospective, non-standardised clinical data in order that our subtypes correspond as closely as possible to the original definitions. Our semiquantitative severity scores of motor and non-motor features were converted into four domains that included one composite for motor (a composite of the individual scores for tremor, bradykinesia, rigidity and postural instability) and one composite for autonomic dysfunction (a composite of the individual scores for urinary symptoms, constipation, symptoms of upper gastrointestinal dysfunction, orthostatic hypotension, sweating abnormalities and erectile dysfunction in males), and two individual scores for RBD and cognitive dysfunction. The corresponding 75th percentile was calculated for each domain and patients were classified at the time of diagnosis into three subtypes:

- Mild motor-predominant. Motor and all non-motor scores < 75th percentile.
- Diffuse malignant. Either motor score > 75th percentile and at least one non-motor score > 75th percentile, or all three non-motor scores > 75th percentile.
- Intermediate. All those individuals not meeting criteria for other subtypes.

As a consequence of adapting the original subtyping system using quantitative assessment tools to a semiquantitative scale with limited categories, groups of patients could have the same score corresponding to the 75th percentile. All those cases with the same score were allocated to the same group (either >75th or <75th percentile group) based on where the majority of them distributed. For example, the 75th percentile for RBD score was 1=mild, and 2 patients with this

score fell into the <75th percentile whilst 22 fell into >75th percentile, therefore all patients with RBD score 1=mild were allocated to >75th percentile.

7.2.4 Neuropathological assessment

Formalin-fixed brain tissue samples were examined and stained using standard protocols and complemented with immunohistochemistry against α-synuclein, Aβ peptide, hyperphosphorilated tau protein, p62 and TAR DNA binding-protein 43 (TDP43). Distribution and severity of Lewy body pathology was assessed following consensus guidelines: representative sections of brainstem, basal forebrain and neocortical areas were graded using semiquantitative scoring and each individual was assigned to a Lewy body subtype (brainstem, limbic or diffuse neocortical) based on Lewy body scores, (11) and a Braak stage (1 - 6) according to the disease staging system proposed by Braak.(7) Additional pathologies were evaluated using current consensus protocols. Severity of neurofibrillary tangle pathology was assessed using specific immunohistochemistry against hyperphosphorylated tau protein and the staging system described by Braak and Braak.(253) Aβ immunostaining was used for the assessment of amyloid β deposition and neuritic plaques and their severity and extent was graded according to the classifications proposed by Thal (254) and the Consortium to establish a registry for Alzheimer's disease (CERAD) protocol.(255) Global Alzheimer's disease neuropathological changes were assessed using the "ABC" scoring system proposed by the National Institute on Aging (NIA) – Alzheimer's Association guidelines, which assign a level of Alzheimer's disease neuropathological changes (Absent, low, intermediate, high) based on the combination of neurofibrillary tangle, amyloid β deposition and neuritic plaque severity.(256) Neuropathological changes were compared between Parkinson's subgroups to assess for potential differences of the neurodegenerative process that could explain the different disease course among PD subtypes.

7.2.5 Statistical analysis

Demographic and clinical features are reported as number (percentage) for categorical variables and median ± standard deviation for continuous variables. Comparisons between groups were performed using Fisher's exact test for categorical variables, and ANOVA for continuous variables as appropriate with Bonferroni correction for multiple pairwise comparisons. Considering the ordinal nature of neuropathology staging systems, global comparisons of histological findings among PD subtypes were performed using the Kruskal-Wallis test.

Multivariable Cox proportional hazard regression models were used to estimate the risk of developing each disease milestone and the risk of death (survival) for each Parkinson's subtype. Other demographic and clinical features previously reported to have prognostic value including age at diagnosis, gender, levodopa response and depression severity were included in the model as explanatory variables. Only those with a relevant association in the univariate analysis that retained statistical significance after adjustment in the multivariate analysis were included. Adjusted HRs and 95% CIs were estimated. Kaplan-Meier curves of disease milestones and survival were plotted for each PD subtype. Visual inspection of Kaplan-Meier curves and plots of scaled Schoenfeld residuals against time were used to assess the proportional hazards assumption. Censoring was considered uninformative. Two-tailed tests were performed and statistical significance was set at $P < 0.05$ and STATA statistical software, version 12 (StataCorp) was used for statistical analysis.

7.3 Results

From 146 potential cases with neuropathological diagnosis of PD, a total of 111 patients (60.4% male) with age at diagnosis of 62.5 ± 11.5 years were included in the final analysis. **Figure 7.1** shows the flow chart of the study.

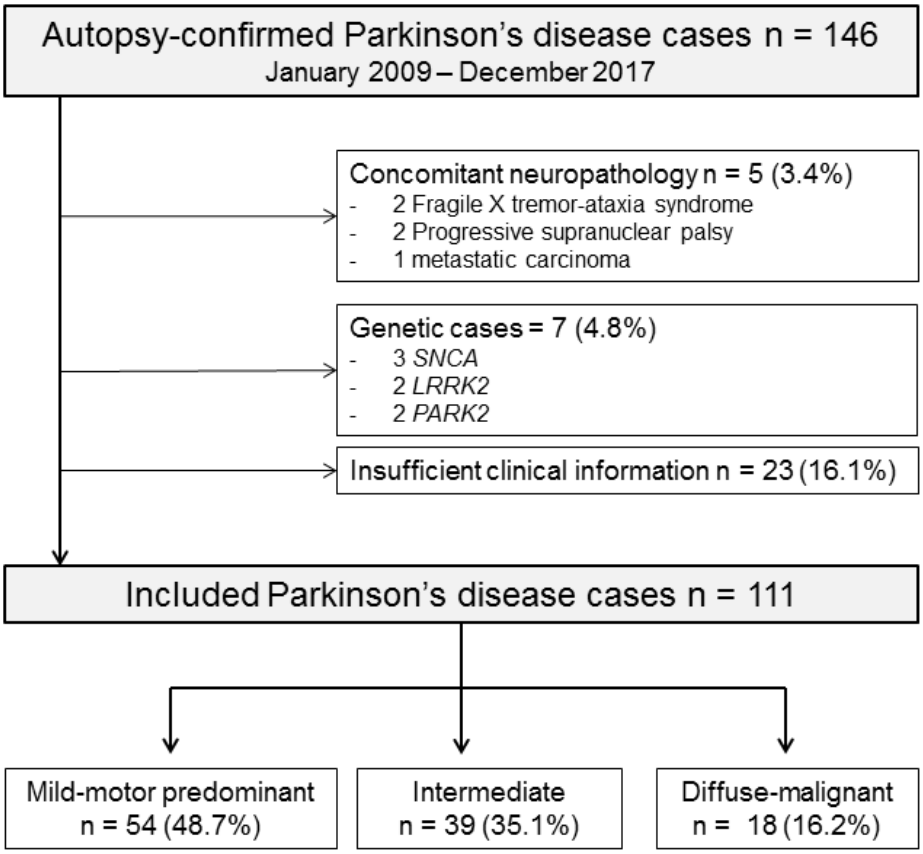


Figure 7.1. Flow chart.

The demographic data and clinical features of the whole group and by Parkinson's subtypes are shown in **Table 7.1**. The QSBB cohort did not differ in age at diagnosis (62.5 ± 11.5 vs 61.1 ± 9.7 years; t = -1.299; P = 0.20), sex (male sex 60.4% vs 65.6%; chi square; P = 0.31) or PD subtype distribution (mild motor-predominant / intermediate / diffuse malignant: 48.7%, 35.1% and 16.2% vs 52.5%, 38.5% and 9.0% respectively; chi square; P = 0.09) compared with the reference PPMI group. Mild motor-predominant subtype patients were significantly younger at diagnosis, had a better response to levodopa and received a higher levodopa equivalent dose. Patients of the diffuse-malignant subtype were older, almost all male, had a poorer response to levodopa and more frequently misdiagnosed with an atypical parkinsonian syndrome in life (see **Table 7.1** for all comparisons).

	Total (n= 111; 100%)	Mild motor-predominant (n= 54; 48.7%)	Intermediate (n= 39; 35.1%)	Diffuse malignant (n= 18; 16.2%)	P value – Significant pairwise comparisons
Age at diagnosis	62.5±11.5	58.2±11.9	65.0±10.4	70.3±6.4	<0.001 MMP vs IM MMP vs DM
Male sex	67 (60.4)	30 (55.6)	20 (51.3)	17 (94.4)	0.003
Clinical diagnosis					<0.001
PD	95 (85.6)	53 (98.2)	32 (82.1)	10 (55.6)	
MSA	11 (9.9)	1 (1.9)	5 (12.8)	5 (27.8)	
PSP	5 (4.5)	0 (0.0)	2 (5.1)	3 (16.7)	
Age at death	78.3±6.4	78.4±6.6	78.2±6.6	78.4±5.6	0.99
Ldopa response					<0.001
Absent	5 (4.4)	2 (3.8)	1 (2.6)	3 (17.7)	
Mild	7 (6.1)	0 (0.0)	1 (2.6)	5 (29.4)	
Moderate	27 (23.7)	7 (13.2)	11 (29.0)	6 (35.3)	
Good	75 (65.8)	44 (83.0)	25 (65.8)	3 (17.7)	
Maximum LED (mg)	899.5±432.3	1016.1±436.9	834.7±394.2	674.1±399.7	0.008 MMP vs DM
Cause of death					1.0
PD-related	94 (84.7)	46 (85.2)	33 (84.6)	15 (83.3)	
PD-unrelated	17 (15.3)	8 (14.8)	6 (15.4)	3 (16.7)	

Table 7.1. Demographic and clinical data by PD subtype.

* Chi square/Fisher exact test for categorical variables and ANOVA for continuous variables as appropriate with Bonferroni correction for pairwise comparisons. Data shown in number (%) or mean ± SD.

DM, diffuse malignant; IM, intermediate; LED, Levodopa equivalent dose; MMP, mild motor-predominant; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

7.3.1 Disease progression and survival in Parkinson's subtypes

PD subtypes showed different rates of deterioration with the diffuse malignant subtype reaching all prognostic milestones earlier in the disease course and having the shortest survival (ANOVA; all P values < 0.001; see **Table 7.2** for all comparisons and **Figure 7.2**). Non-PD related causes of death were similar between groups. Despite differences in disease progression and survival, PD subtypes did not differ in disability at the time of death as judged by the presence of disease milestones and all subtypes reached advanced stages with 94.6% of patients reaching at least one disease milestone (**Table 7.2**).

The risk to develop each milestone was estimated for all subtypes and results were adjusted by other potential relevant variables including age at diagnosis, gender, levodopa response and depression severity although only PD subtype and age at diagnosis remained significant in the multivariate model (**Table 7.3**). The diffuse-malignant group showed an increased risk of development of all disease milestones compared to mild motor-predominant. The probability of survival in the diffuse-malignant group was also reduced with an adjusted HR of 3.65 (95% CI 1.98-6.75; P <0.001) (**Table 7.3** and **Figure 7.3**).

MILESTONES n (%) Time from diagnosis (years)	**Total (n= 111; 100%)**	**Mild motor-predominant (n= 54; 48.7%)**	**Intermediate (n= 39; 35.1%)**	**Diffuse malignant (n= 18; 16.2%)**	**P value – Significant pairwise comparisons**
First milestone	105 (94.6) 9.4±11.6	49 (90.7) 14.3±5.7	38 (97.44) 8.2±5.3	18 (100.0) 3.5±3.2	0.311 <0.001 MMP vs IM MMP vs DM IM vs DM
Falls	90 (81.1) 11.3±7.1	46 (85.2) 15.5±6.0	27 (69.2) 8.6±5.6	17 (94.44) 4.5±3.4	0.056 <0.001 MMP vs IM MMP vs DM IM vs DM
W**heelchair**	62 (55.9) 10.6±15.2	29 (53.7) 16.9±6.7	17 (43.6) 11.8±6.8	16 (88.9) 4.9±3.7	0.004 <0.001 MMP vs IM MMP vs DM IM vs DM
Dementia	64 (57.6) 12.1±7.7	28 (51.9) 17.1±6.3	23 (59.0) 10.6±6.4	13 (72.2) 4.1±3.7	0.307 <0.001 MMP vs IM MMP vs DM IM vs DM
Home care	52 (46.9) 13.3±7.9	21 (38.9) 18.0±7.3	21 (53.9) 12.2±6.8	10 (55.6) 6.0±4.5	0.272 <0.001 MMP vs IM MMP vs DM
Survival from diagnosis (years)	15.8±8.4	20.2±7.8	13.2±6.7	8.1±5.4	<0.001 MMP vs IM MMP vs DM IM vs DM

Table 7.2. Disease progression and survival data by PD subtype.

Data showing frequency (%) and time from diagnosis (mean ± SD) to disease milestones and death by PD subtypes.

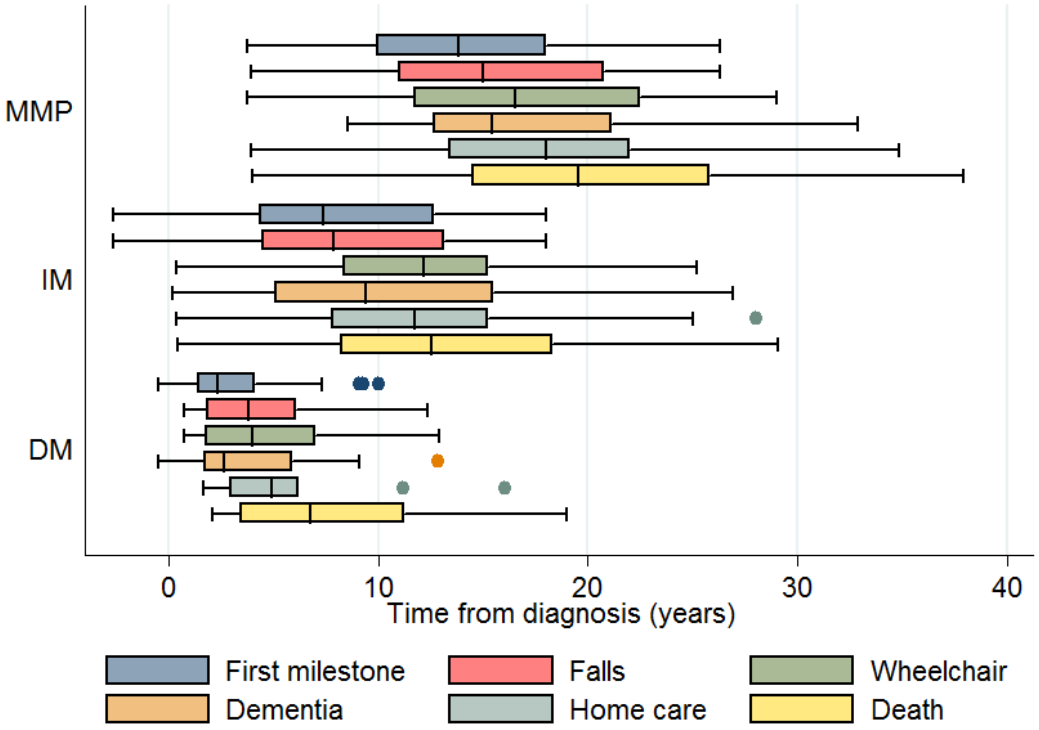


Figure 7.2. Box-and-whisker plots showing time from diagnosis to milestones and death by PD subtypes.

Box represents 25th and 75th percentiles with the line showing the median value. Whiskers represent adjacent values and outliers are represented by dots.

DM, diffuse malignant; IM, intermediate; MMP, mild motor-predominant.

Outcome variable	Crude HR (95% CI)	P value	Adjusted HR (95% CI)	P value
First milestone:				
Intermediate	3.00 (1.86-4.85)	<0.001	2.85 (1.75-4.63)	<0.001
Diffuse malignant	14.02 (7.20-27.29)	<0.001	10.90 (5.51-21.58)	<0.001
Others: severe depression	1.85 (1.07-3.19)	0.03	0.85 (0.48-1.49)	0.57
Others: age at diagnosis	1.09 (1.06-1.12)	<0.001	1.09 (1.06-1.12)	<0.001
Falls:				
Intermediate	2.95 (1.70-5.12)	<0.001	2.53 (1.45-4.43)	0.001
Diffuse malignant	11.96 (6.02-23.78)	<0.001	7.79 (3.84-15.79)	<0.001
Others: severe depression	2.37 (1.23-4.57)	0.01	1.14 (0.56-2.32)	0.72
Others: age at diagnosis	1.11 (1.08-1.14)	<0.001	1.10 (1.07-1.14)	<0.001
Wheelchair:				
Intermediate	5.79 (2.77-12.10)	<0.001	1.19 (0.63-2.24)	0.60
Diffuse malignant	15.17 (5.82-39.51)	<0.001	3.78 (1.74-8.22)	0.001
Others: age at diagnosis	1.10 (1.07-1.14)	<0.001	1.09 (1.06-1.13)	<0.001
Dementia:				
Intermediate	2.42 (1.35-4.32)	0.003	2.24 (1.24-4.02)	0.007
Diffuse malignant	11.0 (4.94-24.47)	<0.001	6.65 (2.91-15.20)	<0.001
Others: severe depression	2.84 (1.51-5.34)	<0.001	1.20 (0.61-2.38)	0.60
Others: age at diagnosis	1.12 (1.08-1.15)	<0.001	1.12 (1.08-1.16)	<0.001
Home care:				
Intermediate	2.04 (1.08-3.83)	0.03	1.83 (0.94-3.55)	0.07
Diffuse malignant	6.66 (2.88-15.41)	<0.001	3.33 (1.35-8.20)	0.009
Others: severe depression	2.46 (1.14-5.29)	0.022	0.55 (0.20-1.51)	0.25
Others: age at diagnosis	1.15 (1.10-1.20)	<0.001	1.14 (1.09-1.18)	<0.001
Survival:				
Intermediate	2.59 (1.66-4.03)	<0.001	2.14 (1.36-3.35)	0.001
Diffuse malignant	6.74 (3.70-12.26)	<0.001	3.65 (1.98-6.75)	<0.001
Others: severe depression	2.49 (1.51-4.10)	<0.001	0.91 (0.54-1.55)	0.74
Others: age at diagnosis	1.15 (1.12-1.18)	<0.001	1.14 (1.11-1.17)	<0.001

Table 7.3. Cox proportional hazard regression models of PD subtypes and other significant variables for milestones and survival.

*Severe depression was defined as depression score > 75th percentile based on severity of depression at time of diagnosis (0 = absent, 1 = mild, 2 = moderate, 3 = severe)**.**

Age at diagnosis, gender, levodopa response and depression severity were included in the model as explanatory variables but only those which retained statistical significance after adjustment in the multivariate analysis were included and shown in this table.

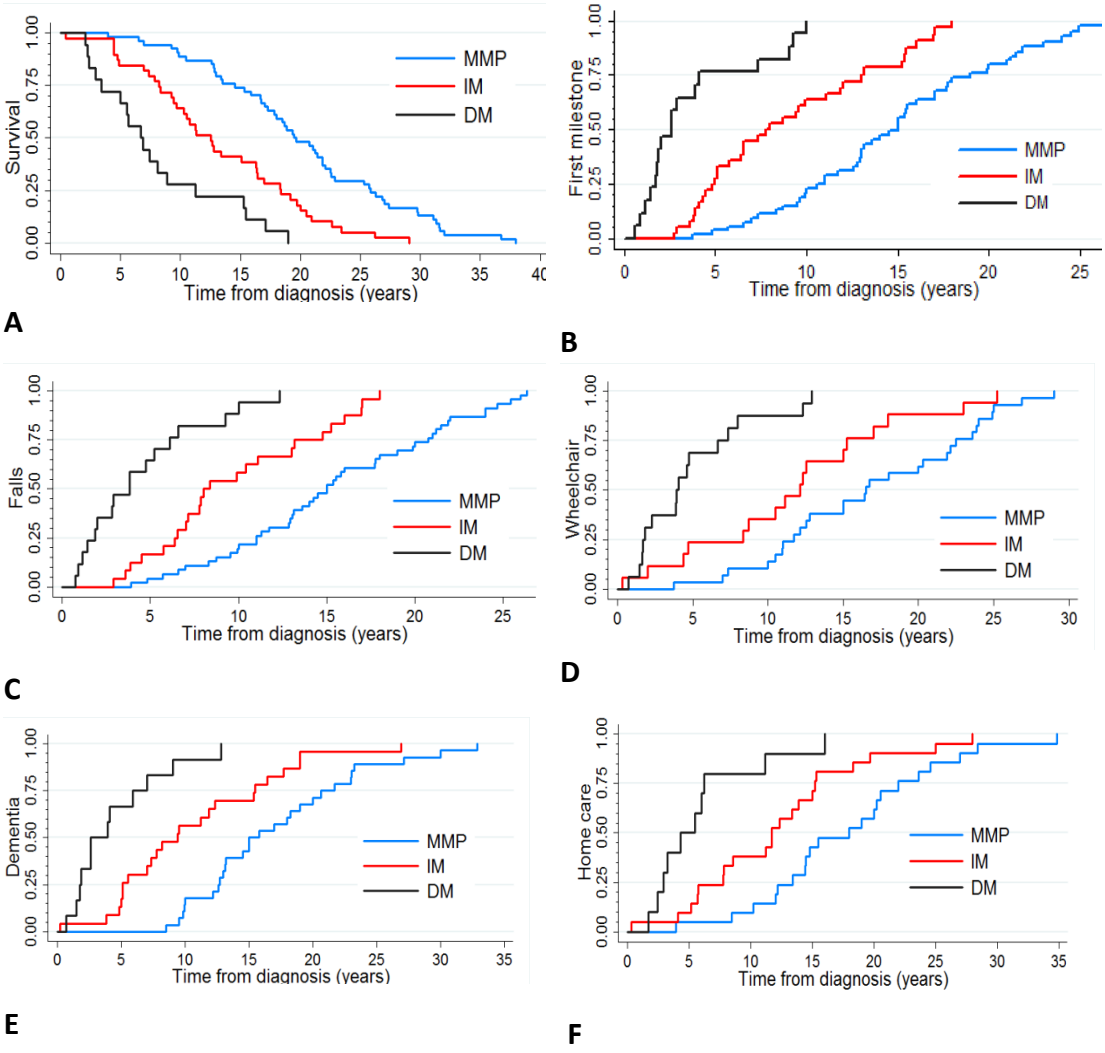


Figure 7.3. Kaplan-Meier curves of cumulative probability of survival and risk of disease milestones.

Kaplan-Meier curves show cumulative probability of survival (A) and cumulative risk of first disease milestone (B), regular falls (C), wheelchair use (D), dementia (E) and care placement (F), among Parkinson's disease subtypes.

DM, diffuse malignant; IM, intermediate; MMP, mild motor-predominant.

7.3.2 Neuropathology in Parkinson's subtypes

Lewy pathology did not show significant differences across PD subtypes measured by Braak and Lewy pathology staging (Kruskal-Wallis test; see **Table 7.4** for specific values). The severity of co-morbid Alzheimer's disease related neuropathological changes was also similar among different PD subtypes measured using several accepted staging systems (**Table 7.4**).

In order to elucidate potential pathogenic mechanisms we analysed the association between neuropathological findings and other demographic characteristics. Neurofibrillary tangle pathology (ANOVA; $F(6,104) = 2.84$; $P = 0.01$), CERAD neuritic plaques (ANOVA; $F(3,107) = 3.56$; $P = 0.02$) and Alzheimer's disease-related pathology (ANOVA; $F(3,107) = 4.12$; $P = 0.008$) showed significant association with increased age at death. Association of neocortical Lewy pathology and disease duration did not reach statistical significance (ANOVA; $F(1, 109) = 3.64$; $P = 0.059$).

Neuropathology	Total	Mild motor-predominant	Intermediate	Diffuse malignant	P value
Braak					
Stage 4	2 (1.8)	0 (0.0)	1 (2.6)	1 (5.6)	
Stage 5	9 (8.1)	4 (7.4)	5 (12.8)	0 (0.0)	0.37
Stage 6	100 (90.1)	50 (92.6)	33 (84.6)	17 (94.4)	
Lewy pathology					
Brainstem	1 (0.9)	0 (0.0)	1 (2.6)	0 (0.0)	
Limbic	16 (14.4)	6 (11.1)	6 (15.4)	4 (22.2)	0.44
Neocortical	94 (84.7)	48 (88.9)	32 (82.1)	14 (77.8)	
Aβ deposition (Thal)					
Phase 0	25 (22.5)	12 (22.2)	8 (20.5)	5 (27.8)	
Phase 1	21 (18.9)	9 (16.7)	7 (18.0)	5 (27.8)	
Phase 2	9 (8.1)	8 (14.8)	0 (0.0)	1 (5.6)	0.35
Phase 3	35 (31.5)	17 (31.5)	14 (35.9)	4 (22.2)	
Phase 4	13 (11.7)	7 (13.0)	5 (12.8)	1 (5.6)	
Phase 5	8 (7.2)	1 (1.9)	5 (12.8)	2 (11.1)	
Neurofibrillary tangle (Braak and Braak)					
Stage 0	6 (5.4)	1 (1.9)	5 (12.8)	0 (0.0)	
Stage I	27 (24.3)	15 (27.8)	8 (20.5)	4 (22.2)	
Stage II	50 (45.1)	25 (46.3)	16 (41.0)	9 (50.0)	
Stage III	19 (17.1)	9 (16.7)	7 (18.0)	3 (16.7)	0.66
Stage IV	6 (5.4)	4 (7.4)	2 (5.1)	0 (0.0)	
Stage V	2 (1.8)	0 (0.0)	0 (0.0)	2 (11.1)	
Stage VI	1 (0.9)	0 (0.0)	1 (2.6)	0 (0.0)	
Neuritic plaque (CERAD)					
Absent	44 (39.6)	22 (40.7)	12 (30.8)	10 (55.6)	
Sparse	39 (35.1)	20 (37.0)	15 (38.5)	4 (22.2)	0.27
Moderate	25 (22.5)	12 (22.2)	10 (25.6)	3 (16.7)	
Frequent	3 (2.7)	0 (0.0)	2 (5.1)	1 (5.6)	
Alzheimer's disease-pathology (NIA)					
Not	28 (25.2)	13 (24.1)	9 (23.1)	6 (33.3)	
Low	63 (56.8)	31 (57.4)	22 (56.4)	10 (55.6)	0.60
Intermediate	18 (16.2)	10 (18.5)	7 (18.0)	1 (5.6)	
High	2 (1.8)	0 (0.0)	1 (2.6)	1 (5.6)	

Table 7.4. Neuropathological findings by PD subtypes.

*P value from comparisons using Kruskal Wallis test.

CERAD, Consortium to Establish a Registry for Alzheimer's Disease score; DM, diffuse malignant; IM, intermediate; MMP, mild motor-predominant; NIA, National Institute on Aging score.

7.4 Discussion

This retrospective study of a large cohort of pathology-confirmed PD patients provides for the first time long-term prognosis and life-course data on new PD subtypes incorporating prognostic NMS and a correlation with neuropathological findings at post-mortem. Our results showed that the classification of patients into subgroups based on clinical data at the time of diagnosis, accurately predicts disease progression and survival. Different rates of progression of Lewy pathology and additional age-related co-morbid neuropathologies (Alzheimer's disease neuropathology) are important determinants of clinical subtypes and are likely to contribute to the clinical heterogeneity.

7.4.1 Parkinson's subtype classification.

Data driven subtype classification studies have the advantage over empirical subtyping in that they exclude a priori assumptions. Multiple subtyping systems have been proposed although there is no consensus on a specific subtype classification over others. To determine the subtype solution most appropriate for our analysis, we reviewed the different cluster subtyping systems recently described in the literature.(237, 238) One of the limitations of this approach is that the results of the clusters sometimes may be difficult to implement in clinical practice. Our study used a classification system based on a previous cluster analysis with an algorithm to translate groups to an individual level. The algorithm suggested by Fereshtehnejad and colleagues included a complex battery of questionnaires and scales that are not regularly measured in clinical practice.(24) We adapted the classification system to a simple composite based on a semiquantitative grading assessment of the clinically relevant variables using non-standardised collected data. Our results demonstrate that cluster subtyping can be easily applicable in clinical practice to individuals even in a

retrospective cohort and reproduced the results in a group of PD patients different from the original PPMI group.

Cluster analysis heavily relies on the number of variables included and recent research has shown that some NMS have important prognostic value and should be considered in subtyping classifications. (25) PPMI provides data on a comprehensive range of motor and non-motor features and two studies using these data have been recently published.(24, 26) Although both studies showed similar subgroups we favoured the scheme proposed by Fereshtehnejad and colleagues because of its applicability at individual level and imaging and biomarker data support. Although no consensus exist and selected variables differ between studies, clinical features used for subtype definition should be clinically relevant, easy to measure in clinical practice, distribute distinctly in subgroups clustering with other relevant variables and carry prognostic implications. In this regard, age at diagnosis has been consistently reported as a key determinant in Parkinson's subtypes (247) but not in the classification system suggested by Fereshtehnejad. Results in our group of patients showed that age at diagnosis differed significantly among Parkinson's subtypes and was the only other additional factor independently associated with faster disease progression and reduced survival in the multivariate regression model in our study. The important role of age as a clinically defining feature is further supported by our neuropathological findings suggesting an association with co-morbid pathologies (see discussion below). Based on this, we propose that age at diagnosis should be included in Parkinson's subtype definition.

7.4.2 Disease progression and prognosis.

Given the clinical heterogeneity of PD, one of the main conditions of a Parkinson's subtype system is its capacity to estimate prognosis. Our results

showed that classification of patients at time of diagnosis based on severity of motor and non-motor features (autonomic dysfunction, RBD and cognitive function) accurately predicts disease progression and survival. Very few studies have assessed the validity of Parkinson's subtypes longitudinally (25, 243) and any firm conclusions are limited by short follow-up periods that make the measure of disease progression in a clinically meaningful way challenging. Our study is the first to provide life-course data on disease progression of new clinical PD subtypes showing that classification of patients at time of diagnosis predicts the development of clinically relevant motor, cognitive and global disability disease milestones. This PD subtype classification also possesses value on estimation of the risk of mortality, as intermediate and diffuse-malignant groups showed reduced survival. As we have demonstrated the feasibility of this classification system using routine clinical data, PD clinical subtyping may be a valid prognostic tool in clinical practice and this knowledge should also be translated in clinical research when designing clinical trials.

7.4.3 Neuropathological correlation and pathophysiological implications of clinical subtypes

Our study also correlated the distribution and severity of neuropathological findings including Lewy pathology, amyloid β deposition, neurofibrillary tangle pathology, neuritic plaques and Alzheimer's disease-related changes, with the clinical subtypes. Only one previous study performed at QSBB using a different group of patients correlated post-mortem findings with PD subtypes although direct comparisons with the current study cannot be drawn as the subtype classification did not include non-motor features.(252) In this previous study, clinical subtypes did not show a clear correlation with neuropathological stages but patients with non-tremor subtype had more severe neocortical Lewy pathology.(252) Although our results showed a different disease rate of clinical progression among PD subtypes, most patients reached advance stages of the

disease without differences between groups in disability at the time of death including presence of dementia, motor disability or care placement.(Figure 7.4) Not surprisingly given the similar level of disability, PD subtypes in our study did not show any differences in the severity or distribution of Lewy pathology as measured by widely accepted neuropathological staging systems. Post-mortem examination in our patients may therefore demonstrate the pathological end-point of the disease and may not be representative of the underlying dynamic neurodegenerative process. Moreover, previous clinicopathological evidence suggests a non-linear progression in the late stages of the disease with patients reaching a common pathological endpoint.(257, 258) Because most of our patients were at this pathological endpoint, if there are topographical differences in Lewy pathology responsible for the clinical heterogeneity during the disease course, they are no longer detectable at post-mortem and we could not demonstrate them among the new Parkinson's subtypes. However, considering the different clinical presentation and rates of clinical progression, one may hypothesise that similar differences may be found in the severity and topographical progression of neuropathology in earlier stages of the disease and subsequently disappear as the disease and the neurodegenerative process progress to advanced stages. Even if one assumes that the anatomical progression of the neuropathological process was the same for different Parkinson's subtypes, our results show that the rate of pathology progression is different between them, as different Parkinson's subtypes reached similar advanced neuropathological stages despite considerable different disease duration. This same conclusion was reached by Kempster and colleagues in previous clinicopathological studies correlating clinical progression with neuropathological findings in PD patients divided in groups by age at death (257) and patterns of levodopa response.(258)

Taken together, both studies showed that despite a heterogeneous disease course during early-middle stages, the advanced stages of the disease are clinically very similar, with accumulation of disability in a similar time course followed by death without any differences on neuropathological findings at post-mortem.(**Figure 7.4**) A non-linear progression may govern the terminal stages of the neurodegenerative process and age and other co-morbid pathologies may play a synergistic effect.(257, 258)

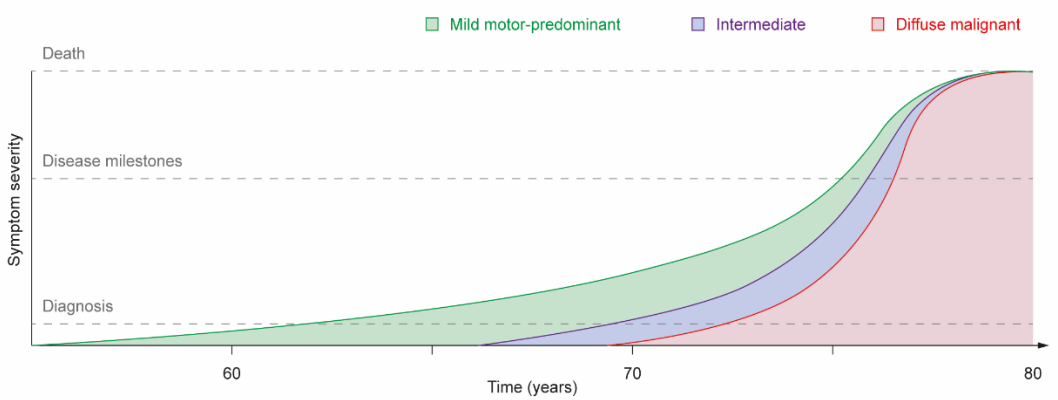


Figure 7.4. Schematic representation of the clinical course illustrating symptom severity over time for PD subtypes.

In the early-middle stages, PD has a very heterogeneous course with different rates of progression and levels of disability among Parkinson's subtypes. In the final stages of the diseases, there is an exponential clinical (and neuropathological) progression with all PD patients reaching a similar end-point characterised by severe disability followed by death at the same age. At this terminal stage of the disease, postmortem examination shows extensive and severe neuropathological changes with no significant differences among Parkinson's subtypes.

This interaction between different pathologies is particularly evident in the case of dementia in PD patients, where numerous neuropathological studies have demonstrated a synergistic effect between Lewy pathology, neurofibrillary tau pathology and Aβ deposition and their clinical impact in the form of cognitive impairment may be influenced by age.(259-262) Analysis of co-morbid pathology in our cases showed no significant differences in Alzheimer's disease related neuropathology among PD subtypes which suggests a more rapid deposition in the group with shorter disease duration (diffuse malignant) although age seems to be an additional important determinant as discussed below.

We also attempted to elucidate any underlying pathophysiological differences in the Parkinson's subgroups by further assessing any potential associations in the reverse direction, from neuropathological findings to other clinical features including the effect of age and disease duration. In our patients, Lewy body pathology seems a disease-related process (as neocortical Lewy stages were associated with disease duration) whilst Alzheimer's disease-related pathology showed a significant association with age at death, more consistent with an age-dependent process.(**Figure 7.5**) Halliday *et al* used a similar reverse approach, evaluating pathologically distinct Parkinson's subgroups and correlating them with clinical findings.(261) Three different patterns of pathology progression were identified: a group with Lewy pathology progressing anatomically as described by Braak (7) who presented with young onset and long disease duration, a second group with early severe neocortical Lewy pathology presenting with dementia and a third group with marked cortical Lewy pathology associated with Alzheimer's disease-related and vascular changes with older onset and complex disease course.

Combining our findings with results from previous clinicopathological studies, several conclusions with regards to the mechanisms underlying the clinical and pathological variability in PD can be made (**Figure 7.5**).(257-259, 261)

- Young patients presenting with a more classical Lewy body phenotype with mild motor and non-motor symptoms (mild motor-predominant) show a slow disease course and neuropathological progression before they reach the terminal stages of the disease. Disease duration seems to be the main determinant of clinical and pathological progression in this subgroup.
- Additional factors are likely to contribute to the more rapid spread of Lewy pathology throughout the brain in diffuse malignant subtypes. Moreover, other age-related pathologies contribute to the clinical picture in older patients with a more complex disease course (corresponding to the diffuse malignant subgroup) and more rapid clinical and pathological progression with a potential synergistic effect between co-morbid pathologies.
- There may also be topographical differences in Lewy pathology distribution at the early stages of the disease, with those with an initial cognitive presentation consistent with Lewy body dementia showing early involvement of cortical areas(261) although these patients were not included neither in our study nor the PPMI cohort.
- So far no precise correlations have been demonstrated between neuropathological findings and specific Parkinson's subtypes, and the effect of Lewy body and co-morbid pathologies on other non-motor symptoms apart from cognition remains poorly understood. The mechanisms by which these and other factors can modify the clinical and pathological progression of the disease to account for its marked heterogeneity remain to be elucidated.

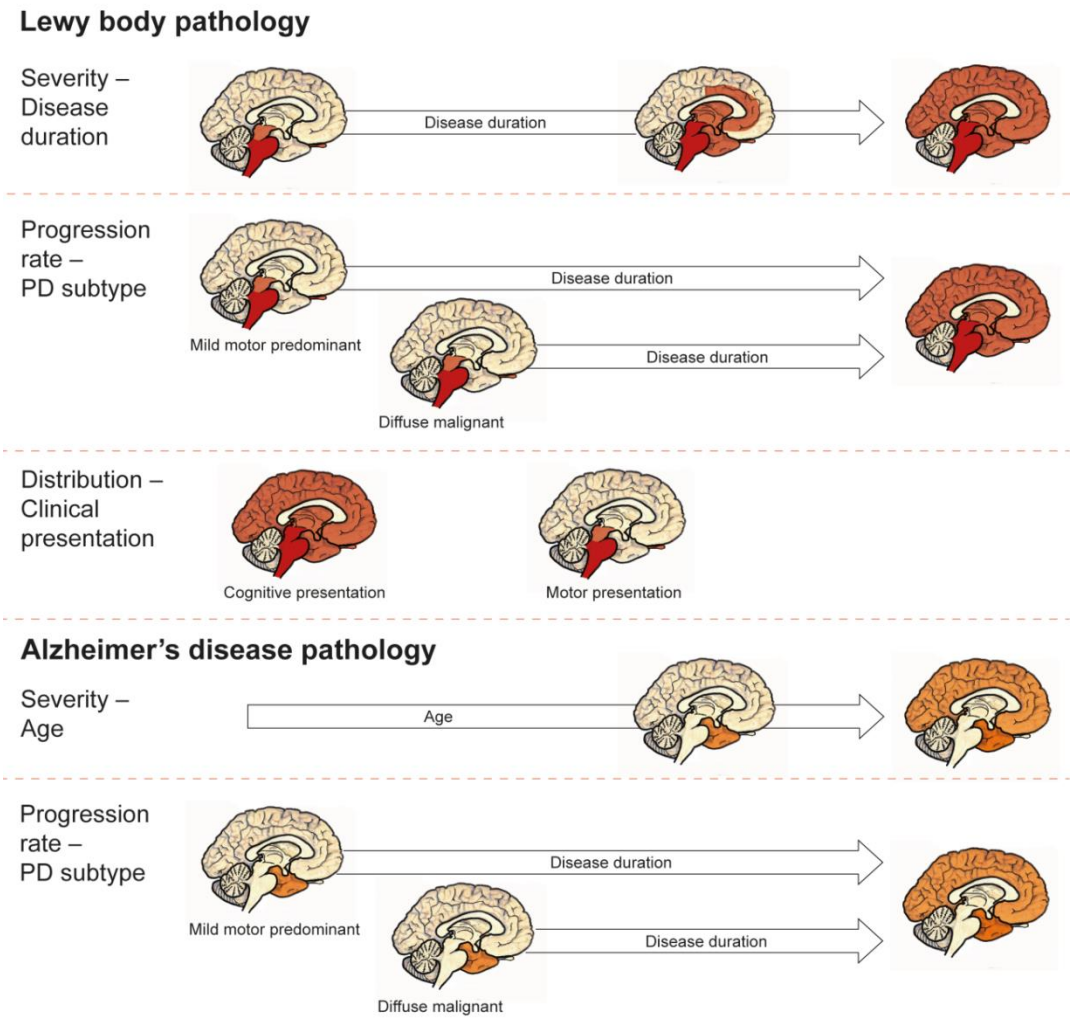


Figure 7.5. Contributing factors of neuropathological heterogeneity in PD.

Lewy pathology seems directly related to the duration of the disease although other factors may modulate this association. In this regard, Parkinson's subtypes seem to have a different neuropathological progression rate, as all cases reach advanced stages despite significant differences in disease duration. Distribution of Lewy pathology is also variable and at least a proportion of those patients presenting with early dementia shows diffuse neocortical Lewy pathology since the onset, compared with the typical caudo-rostral pattern of progression seen in the majority of patients. Other co-morbid pathologies are likely to contribute to the clinical heterogeneity. Particularly important in elderly patients is the presence of Alzheimer's disease pathology which is directly correlated with age at death. The underlying mechanisms by which these factors influence the neuropathological process and the impact of other co-morbid pathologies (cerebrovascular disease, cerebral amyloid angiopathy) remain to be elucidated.

The main strengths of this study are the presence of longitudinal regular clinical data, post-mortem diagnostic confirmation and detailed neuropathological findings. The retrospective nature of the study and the assessment by different professionals without clear methodological homogeneity (including the lack of validated questionnaires or investigations such as cardiovascular autonomic function tests or polysomnography) may potentially account for some limitations in the accuracy of the recording of the symptoms. To limit this potential bias, strict exclusion criteria were used and only the most recent cases with detailed and regular clinical information from hospital specialists were selected. Despite this and the fact that clinical information at the time of diagnosis was documented in detail for all included cases, we acknowledge that some of the symptoms, particularly mild non-motor symptoms without significant impact on clinical practice, may have been underreported. As discussed above, the advanced clinical and pathological stages of our patients may have limited detection of any potential neuropathological topographic differences in earlier stages of the disease.

In summary, our study corroborated that clinical subtyping of PD patients based on motor symptoms, RBD, autonomic and cognitive dysfunction data at the time of diagnosis provides accurate long term estimation on disease progression and survival. Therefore, clinical subtyping is feasible in clinical practice, has important clinical implications and provides accurate prognostication. Subtyping classification will need further development in order to define Parkinson's subgroups and the relevant variables that need to be included. We propose that age at diagnosis should be considered in future classification systems as our results and previous literature have shown its important prognostic implications and the influence that it may cause on the underlying pathological process.

To date, no clear correlation has been established between Parkinson's subtypes and neuropathological findings, although this has only been attempted by very few studies and results may be limited by the advanced

stage of the disease in the cases assessed. However, our study supports the evidence suggesting that different pathologies with different progression rates may contribute to the clinical heterogeneity. In this regard, clinical and pathological progression seem associated with disease duration in those young patients with a more pure Lewy body phenotype, while age at diagnosis influences the progression of co-morbid pathologies determining the complex disease course and disability in older patients. Further studies of longitudinal cohorts with post-mortem examination at different disease stages are warranted to confirm these results and assess any pathological differences underlying PD clinical subtypes.

Summary of findings and future work

PD has been traditionally considered a predominantly motor neurodegenerative disorder due to dopaminergic deficits in the nigrostriatal system. Over the last two decades, a wide range of non-motor features associated with the disease have been recognised. Understanding their underlying pathophysiological mechanisms and anatomical areas involved has provided valuable insights on clinical progression, clinical heterogeneity and mechanisms of disease pathogenesis. The traditional concept of PD has been gradually replaced by a more complex model encompassing a slowly neurodegenerative disorder that starts many years before the clinical diagnosis, involving multiple neuroanatomical areas of the central and peripheral nervous system, affecting dopaminergic and non-dopaminergic pathways that manifests with a wide range of motor and non-motor symptoms. Despite significant advances in recent years, a clear clinico-anatomical correlation, a full understanding of the pathogenic mechanisms and potential influence that some non-motor features may have in the clinical progression of PD or its associated neurodegenerative process remain to be fully elucidated. Advances in these areas will provide essential steps in the development of individualised symptomatic and disease modifying treatments. Through different projects using clinical, epidemiological, imaging, physiological and histopathological methods, this thesis provides further evidence that improves the understanding of the bidirectional influence between some of the non-motor features and the pathogenic mechanisms of PD.

The first part of the thesis explores the underlying pathophysiological mechanisms and neuroanatomical correlations of hypothalamic dysfunction, circadian abnormalities and constipation in PD.

The first project is a case-control clinicopathological study of the hypothalamus in PD. The PVN, INF and SON of the hypothalamus play a key regulatory role in autonomic function, body weight homeostasis and blood pressure control

respectively. These functions are known to be disrupted in patients with PD leading to symptoms such as AutD, weight changes and orthostatic hypotension. The aim of this study was to provide a detailed histological assessment of these hypothalamic nuclei and a clinical correlation in patients with PD and severe symptoms of AutD, weight loss and orthostatic hypotension, patients with PD without these symptoms, subjects with incidental Lewy body disease and healthy controls. Histological analysis showed Lewy pathology in all three nuclei of all PD patients and it was also found in a proportion of incidental cases suggesting that hypothalamic histological involvement can occur at early stages (even pre-clinical phase) and progressively affects the PVN, INF and SON in PD. TH immunohistochemistry did not show any differences between groups suggesting that hypothalamic dopaminergic systems are spared. No direct correlation between these histological findings and clinical symptoms of AutD, weight loss and orthostatic hypotension could be demonstrated in our patients and this may be in part due to the multifactorial origin of these symptoms. Our findings and previous evidence from imaging studies suggest that hypothalamic dysfunction is an early feature in PD although its clinical role is still to be fully elucidated. It is likely that the hypothalamus may be responsible, at least in part, for the potential symptomatology associated to disruption of these functions, although simultaneous involvement of other central and peripheral structures in these physiological systems may be more clinically relevant. Therefore, the clinical impact of hypothalamic dysfunction in PD should be ascertained and future research should focus on establishing a better clinical correlation of the hypothalamic involvement. Elucidation of the potential neurotransmitters and neurohormones impaired in hypothalamic dysfunction in PD could provide new therapeutic targets for symptomatic therapies for non-motor features.

In the second study we aimed to identify the neuroanatomical site of circadian dysfunction in PD by performing a neuropathological analysis of the SCN and pineal gland in patients with PD, patients with other neurodegenerative parkinsonian conditions with potential circadian dysfunction (MSA and PSP) and

healthy controls. Results showed a clear distinct pattern of neuropathological involvement of the circadian system in PD and also PSP, suggesting that the neuroanatomical site of circadian disruption is located in the SCN (rather than the pineal gland). Neuropathological involvement of the circadian system is not a common feature of neurodegenerative diseases as in patients with MSA there was no evidence of α-synuclein deposition in either the SCN or pineal gland and abnormalities of circadian rhythms may be secondary to disruption of other functional systems in this disease. Circadian function plays an important role in PD symptomatology, is crucial for general wellbeing and has been suggested to contribute to PD-related neurodegeneration. Based on our findings, the SCN seems the more likely neuroanatomical therapeutical target. Future research should attempt to elucidate the underlying molecular mechanisms responsible for circadian dysfunction and comparison to other neurodegenerative disorders with similar problems, such as Alzheimer's disease, may reveal common dysregulated pathways. Resynchronisation of the circadian function in patients with PD could potentially, not only provide symptomatic benefit, but also affect the neurodegenerative process. In addition to more rigorous clinical trials to further evaluate the therapeutic value of melatonin or bright light therapy, future research should explore the potential use of alternative pathways to re-establish circadian function such as regulation of expression of clock genes, enhance norepinephrine transmission to facilitate endogenous synthesis of melatonin or how behavioural interventions can re-train other biological clocks to control circadian rhythms.

Lastly, in the third project of the first part of the thesis, a pathophysiological characterisation of constipation in PD was performed using clinical, radiological and electrophysiological techniques. Participants with PD and subjective complaints of constipation underwent a systematic assessment including validated clinical questionnaires, colonic transit studies, high-resolution ARM and MRI defecography. Results showed a discrepancy between patient perception of the symptoms and the definitions and severity questionnaires commonly used in clinical practice which reflects the lack of specific diagnostic

tools for the assessment of this symptom in PD. Our results showed a heterogeneous pattern of pathophysiological abnormalities suggesting multiple overlapping mechanisms contributing to the symptomatology involving both slow colonic motility and, more frequently, abnormalities of anorectal sensory (rectal hyposensitivity) and motor function (defecatory dyssynergia). Our findings have important clinical implications in the assessment and management of constipation in patients with PD. No specific clinical tools have been developed to evaluate constipation in PD and our results revealed that available general questionnaires do not accurately reflect patient perception of the symptoms. Future research efforts should consider this subjective-based character in the development and validation of specific clinical questionnaires to assess constipation in PD and evaluate symptoms reflecting the multiple potential pathophysiological mechanisms. As a consequence of the complex overlapping pathophysiology and poor correlation between different investigations a battery of tests including at least one investigation assessing colonic transit and two evaluating anorectal function should be included for a comprehensive pathophysiological evaluation in those with refractory symptoms. Results of this systematic assessment should guide the multidisciplinary management of the symptoms with dietary modifications, laxatives and biofeedback therapy as appropriate according to the underlying pathophysiological abnormalities. The evidence supporting the use of these treatments, particularly for the symptomatic management of anorectal dysfunction, is provided by studies on patients with idiopathic functional constipation and only results from small studies or case series is available for PD. Therefore, further studies are warranted to evaluate the symptomatic benefit of botulinum toxin, apomorphine injections, biofeedback and other potential therapies in PD patients with well-established defecatory dysfunction.

Whilst the first part of the thesis investigated how neurodegeneration in PD is responsible of non-motor features, the second part of the thesis focuses on the reverse direction of this relationship: the effect that abnormalities of glucose metabolism can have on the pathogenic process of PD as a predisposing factor,

and the impact that AutD may have on the clinical phenotype, disease course and prognosis.

A retrospective record-linkage cohort study was performed to evaluate the subsequent risk of PD in patients with T2DM as epidemiological data on this association remain equivocal despite converging biological evidence. English national hospital admissions and mortality data between 1999-2011 were analysed in conjunction. Participants without PD and either T2DM (exposed cohort) or minor medical conditions and surgical procedures (reference cohort) were identified by diagnostic codes during hospital admissions. Both cohorts were subsequently searched for any subsequent hospital admission with a coded diagnosis of PD until death or end of data collection. Results showed that patients with T2DM had a 32% increase in the risk of subsequent diagnosis of PD (HR 1.32; 95% CI 1.29-1.35) particularly in younger individuals and those with complicated T2DM in what constitutes the largest cohort study by far on this association. Results were adjusted for multiple confounders and sensitivity analyses did not substantially changed the association. These results support the link between these two conditions which may be the result of genetic predisposition and / or disrupted shared pathogenic pathways with potential clinical and therapeutic implications. Previous observational studies showed striking conflicting results, particularly between case-control and cohort studies, and future epidemiological efforts should focus on elucidating the role of other potential confounding and modifying factors of this association. The conflicting results among populations (whether explained by genetic or environmental differences), the role of diet or antidiabetic medications are far from understood and large cohort studies with detailed documentation should further explore the effect of these variables. Further research into the pathogenic molecular mechanisms linking these conditions is currently on going although the ultimately deleterious effect of altered insulin signalling on the central nervous system in PD remains to be elucidated. Whether a consequence on increased co-morbid cerebrovascular damage, more severe α-synuclein aggregation or cell loss and gliosis secondary to metabolic disruption, future

neuropathological studies could shed some light on this question. Better understanding of the shared pathogenesis will open up new therapeutic avenues with repurposing of diabetic drugs for a better control of brain glucose metabolism abnormalities in PD. Clinical trials should also evaluate whether a better glycaemic control on those with concomitant T2DM and PD would provide an improvement in the disease course.

Despite growing evidence consistently reporting that AutD is a poor prognostic feature in other synucleinopathies this association has only been assessed in a few studies in patients with PD with conclusions limited by lack of diagnostic confirmation, short follow up periods and restricted assessment to particular autonomic symptoms. A retrospective review of a cohort of 100 consecutive cases with autopsy confirmed diagnosis of PD from the QSBB archive evaluated the effect of AutD on clinical progression throughout disease course and survival. AutD was associated with male sex, older age, poor levodopa response and postural abnormalities which has important clinical implications for PD subgroup definition. Multivariable Cox proportional hazards models showed that earlier AutD was an independent predictor of more rapid disease progression, disability and shorter survival in PD patients. Older age and poor levodopa response were the other two variables associated with poor prognosis in the survival model. Earlier AutD was not associated with a more diffuse neurodegeneration on neuropathological assessment, although a dedicated evaluation of autonomic structures was not performed, suggesting that the poor prognosis may be direct consequence of increased comorbidities secondary to autonomic symptoms. Although AutD has been traditionally considered a result of peripheral involvement in PD, accumulating evidence indicate a prominent role of central autonomic structures. It is therefore imperative to perform a detailed and systematic neuropathological assessment of the central autonomic centres in a large group of patients with precise clinical correlations in order to delineate their contribution to AutD in PD. However our results indicate that the negative prognosis may be associated to increased comorbidities as a result of the autonomic symptoms, raising the

possibility that a better symptomatic control could potentially improve the prognosis in these individuals. As drugs and therapeutic interventions for autonomic symptoms are already available, future clinical trials should explore the effect of a stricter symptomatic control of AutD on disease course and survival.

In the final project of this thesis and following from the results of the previous study on AutD in PD, we evaluated how new PD subtyping incorporating non-motor features can estimate disease course and survival, and correlated PD subtypes with neuropathological findings. We performed a retrospective cohort study including 111 patients with an autopsy-confirmed diagnosis of PD from the QSBB archive. Using a previous cluster classification system incorporating relevant non-motor features with prognostic implications, patients were divided at the time of diagnosis based on severity of motor and non-motor symptoms including RBD, cognitive and autonomic function. The three resulting subtypes were diffuse malignant (with severe motor and non-motor symptoms), intermediate and mild-motor predominant (with mild motor and non-motor symptoms). Disease-course clinical data were reported for each of the PD subtypes with the diffuse malignant group developing disability milestones earlier in the disease course and with shorter survival. Multivariable Cox proportional hazard regression models showed a higher risk of developing each disease milestone and higher risk of death for the diffuse malignant subtype. Because age was the only additional significant variable in the survival predictor model and because it is an important determinant on the severity of additional neuropathologies, we propose that should be included in PD subtype definition. Lewy pathology and Alzheimer's disease-related neuropathology showed different progression rates for each subtype and the latter was also correlated with age at death suggesting that neuropathology is a significant determinant of PD heterogeneity and subtypes. Because we used routine clinical data for subtype classification our results demonstrate that PD subtyping is easy to implement in clinical practice and can be useful for long-term prognostication in clinical settings. Before any further research efforts on

PD subtypes are made, general consensus should be reached regarding the classification system to be implemented. Despite methodological differences, most studies tend to agree that there is a group of patients with young onset, mild motor symptoms and slow progression, one with older onset, rapid disease progression and complicated with severe non-motor features and an intermediate group. The variables to classify patients into these subtypes should be clinically relevant, carry prognostic implications and easy to evaluate in clinical practice. Moreover, our results suggest that PD heterogeneity and subtypes are secondary to underlying pathophysiological differences. Further clinico-pathological studies of large cohorts with post-mortem examination at different stages of the disease should be able to further delineate any potential neuropathological differences among PD subtypes. Further understanding of the pathophysiological differences among subtypes will provide the basis for future individualised and more effective treatment. Before that becomes a reality, PD subtyping should be implemented in current clinical research as it is likely that these different pathophysiologies will translate in different responses to therapies as seen in the levodopa response disparity among subtypes in our study.

This thesis provides further evidence to advance the understanding of the bidirectional association between PD and non-motor features. The studies described here provide new data on neuroanatomical correlation and pathogenic mechanisms of some of the non-motor features in PD. Further elucidation of the specific underlying molecular mechanisms will eventually lead to improved symptomatic therapies. Findings from this thesis also revealed valuable insights on how some non-motor features play a relevant role on clinical progression, clinical heterogeneity and mechanisms of disease pathogenesis in PD. Future research should focus on a better understanding of these mechanisms which may reveal new therapeutic avenues, drug repurposing and open up the possibility of individualised therapies based on pathophysiology that could potentially modify the disease course.

Acknowledgements

My sincere thanks and huge gratitude to my supervisor Professor Thomas Warner who gave me the opportunity to take my first steps in clinical research and entrusted me with developing all my research ideas into projects. His invaluable advice and unconditional support helped me throughout my PhD and it has been a pleasure to develop my clinical and research skills under his supervision.

I am truly indebted to Professor Janice Holton for her patient introduction to neuropathology and all her time spent on discussions over a microscope. Special thanks to Professor Andrew Lees for sharing his knowledge and expertise (whether on movement disorders or on life) and for his always sharp comments on my studies. I would also like to thank Professor Kailash Bhatia for reminding me of the importance of a detailed neurological examination in any diagnostic process and enlightening me on complex movement disorders.

Warm thanks and gratitude also go to my colleagues the clinical research fellows from the Reta Lila Weston Institute. Special thanks to Dr Helen Ling and Dr Alastair Noyce for their inspiration, support and advice, and to Dr Pedro Barbosa for taking this trip together. I also wish to thank Iliyana Komsiyska, Karen Shaw, Dr Daniela Hansen, Dr Sam Shribman, Dr Marcos Oliveira, Robert Courtney, Kate Strand, Nuria, Abi, Isabel and so many others at the QSBB who helped me in many different ways over these years and made my time spent here a memorable experience.

I have had the privilege of collaborating with numerous brilliant colleagues, within and outside UCL, and without their contribution this thesis would not have been possible: Dr Julia Pakpoor and Dr Raph Goldacre from the Health-Care Epidemiology Unit at the University of Oxford, Dr Valentina Passananti, Dr Natalia Zárate López and Dr Anton Emmanuel from the Gastrointestinal Physiology Unit at UCL Hospital, and Dr Djordje Gveric from the Parkinson's UK Brain Bank.

This piece of research would have not been possible without the generous support, trust and participation from all the patients and family members. The Martín Escudero Foundation and trustees of the Reta Lila Weston Institute kindly supported this research.

Most of all, I would like to thank my parents, Félix and Gloria, Gonzalo and Áine whose support and dedication in numerous ways is hard to put in to words.

Bibliography

1. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2018;17(11):939-53.

2. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol. 2006;5(6):525-35.

3. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992;55(3):181-4.

4. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol. 2009;8(12):1150-7.

5. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: Review of the literature. Mov Disord. 2017;32(11):1504-23.

6. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839-40.

7. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197-211.

8. Burke RE, Dauer WT, Vonsattel JP. A critical evaluation of the Braak staging scheme for Parkinson's disease. Ann Neurol. 2008;64(5):485-91.

9. Jellinger KA. A critical reappraisal of current staging of Lewy-related pathology in human brain. Acta Neuropathol. 2008;116(1):1-16.

10. Halliday G, McCann H, Shepherd C. Evaluation of the Braak hypothesis: how far can it explain the pathogenesis of Parkinson's disease? Expert Rev Neurother. 2012;12(6):673-86.

11. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017;89(1):88-100.

12. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain. 1991;114 (Pt 5):2283-301.

13. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol. 2010;67(6):715-25.

14. Chaudhuri KR, Martinez-Martin P, Schapira AH, Stocchi F, Sethi K, Odin P, et al. International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson's disease: the NMSQuest study. Mov Disord. 2006;21(7):916-23.

15. Chaudhuri KR, Healy DG, Schapira AH. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol. 2006;5(3):235-45.

16. Jellinger KA. Neuropathobiology of non-motor symptoms in Parkinson disease. J Neural Transm. 2015.

17. Seppi K, Weintraub D, Coelho M, Perez-Lloret S, Fox SH, Katzenschlager R, et al. The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for the non-motor symptoms of Parkinson's disease. Mov Disord. 2011;26 Suppl 3:S42-80.

18. Schrag A, Horsfall L, Walters K, Noyce A, Petersen I. Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. Lancet Neurol. 2015;14(1):57-64.

19. Ferrer I, Martinez A, Blanco R, Dalfo E, Carmona M. Neuropathology of sporadic Parkinson disease before the appearance of parkinsonism: preclinical Parkinson disease. Journal of neural transmission (Vienna, Austria : 1996). 2011;118(5):821-39.

20. Salat D, Noyce AJ, Schrag A, Tolosa E. Challenges of modifying disease progression in prediagnostic Parkinson's disease. Lancet Neurol. 2016.

21. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. Ann N Y Acad Sci. 2009;1170:615-22.

22. Adler CH, Beach TG. Neuropathological basis of nonmotor manifestations of Parkinson's disease. Mov Disord. 2016;31(8):1114-9.

23. Greenland JC, Williams-Gray CH, Barker RA. The clinical heterogeneity of Parkinson's disease and its therapeutic implications. Eur J Neurosci. 2018.

24. Fereshtehnejad SM, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. Brain. 2017;140(7):1959-76.

25. Fereshtehnejad SM, Romenets SR, Anang JB, Latreille V, Gagnon JF, Postuma RB. New Clinical Subtypes of Parkinson Disease and Their Longitudinal Progression: A Prospective Cohort Comparison With Other Phenotypes. JAMA neurology. 2015;72(8):863-73.

26. Erro R, Picillo M, Vitale C, Palladino R, Amboni M, Moccia M, et al. Clinical clusters and dopaminergic dysfunction in de-novo Parkinson disease. Parkinsonism Relat Disord. 2016;28:137-40.

27. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591-601.

28. Malek N, Lawton MA, Grosset KA, Bajaj N, Barker RA, Ben-Shlomo Y, et al. Utility of the new Movement Disorder Society clinical diagnostic criteria for Parkinson's disease applied retrospectively in a large cohort study of recent onset cases. Parkinsonism Relat Disord. 2017;40:40-6.

29. Ferguson AV, Latchford KJ, Samson WK. The paraventricular nucleus of the hypothalamus - a potential target for integrative treatment of autonomic dysfunction. Expert Opin Ther Targets. 2008;12(6):717-27.

30. Cersosimo MG, Benarroch EE. Central control of autonomic function and involvement in neurodegenerative disorders. Handb Clin Neurol. 2013;117:45-57.

31. Cunningham JT, Bruno SB, Grindstaff RR, Grindstaff RJ, Higgs KH, Mazzella D, et al. Cardiovascular regulation of supraoptic vasopressin neurons. Prog Brain Res. 2002;139:257-73.

32. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. Neuron. 2002;36(2):199-211.

33. Benarroch EE. Neural control of feeding behavior: Overview and clinical correlations. Neurology. 2010;74(20):1643-50.

34. Asahina M, Vichayanrat E, Low DA, Iodice V, Mathias CJ. Autonomic dysfunction in parkinsonian disorders: assessment and pathophysiology. J Neurol Neurosurg Psychiatry. 2013;84(6):674-80.

35. Coon EA, Cutsforth-Gregory JK, Benarroch EE. Neuropathology of autonomic dysfunction in synucleinopathies. Mov Disord. 2018;33(3):349-58.

36. Espay AJ, LeWitt PA, Hauser RA, Merola A, Masellis M, Lang AE. Neurogenic orthostatic hypotension and supine hypertension in Parkinson's disease and related synucleinopathies: prioritisation of treatment targets. Lancet Neurol. 2016;15(9):954-66.

37. Goldstein DS, Holmes C, Sharabi Y, Wu T. Survival in synucleinopathies: A prospective cohort study. Neurology. 2015;85(18):1554-61.

38. Velseboer DC, de Haan RJ, Wieling W, Goldstein DS, de Bie RM. Prevalence of orthostatic hypotension in Parkinson's disease: a systematic review and meta-analysis. Parkinsonism Relat Disord. 2011;17(10):724-9.

39. Ikeda K, Kashihara H, Tamura M, Kano O, Iwamoto K, Iwasaki Y. Body mass index and the risk of Parkinson disease. Neurology. 2007;68(24):2156; author reply -7.

40. Chen H, Zhang SM, Hernan MA, Willett WC, Ascherio A. Weight loss in Parkinson's disease. Ann Neurol. 2003;53(5):676-9.

41. van der Marck MA, Dicke HC, Uc EY, Kentin ZH, Borm GF, Bloem BR, et al. Body mass index in Parkinson's disease: a meta-analysis. Parkinsonism Relat Disord. 2012;18(3):263-7.

42. Wills AA, Perez A, Wang J, Su X, Morgan J, Rajan SS, et al. Association Between Change in Body Mass Index, Unified Parkinson's Disease Rating Scale Scores, and Survival Among Persons With Parkinson Disease: Secondary Analysis of Longitudinal Data From NINDS Exploratory Trials in Parkinson Disease Long-term Study 1. JAMA neurology. 2016:1-8.

43. Delikanaki-Skaribas E, Trail M, Wong WW, Lai EC. Daily energy expenditure, physical activity, and weight loss in Parkinson's disease patients. Mov Disord. 2009;24(5):667-71.

44. Montaurier C, Morio B, Bannier S, Derost P, Arnaud P, Brandolini-Bunlon M, et al. Mechanisms of body weight gain in patients with Parkinson's disease after subthalamic stimulation. Brain. 2007;130(Pt 7):1808-18.

45. Breen DP, Nombela C, Vuono R, Jones PS, Fisher K, Burn DJ, et al. Hypothalamic volume loss is associated with reduced melatonin output in Parkinson's disease. Mov Disord. 2016.

46. Politis M, Piccini P, Pavese N, Koh SB, Brooks DJ. Evidence of dopamine dysfunction in the hypothalamus of patients with Parkinson's disease: an in vivo 11C-raclopride PET study. Exp Neurol. 2008;214(1):112-6.

47. Pavese N, Rivero-Bosch M, Lewis SJ, Whone AL, Brooks DJ. Progression of monoaminergic dysfunction in Parkinson's disease: a longitudinal 18F-dopa PET study. Neuroimage. 2011;56(3):1463-8.

48. Langston JW, Forno LS. The hypothalamus in Parkinson disease. Ann Neurol. 1978;3(2):129-33.

49. Ansorge O, Daniel SE, Pearce RK. Neuronal loss and plasticity in the supraoptic nucleus in Parkinson's disease. Neurology. 1997;49(2):610-3.

50. Purba JS, Hofman MA, Swaab DF. Decreased number of oxytocin-immunoreactive neurons in the paraventricular nucleus of the hypothalamus in Parkinson's disease. Neurology. 1994;44(1):84-9.

51. Matzuk MM, Saper CB. Preservation of hypothalamic dopaminergic neurons in Parkinson's disease. Ann Neurol. 1985;18(5):552-5.

52. Uc EY, Struck LK, Rodnitzky RL, Zimmerman B, Dobson J, Evans WJ. Predictors of weight loss in Parkinson's disease. Mov Disord. 2006;21(7):930-6.

53. O'Sullivan SS, Massey LA, Williams DR, Silveira-Moriyama L, Kempster PA, Holton JL, et al. Clinical outcomes of progressive supranuclear palsy and multiple system atrophy. Brain. 2008;131(Pt 5):1362-72.

54. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.

55. Gibb WR, Lees AJ. The significance of the Lewy body in the diagnosis of idiopathic Parkinson's disease. Neuropathol Appl Neurobiol. 1989;15(1):27-44.

56. DelleDonne A, Klos KJ, Fujishiro H, Ahmed Z, Parisi JE, Josephs KA, et al. Incidental Lewy body disease and preclinical Parkinson disease. Arch Neurol. 2008;65(8):1074-80.

57. Dickson DW, Fujishiro H, DelleDonne A, Menke J, Ahmed Z, Klos KJ, et al. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol. 2008;115(4):437-44.

58. Iacono D, Geraci-Erck M, Rabin ML, Adler CH, Serrano G, Beach TG, et al. Parkinson disease and incidental Lewy body disease: Just a question of time? Neurology. 2015;85(19):1670-9.

59. Kremer HP, Bots GT. Lewy bodies in the lateral hypothalamus: do they imply neuronal loss? Mov Disord. 1993;8(3):315-20.

60. Dayan E, Sklerov M, Browner N. Disrupted hypothalamic functional connectivity in patients with PD and autonomic dysfunction. Neurology. 2018;90(23):e2051-e8.

61. Ferrer I, Lopez-Gonzalez I, Carmona M, Dalfo E, Pujol A, Martinez A. Neurochemistry and the non-motor aspects of PD. Neurobiol Dis. 2012;46(3):508-26.

62. Saper CB. The central circadian timing system. Curr Opin Neurobiol. 2013;23(5):747-51.

63. An S, Harang R, Meeker K, Granados-Fuentes D, Tsai CA, Mazuski C, et al. A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. Proc Natl Acad Sci U S A. 2013;110(46):E4355-61.

64. Duguay D, Cermakian N. The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 2009;26(8):1479-513.

65. Benarroch EE. Suprachiasmatic nucleus and melatonin: reciprocal interactions and clinical correlations. Neurology. 2008;71(8):594-8.

66. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. Sleep Med Rev. 2005;9(1):11-24.

67. Roenneberg T, Merrow M. The Circadian Clock and Human Health. Curr Biol. 2016;26(10):R432-43.

68. Karatsoreos IN. Effects of circadian disruption on mental and physical health. Curr Neurol Neurosci Rep. 2012;12(2):218-25.

69. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. Science. 2010;330(6009):1349-54.

70. Savvidis C, Koutsilieris M. Circadian rhythm disruption in cancer biology. Mol Med. 2012;18:1249-60.

71. Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, et al. Crosstalk between the circadian clock circuitry and the immune system. Chronobiol Int. 2013;30(7):870-88.

72. Bonny O, Firsov D. Circadian regulation of renal function and potential role in hypertension. Curr Opin Nephrol Hypertens. 2013;22(4):439-44.

73. Portaluppi F, Tiseo R, Smolensky MH, Hermida RC, Ayala DE, Fabbian F. Circadian rhythms and cardiovascular health. Sleep Med Rev. 2012;16(2):151-66.

74. Meyer C, Muto V, Jaspar M, Kusse C, Lambot E, Chellappa SL, et al. Seasonality in human cognitive brain responses. Proc Natl Acad Sci U S A. 2016;113(11):3066-71.

75. Jagannath A, Peirson SN, Foster RG. Sleep and circadian rhythm disruption in neuropsychiatric illness. Curr Opin Neurobiol. 2013;23(5):888-94.

76. Videnovic A, Lazar AS, Barker RA, Overeem S. 'The clocks that time us'--circadian rhythms in neurodegenerative disorders. Nat Rev Neurol. 2014;10(12):683-93.

77. Breen DP, Vuono R, Nawarathna U, Fisher K, Shneerson JM, Reddy AB, et al. Sleep and circadian rhythm regulation in early Parkinson disease. JAMA neurology. 2014;71(5):589-95.

78. Videnovic A, Noble C, Reid KJ, Peng J, Turek FW, Marconi A, et al. Circadian melatonin rhythm and excessive daytime sleepiness in Parkinson disease. JAMA neurology. 2014;71(4):463-9.

79. Niwa F, Kuriyama N, Nakagawa M, Imanishi J. Circadian rhythm of rest activity and autonomic nervous system activity at different stages in Parkinson's disease. Auton Neurosci. 2011;165(2):195-200.

80. van Hilten JJ, Kabel JF, Middelkoop HA, Kramer CG, Kerkhof GA, Roos RA. Assessment of response fluctuations in Parkinson's disease by ambulatory wrist activity monitoring. Acta Neurol Scand. 1993;87(3):171-7.

81. van Hilten JJ, Middelkoop HA, Kerkhof GA, Roos RA. A new approach in the assessment of motor activity in Parkinson's disease. J Neurol Neurosurg Psychiatry. 1991;54(11):976-9.

82. Bonuccelli U, Del Dotto P, Lucetti C, Petrozzi L, Bernardini S, Gambaccini G, et al. Diurnal motor variations to repeated doses of levodopa in Parkinson's disease. Clin Neuropharmacol. 2000;23(1):28-33.

83. Mendoza J, Challet E. Circadian insights into dopamine mechanisms. Neuroscience. 2014;282:230-42.

84. Struck LK, Rodnitzky RL, Dobson JK. Circadian fluctuations of contrast sensitivity in Parkinson's disease. Neurology. 1990;40(3 Pt 1):467-70.

85. Haapaniemi TH, Pursiainen V, Korpelainen JT, Huikuri HV, Sotaniemi KA, Myllyla VV. Ambulatory ECG and analysis of heart rate variability in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2001;70(3):305-10.

86. Ejaz AA, Sekhon IS, Munjal S. Characteristic findings on 24-h ambulatory blood pressure monitoring in a series of patients with Parkinson's disease. Eur J Intern Med. 2006;17(6):417-20.

87. Plaschke M, Trenkwalder P, Dahlheim H, Lechner C, Trenkwalder C. Twenty-four-hour blood pressure profile and blood pressure responses to head-up tilt tests in Parkinson's disease and multiple system atrophy. J Hypertens. 1998;16(10):1433-41.

88. Zhong G, Bolitho S, Grunstein R, Naismith SL, Lewis SJ. The relationship between thermoregulation and REM sleep behaviour disorder in Parkinson's disease. PLoS One. 2013;8(8):e72661.

89. Benarroch EE, Schmeichel AM, Sandroni P, Low PA, Parisi JE. Differential involvement of hypothalamic vasopressin neurons in multiple system atrophy. Brain. 2006;129(Pt 10):2688-96.

90. Ozawa T, Soma Y, Yoshimura N, Fukuhara N, Tanaka M, Tsuji S. Reduced morning cortisol secretion in patients with multiple system atrophy. Clin Auton Res. 2001;11(4):271-2.

91. Ozawa T, Tanaka H, Nakano R, Sato M, Inuzuka T, Soma Y, et al. Nocturnal decrease in vasopressin secretion into plasma in patients with multiple system atrophy. J Neurol Neurosurg Psychiatry. 1999;67(4):542-5.

92. Pierangeli G, Provini F, Maltoni P, Barletta G, Contin M, Lugaresi E, et al. Nocturnal body core temperature falls in Parkinson's disease but not in Multiple-System Atrophy. Mov Disord. 2001;16(2):226-32.

93. Suzuki K, Miyamoto T, Miyamoto M, Hirata K. The core body temperature rhythm is altered in progressive supranuclear palsy. Clin Auton Res. 2009;19(1):65-8.

94. Walsh CM, Ruoff L, Varbel J, Walker K, Grinberg LT, Boxer AL, et al. Rest-activity rhythm disruption in progressive supranuclear palsy. Sleep Med. 2016;22:50-6.

95. Schmidt C, Berg D, Prieur S, Junghanns S, Schweitzer K, Globas C, et al. Loss of nocturnal blood pressure fall in various extrapyramidal syndromes. Mov Disord. 2009;24(14):2136-42.

96. Ondo WG. Sleep/wake problems in Parkinson's disease: pathophysiology and clinicopathologic correlations. Journal of neural transmission (Vienna, Austria : 1996). 2014;121 Suppl 1:S3-13.

97. Cai Y, Liu S, Sothern RB, Xu S, Chan P. Expression of clock genes Per1 and Bmal1 in total leukocytes in health and Parkinson's disease. Eur J Neurol. 2010;17(4):550-4.

98. Ding H, Liu S, Yuan Y, Lin Q, Chan P, Cai Y. Decreased expression of Bmal2 in patients with Parkinson's disease. Neurosci Lett. 2011;499(3):186-8.

99. Lin Q, Ding H, Zheng Z, Gu Z, Ma J, Chen L, et al. Promoter methylation analysis of seven clock genes in Parkinson's disease. Neurosci Lett. 2012;507(2):147-50.

100. Bolitho SJ, Naismith SL, Rajaratnam SM, Grunstein RR, Hodges JR, Terpening Z, et al. Disturbances in melatonin secretion and circadian sleep-wake regulation in Parkinson disease. Sleep Med. 2014;15(3):342-7.

101. Hartmann A, Veldhuis JD, Deuschle M, Standhardt H, Heuser I. Twenty-four hour cortisol release profiles in patients with Alzheimer's and Parkinson's disease compared to normal controls: ultradian secretory pulsatility and diurnal variation. Neurobiol Aging. 1997;18(3):285-9.

102. Djamshidian A, O'Sullivan SS, Papadopoulos A, Bassett P, Shaw K, Averbeck BB, et al. Salivary cortisol levels in Parkinson's disease and its

correlation to risk behaviour. J Neurol Neurosurg Psychiatry. 2011;82(10):1107-11.

103. Wang JL, Lim AS, Chiang WY, Hsieh WH, Lo MT, Schneider JA, et al. Suprachiasmatic neuron numbers and rest-activity circadian rhythms in older humans. Ann Neurol. 2015;78(2):317-22.

104. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology. 2005;65(12):1863-72.

105. Trojanowski JQ, Revesz T. Proposed neuropathological criteria for the post mortem diagnosis of multiple system atrophy. Neuropathol Appl Neurobiol. 2007;33(6):615-20.

106. Williams DR, Lees AJ. Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges. Lancet Neurol. 2009;8(3):270-9.

107. Kondratova AA, Kondratov RV. The circadian clock and pathology of the ageing brain. Nat Rev Neurosci. 2012;13(5):325-35.

108. Fertl E, Auff E, Doppelbauer A, Waldhauser F. Circadian secretion pattern of melatonin in Parkinson's disease. J Neural Transm Park Dis Dement Sect. 1991;3(1):41-7.

109. Bordet R, Devos D, Brique S, Touitou Y, Guieu JD, Libersa C, et al. Study of circadian melatonin secretion pattern at different stages of Parkinson's disease. Clin Neuropharmacol. 2003;26(2):65-72.

110. Critchley PH, Malcolm GP, Malcolm PN, Gibb WR, Arendt J, Parkes JD. Fatigue and melatonin in Parkinson's disease. J Neurol Neurosurg Psychiatry. 1991;54(1):91-2.

111. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. Science. 2016;354(6315):1004-8.

112. Videnovic A, Willis GL. Circadian system - A novel diagnostic and therapeutic target in Parkinson's disease? Mov Disord. 2016;31(3):260-9.

113. Rodrigues TM, Castro Caldas A, Ferreira JJ. Pharmacological interventions for daytime sleepiness and sleep disorders in Parkinson's disease: Systematic review and meta-analysis. Parkinsonism Relat Disord. 2016;27:25-34.

114. Dowling GA, Mastick J, Colling E, Carter JH, Singer CM, Aminoff MJ. Melatonin for sleep disturbances in Parkinson's disease. Sleep Med. 2005;6(5):459-66.

115. Medeiros CA, Carvalhedo de Bruin PF, Lopes LA, Magalhaes MC, de Lourdes Seabra M, de Bruin VM. Effect of exogenous melatonin on sleep and motor dysfunction in Parkinson's disease. A randomized, double blind, placebo-controlled study. J Neurol. 2007;254(4):459-64.

116. Rutten S, Vriend C, van den Heuvel OA, Smit JH, Berendse HW, van der Werf YD. Bright light therapy in Parkinson's disease: an overview of the background and evidence. Parkinsons Dis. 2012;2012:767105.

117. Videnovic A, Klerman EB, Wang W, Marconi A, Kuhta T, Zee PC. Timed Light Therapy for Sleep and Daytime Sleepiness Associated With Parkinson Disease: A Randomized Clinical Trial. JAMA neurology. 2017;74(4):411-8.

118. Paus S, Schmitz-Hubsch T, Wullner U, Vogel A, Klockgether T, Abele M. Bright light therapy in Parkinson's disease: a pilot study. Mov Disord. 2007;22(10):1495-8.

119. Adams-Carr KL, Bestwick JP, Shribman S, Lees A, Schrag A, Noyce AJ. Constipation preceding Parkinson's disease: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2016;87(7):710-6.

120. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, et al. Frequency of bowel movements and the future risk of Parkinson's disease. Neurology. 2001;57(3):456-62.

121. Beach TG, Adler CH, Sue LI, Vedders L, Lue L, White Iii CL, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 2010;119(6):689-702.

122. Sanchez-Ferro A, Rabano A, Catalan MJ, Rodriguez-Valcarcel FC, Diez SF, Herreros-Rodriguez J, et al. In vivo gastric detection of alpha-synuclein inclusions in Parkinson's disease. Mov Disord. 2015;30(4):517-24.

123. Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen JM, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One. 2010;5(9):e12728.

124. Ruffmann C, Parkkinen L. Gut Feelings About alpha-Synuclein in Gastrointestinal Biopsies: Biomarker in the Making? Mov Disord. 2016;31(2):193-202.

125. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. Journal of neural transmission (Vienna, Austria : 1996). 2003;110(5):517-36.

126. Svensson E, Horvath-Puho E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. Ann Neurol. 2015;78(4):522-9.

127. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord. 2015;30(3):350-8.

128. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS One. 2011;6(12):e28032.

129. Lionnet A, Leclair-Visonneau L, Neunlist M, Murayama S, Takao M, Adler CH, et al. Does Parkinson's disease start in the gut? Acta Neuropathol. 2018;135(1):1-12.

130. Shannon K, Vanden Berghe P. The enteric nervous system in PD: gateway, bystander victim, or source of solutions. Cell Tissue Res. 2018;373(1):313-26.

131. Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. Lancet Neurol. 2015;14(6):625-39.

132. Knudsen K, Krogh K, Ostergaard K, Borghammer P. Constipation in parkinson's disease: Subjective symptoms, objective markers, and new perspectives. Mov Disord. 2017;32(1):94-105.

133. Coffin B, Causse C. Constipation assessment scales in adults: a literature review including the new Bowel Function Index. Expert review of gastroenterology & hepatology. 2011;5(5):601-13.

134. Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. Parkinsonism Relat Disord. 2011;17(1):10-5.

135. Kim JS, Sung HY, Lee KS, Kim YI, Kim HT. Anorectal dysfunctions in Parkinson's disease. J Neurol Sci. 2011;310(1-2):144-51.

136. Kim ER, Rhee PL. How to interpret a functional or motility test - colon transit study. J Neurogastroenterol Motil. 2012;18(1):94-9.

137. Skardoon GR, Khera AJ, Emmanuel AV, Burgell RE. Review article: dyssynergic defaecation and biofeedback therapy in the pathophysiology and management of functional constipation. Aliment Pharmacol Ther. 2017;46(4):410-23.

138. Carrington EV, Scott SM, Bharucha A, Mion F, Remes-Troche JM, Malcolm A, et al. Expert consensus document: Advances in the evaluation of anorectal function. Nature reviews Gastroenterology & hepatology. 2018;15(5):309-23.

139. Lee TH, Bharucha AE. How to Perform and Interpret a High-resolution Anorectal Manometry Test. J Neurogastroenterol Motil. 2016;22(1):46-59.

140. Ashraf W, Pfeiffer RF, Quigley EM. Anorectal manometry in the assessment of anorectal function in Parkinson's disease: a comparison with chronic idiopathic constipation. Mov Disord. 1994;9(6):655-63.

141. Sung HY, Choi MG, Kim YI, Lee KS, Kim JS. Anorectal manometric dysfunctions in newly diagnosed, early-stage Parkinson's disease. J Clin Neurol. 2012;8(3):184-9.

142. Yu T, Wang Y, Wu G, Xu Q, Tang Y, Lin L. High-resolution Anorectal Manometry in Parkinson Disease With Defecation Disorder: A Comparison With Functional Defecation Disorder. J Clin Gastroenterol. 2016;50(7):566-71.

143. Bassotti G, Maggio D, Battaglia E, Giulietti O, Spinozzi F, Reboldi G, et al. Manometric investigation of anorectal function in early and late stage Parkinson's disease. J Neurol Neurosurg Psychiatry. 2000;68(6):768-70.

144. Rao SS, Ozturk R, Laine L. Clinical utility of diagnostic tests for constipation in adults: a systematic review. Am J Gastroenterol. 2005;100(7):1605-15.

145. Su A, Gandhy R, Barlow C, Triadafilopoulos G. Utility of high-resolution anorectal manometry and wireless motility capsule in the evaluation of patients with Parkinson's disease and chronic constipation. BMJ open gastroenterology. 2016;3(1):e000118.

146. Frank L, Kleinman L, Farup C, Taylor L, Miner P, Jr. Psychometric validation of a constipation symptom assessment questionnaire. Scand J Gastroenterol. 1999;34(9):870-7.

147. Krogh K, Christensen P, Sabroe S, Laurberg S. Neurogenic bowel dysfunction score. Spinal Cord. 2006;44(10):625-31.

148. Krogh K, Ostergaard K, Sabroe S, Laurberg S. Clinical aspects of bowel symptoms in Parkinson's disease. Acta Neurol Scand. 2008;117(1):60-4.

149. Evans RC, Kamm MA, Hinton JM, Lennard-Jones JE. The normal range and a simple diagram for recording whole gut transit time. Int J Colorectal Dis. 1992;7(1):15-7.

150. Carrington EV, Brokjaer A, Craven H, Zarate N, Horrocks EJ, Palit S, et al. Traditional measures of normal anal sphincter function using high-resolution anorectal manometry (HRAM) in 115 healthy volunteers. Neurogastroenterol Motil. 2014;26(5):625-35.

151. Heinrich H, Sauter M, Fox M, Weishaupt D, Halama M, Misselwitz B, et al. Assessment of Obstructive Defecation by High-Resolution Anorectal Manometry Compared With Magnetic Resonance Defecography. Clin Gastroenterol Hepatol. 2015;13(7):1310-7.e1.

152. Knudsen K, Fedorova TD, Bekker AC, Iversen P, Ostergaard K, Krogh K, et al. Objective Colonic Dysfunction is Far more Prevalent than Subjective Constipation in Parkinson's Disease: A Colon Transit and Volume Study. J Parkinsons Dis. 2017;7(2):359-67.

153. Miller LE, Ibarra A, Ouwehand AC. Normative Values for Colonic Transit Time and Patient Assessment of Constipation in Adults With Functional Constipation: Systematic Review With Meta-Analysis. Clin Med Insights Gastroenterol. 2017;11:1179552217729343.

154. Sakakibara R, Shinotoh H, Uchiyama T, Sakuma M, Kashiwado M, Yoshiyama M, et al. Questionnaire-based assessment of pelvic organ dysfunction in Parkinson's disease. Auton Neurosci. 2001;92(1-2):76-85.

155. Edwards LL, Pfeiffer RF, Quigley EM, Hofman R, Balluff M. Gastrointestinal symptoms in Parkinson's disease. Mov Disord. 1991;6(2):151-6.

156. Jost WH, Schrank B. Defecatory disorders in de novo Parkinsonians--colonic transit and electromyogram of the external anal sphincter. Wiener klinische Wochenschrift. 1998;110(15):535-7.

157. Sakakibara R, Odaka T, Uchiyama T, Asahina M, Yamaguchi K, Yamaguchi T, et al. Colonic transit time and rectoanal videomanometry in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2003;74(2):268-72.

158. Edwards LL, Quigley EM, Harned RK, Hofman R, Pfeiffer RF. Characterization of swallowing and defecation in Parkinson's disease. Am J Gastroenterol. 1994;89(1):15-25.

159. Mathers SE, Kempster PA, Law PJ, Frankel JP, Bartram CI, Lees AJ, et al. Anal sphincter dysfunction in Parkinson's disease. Arch Neurol. 1989;46(10):1061-4.

160. Edwards LL, Quigley EM, Harned RK, Hofman R, Pfeiffer RF. Defecatory function in Parkinson's disease: response to apomorphine. Ann Neurol. 1993;33(5):490-3.

161. Cadeddu F, Bentivoglio AR, Brandara F, Marniga G, Brisinda G, Maria G. Outlet type constipation in Parkinson's disease: results of botulinum toxin treatment. Aliment Pharmacol Ther. 2005;22(10):997-1003.

162. Ashraf W, Pfeiffer RF, Park F, Lof J, Quigley EM. Constipation in Parkinson's disease: objective assessment and response to psyllium. Mov Disord. 1997;12(6):946-51.

163. Jost WH, Schimrigk K. Constipation in Parkinson's disease. Klin Wochenschr. 1991;69(20):906-9.

164. Cersosimo MG, Benarroch EE. Pathological correlates of gastrointestinal dysfunction in Parkinson's disease. Neurobiol Dis. 2012;46(3):559-64.

165. Gladman MA, Lunniss PJ, Scott SM, Swash M. Rectal hyposensitivity. Am J Gastroenterol. 2006;101(5):1140-51.

166. Burgell RE, Scott SM. Rectal hyposensitivity. J Neurogastroenterol Motil. 2012;18(4):373-84.

167. Stocchi F, Badiali D, Vacca L, D'Alba L, Bracci F, Ruggieri S, et al. Anorectal function in multiple system atrophy and Parkinson's disease. Mov Disord. 2000;15(1):71-6.

168. Reiner CS, Tutuian R, Solopova AE, Pohl D, Marincek B, Weishaupt D. MR defecography in patients with dyssynergic defecation: spectrum of imaging findings and diagnostic value. Br J Radiol. 2011;84(998):136-44.

169. Southwell BR, Clarke MC, Sutcliffe J, Hutson JM. Colonic transit studies: normal values for adults and children with comparison of radiological and scintigraphic methods. Pediatr Surg Int. 2009;25(7):559-72.

170. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the brain: sources, localization and functions. Mol Neurobiol. 2013;47(1):145-71.

171. Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: A new target for disease modification? Prog Neurobiol. 2016;145-146:98-120.

172. Bassil F, Fernagut PO, Bezard E, Meissner WG. Insulin, IGF-1 and GLP-1 signaling in neurodegenerative disorders: targets for disease modification? Prog Neurobiol. 2014;118:1-18.

173. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol. 2004;3(3):169-78.

174. Santiago JA, Potashkin JA. Shared dysregulated pathways lead to Parkinson's disease and diabetes. Trends Mol Med. 2013;19(3):176-86.

175. Aviles-Olmos I, Limousin P, Lees A, Foltynie T. Parkinson's disease, insulin resistance and novel agents of neuroprotection. Brain. 2013;136(Pt 2):374-84.

176. Sandyk R. The relationship between diabetes mellitus and Parkinson's disease. Int J Neurosci. 1993;69(1-4):125-30.

177. Marques A, Dutheil F, Durand E, Rieu I, Mulliez A, Fantini ML, et al. Glucose dysregulation in Parkinson's disease: Too much glucose or not enough insulin? Parkinsonism Relat Disord. 2018;55:122-7.

178. Cereda E, Barichella M, Cassani E, Caccialanza R, Pezzoli G. Clinical features of Parkinson disease when onset of diabetes came first: A case-control study. Neurology. 2012;78(19):1507-11.

179. Pagano G, Polychronis S, Wilson H, Giordano B, Ferrara N, Niccolini F, et al. Diabetes mellitus and Parkinson disease. Neurology. 2018;90(19):e1654-e62.

180. Mohamed Ibrahim N, Ramli R, Koya Kutty S, Shah SA. Earlier onset of motor complications in Parkinson's patients with comorbid diabetes mellitus. Mov Disord. 2018;33(12):1967-8.

181. Mollenhauer B, Zimmermann J, Sixel-Doring F, Focke NK, Wicke T, Ebentheuer J, et al. Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the De Novo Parkinson Cohort (DeNoPa). Mov Disord. 2018.

182. Bohnen NI, Kotagal V, Muller ML, Koeppe RA, Scott PJ, Albin RL, et al. Diabetes mellitus is independently associated with more severe cognitive impairment in Parkinson disease. Parkinsonism Relat Disord. 2014;20(12):1394-8.

183. Chung SJ, Jeon S, Yoo HS, Kim G, Oh JS, Kim JS, et al. Detrimental effect of type 2 diabetes mellitus in a large case series of Parkinson's disease. Parkinsonism Relat Disord. 2018.

184. Kotagal V, Albin RL, Muller ML, Koeppe RA, Frey KA, Bohnen NI. Diabetes is associated with postural instability and gait difficulty in Parkinson disease. Parkinsonism Relat Disord. 2013;19(5):522-6.

185. Giuntini M, Baldacci F, Del Prete E, Bonuccelli U, Ceravolo R. Diabetes is associated with postural and cognitive domains in Parkinson's disease. Results from a single-center study. Parkinsonism Relat Disord. 2014;20(6):671-2.

186. Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol. 2012;72(6):893-901.

187. Lu L, Fu DL, Li HQ, Liu AJ, Li JH, Zheng GQ. Diabetes and risk of Parkinson's disease: an updated meta-analysis of case-control studies. PLoS One. 2014;9(1):e85781.

188. Cereda E, Barichella M, Pedrolli C, Klersy C, Cassani E, Caccialanza R, et al. Diabetes and risk of Parkinson's disease: a systematic review and meta-analysis. Diabetes Care. 2011;34(12):2614-23.

189. Yue X, Li H, Yan H, Zhang P, Chang L, Li T. Risk of Parkinson Disease in Diabetes Mellitus: An Updated Meta-Analysis of Population-Based Cohort Studies. Medicine (Baltimore). 2016;95(18):e3549.

190. Xu Q, Park Y, Huang X, Hollenbeck A, Blair A, Schatzkin A, et al. Diabetes and risk of Parkinson's disease. Diabetes Care. 2011;34(4):910-5.

191. Hu G, Jousilahti P, Bidel S, Antikainen R, Tuomilehto J. Type 2 diabetes and the risk of Parkinson's disease. Diabetes Care. 2007;30(4):842-7.

192. Palacios N, Gao X, McCullough ML, Jacobs EJ, Patel AV, Mayo T, et al. Obesity, diabetes, and risk of Parkinson's disease. Mov Disord. 2011;26(12):2253-9.

193. Santiago JA, Potashkin JA. Integrative network analysis unveils convergent molecular pathways in Parkinson's disease and diabetes. PLoS One. 2013;8(12):e83940.

194. De Pablo-Fernandez E, Sierra-Hidalgo F, Benito-Leon J, Bermejo-Pareja F. Association between Parkinson's disease and diabetes: Data from NEDICES study. Acta Neurol Scand. 2017;136(6):732-6.

195. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol. 2018;14(3):168-81.

196. Moran C, Beare R, Phan TG, Bruce DG, Callisaya ML, Srikanth V. Type 2 diabetes mellitus and biomarkers of neurodegeneration. Neurology. 2015;85(13):1123-30.

197. Petrou M, Davatzikos C, Hsieh M, Foerster BR, Albin RL, Kotagal V, et al. Diabetes, Gray Matter Loss, and Cognition in the Setting of Parkinson Disease. Acad Radiol. 2016;23(5):577-81.

198. Ong M, Foo H, Chander RJ, Wen MC, Au WL, Sitoh YY, et al. Influence of diabetes mellitus on longitudinal atrophy and cognition in Parkinson's disease. J Neurol Sci. 2017;377:122-6.

199. Brauer R, Bhaskaran K, Chaturvedi N, Dexter DT, Smeeth L, Douglas I. Glitazone Treatment and Incidence of Parkinson's Disease among People with Diabetes: A Retrospective Cohort Study. PLoS Med. 2015;12(7):e1001854.

200. Lin HL, Lin HC, Tseng YF, Chao JC, Hsu CY. Association of thiazolidinedione with a lower risk of Parkinson's disease in a population with newly-diagnosed diabetes mellitus. Ann Med. 2018;50(5):430-6.

201. Brakedal B, Flones I, Reiter SF, Torkildsen O, Dolle C, Assmus J, et al. Glitazone use associated with reduced risk of Parkinson's disease. Mov Disord. 2017;32(11):1594-9.

202. Pioglitazone in early Parkinson's disease: a phase 2, multicentre, double-blind, randomised trial. Lancet Neurol. 2015;14(8):795-803.

203. Athauda D, Foltynie T. The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: mechanisms of action. Drug discovery today. 2016;21(5):802-18.

204. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet. 2017;390(10103):1664-75.

205. National Institute for Health and Care Excellence. Parkinson's disease in adults. NICE clinical guideline NG71 London: National Institute for Health and Care Excellence; 2017 [updated July 2017. Available from: https://www.nice.org.uk/guidance/NG71.

206. Gelpi E, Navarro-Otano J, Tolosa E, Gaig C, Compta Y, Rey MJ, et al. Multiple organ involvement by alpha-synuclein pathology in Lewy body disorders. Mov Disord. 2014;29(8):1010-8.

207. Cersosimo MG, Benarroch EE. Autonomic involvement in Parkinson's disease: pathology, pathophysiology, clinical features and possible peripheral biomarkers. J Neurol Sci. 2012;313(1-2):57-63.

208. Proulx M, de Courval FP, Wiseman MA, Panisset M. Salivary production in Parkinson's disease. Mov Disord. 2005;20(2):204-7.

209. Bagheri H, Damase-Michel C, Lapeyre-Mestre M, Cismondo S, O'Connell D, Senard JM, et al. A study of salivary secretion in Parkinson's disease. Clin Neuropharmacol. 1999;22(4):213-5.

210. McDonald C, Winge K, Burn DJ. Lower urinary tract symptoms in Parkinson's disease: Prevalence, aetiology and management. Parkinsonism Relat Disord. 2017;35:8-16.

211. Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. Lancet Neurol. 2015;14(7):720-32.

212. Sakakibara R, Uchiyama T, Yamanishi T, Kishi M. Genitourinary dysfunction in Parkinson's disease. Mov Disord. 2010;25(1):2-12.

213. Post B, Merkus MP, de Haan RJ, Speelman JD. Prognostic factors for the progression of Parkinson's disease: a systematic review. Mov Disord. 2007;22(13):1839-51; quiz 988.

214. van der Heeden JF, Marinus J, Martinez-Martin P, Rodriguez-Blazquez C, Geraedts VJ, van Hilten JJ. Postural instability and gait are associated with severity and prognosis of Parkinson disease. Neurology. 2016;86(24):2243-50.

215. Macleod AD, Taylor KS, Counsell CE. Mortality in Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014;29(13):1615-22.

216. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008;71(9):670-6.

217. Watanabe H, Saito Y, Terao S, Ando T, Kachi T, Mukai E, et al. Progression and prognosis in multiple system atrophy: an analysis of 230 Japanese patients. Brain. 2002;125(Pt 5):1070-83.

218. Low PA, Reich SG, Jankovic J, Shults CW, Stern MB, Novak P, et al. Natural history of multiple system atrophy in the USA: a prospective cohort study. Lancet Neurol. 2015;14(7):710-9.

219. Coon EA, Sletten DM, Suarez MD, Mandrekar JN, Ahlskog JE, Bower JH, et al. Clinical features and autonomic testing predict survival in multiple system atrophy. Brain. 2015;138(Pt 12):3623-31.

220. Tada M, Onodera O, Tada M, Ozawa T, Piao YS, Kakita A, et al. Early development of autonomic dysfunction may predict poor prognosis in patients with multiple system atrophy. Arch Neurol. 2007;64(2):256-60.

221. Petrovic IN, Ling H, Asi Y, Ahmed Z, Kukkle PL, Hazrati LN, et al. Multiple system atrophy-parkinsonism with slow progression and prolonged survival: a diagnostic catch. Mov Disord. 2012;27(9):1186-90.

222. Stubendorff K, Aarsland D, Minthon L, Londos E. The impact of autonomic dysfunction on survival in patients with dementia with Lewy bodies and Parkinson's disease with dementia. PLoS One. 2012;7(10):e45451.

223. Gray WK, Wood BH, Walker RW. Do autonomic function tests in people with Parkinson's disease predict survival rates at 7 years follow-up? Mov Disord. 2009;24(16):2432-4.

224. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. Neurology. 2001;57(8):1497-9.

225. Magalhaes M, Wenning GK, Daniel SE, Quinn NP. Autonomic dysfunction in pathologically confirmed multiple system atrophy and idiopathic Parkinson's disease--a retrospective comparison. Acta Neurol Scand. 1995;91(2):98-102.

226. Koga S, Aoki N, Uitti RJ, van Gerpen JA, Cheshire WP, Josephs KA, et al. When DLB, PD, and PSP masquerade as MSA: an autopsy study of 134 patients. Neurology. 2015;85(5):404-12.

227. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord. 2010;25(15):2649-53.

228. Postuma RB, Gagnon JF, Pelletier A, Montplaisir J. Prodromal autonomic symptoms and signs in Parkinson's disease and dementia with Lewy bodies. Mov Disord. 2013;28(5):597-604.

229. Kaufmann H, Nahm K, Purohit D, Wolfe D. Autonomic failure as the initial presentation of Parkinson disease and dementia with Lewy bodies. Neurology. 2004;63(6):1093-5.

230. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain. 2002;125(Pt 4):861-70.

231. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. Neurology. 2016;86(6):566-76.

232. de Lau LM, Verbaan D, Marinus J, van Hilten JJ. Survival in Parkinson's disease. Relation with motor and non-motor features. Parkinsonism Relat Disord. 2014;20(6):613-6.

233. Merola A, Romagnolo A, Rosso M, Suri R, Berndt Z, Maule S, et al. Autonomic dysfunction in Parkinson's disease: A prospective cohort study. Mov Disord. 2018;33(3):391-7.

234. McDonald C, Newton JL, Burn DJ. Orthostatic hypotension and cognitive impairment in Parkinson's disease: Causation or association? Mov Disord. 2016;31(7):937-46.

235. Udow SJ, Robertson AD, MacIntosh BJ, Espay AJ, Rowe JB, Lang AE, et al. 'Under pressure': is there a link between orthostatic hypotension and cognitive impairment in alpha-synucleinopathies? J Neurol Neurosurg Psychiatry. 2016;87(12):1311-21.

236. Centi J, Freeman R, Gibbons CH, Neargarder S, Canova AO, Cronin-Golomb A. Effects of orthostatic hypotension on cognition in Parkinson disease. Neurology. 2017;88(1):17-24.

237. Marras C, Lang A. Parkinson's disease subtypes: lost in translation? J Neurol Neurosurg Psychiatry. 2013;84(4):409-15.

238. Fereshtehnejad SM, Postuma RB. Subtypes of Parkinson's Disease: What Do They Tell Us About Disease Progression? Curr Neurol Neurosci Rep. 2017;17(4):34.

239. Allcock LM, Kenny RA, Burn DJ. Clinical phenotype of subjects with Parkinson's disease and orthostatic hypotension: autonomic symptom and demographic comparison. Mov Disord. 2006;21(11):1851-5.

240. Muller B, Larsen JP, Wentzel-Larsen T, Skeie GO, Tysnes OB. Autonomic and sensory symptoms and signs in incident, untreated Parkinson's disease: frequent but mild. Mov Disord. 2011;26(1):65-72.

241. van Rooden SM, Colas F, Martinez-Martin P, Visser M, Verbaan D, Marinus J, et al. Clinical subtypes of Parkinson's disease. Mov Disord. 2011;26(1):51-8.

242. Anang JB, Gagnon JF, Bertrand JA, Romenets SR, Latreille V, Panisset M, et al. Predictors of dementia in Parkinson disease: a prospective cohort study. Neurology. 2014;83(14):1253-60.

243. de Lau LM, Verbaan D, van Rooden SM, Marinus J, van Hilten JJ. Relation of clinical subtypes in Parkinson's disease with survival. Mov Disord. 2014;29(1):150-1.

244. Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol. 1997;38 Suppl 2:2-7.

245. Foltynie T, Brayne C, Barker RA. The heterogeneity of idiopathic Parkinson's disease. J Neurol. 2002;249(2):138-45.

246. Jankovic J, McDermott M, Carter J, Gauthier S, Goetz C, Golbe L, et al. Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. Neurology. 1990;40(10):1529-34.

247. van Rooden SM, Heiser WJ, Kok JN, Verbaan D, van Hilten JJ, Marinus J. The identification of Parkinson's disease subtypes using cluster analysis: a systematic review. Mov Disord. 2010;25(8):969-78.

248. Marras C, Chaudhuri KR. Nonmotor features of Parkinson's disease subtypes. Mov Disord. 2016;31(8):1095-102.

249. Lawton M, Ben-Shlomo Y, May MT, Baig F, Barber TR, Klein JC, et al. Developing and validating Parkinson's disease subtypes and their motor and cognitive progression. J Neurol Neurosurg Psychiatry. 2018;89(12):1279-87.

250. The Parkinson Progression Marker Initiative (PPMI). Prog Neurobiol. 2011;95(4):629-35.

251. Lewis SJ, Foltynie T, Blackwell AD, Robbins TW, Owen AM, Barker RA. Heterogeneity of Parkinson's disease in the early clinical stages using a data driven approach. J Neurol Neurosurg Psychiatry. 2005;76(3):343-8.

252. Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ. A clinico-pathological study of subtypes in Parkinson's disease. Brain. 2009;132(Pt 11):2947-57.

253. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112(4):389-404.

254. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58(12):1791-800.

255. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology. 1991;41(4):479-86.

256. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012;123(1):1-11.

257. Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. Brain. 2010;133(Pt 6):1755-62.

258. Kempster PA, Williams DR, Selikhova M, Holton J, Revesz T, Lees AJ. Patterns of levodopa response in Parkinson's disease: a clinico-pathological study. Brain. 2007;130(Pt 8):2123-8.

259. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain. 2011;134(Pt 5):1493-505.

260. Lashley T, Holton JL, Gray E, Kirkham K, O'Sullivan SS, Hilbig A, et al. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol. 2008;115(4):417-25.

261. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. Acta Neuropathol. 2008;115(4):409-15.

262. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol. 2012;72(4):587-98.

Appendix

Appendix 1. Queen Square Brain Bank donation program

The QSBB has a unique brain tissue collection comprising more than 2000 brains. Although it was initially established as a brain bank for PD and atypical parkinsonian conditions and has become one of the leading centres in clinico-pathological research in these conditions, the collection has developed to include brain donations from patients with other neurological conditions including dementia and dystonia.

Detailed clinical information is stored according to GDPR regulations and tissue is stored under a Human Tissue Authority licence (Licence number 12198). Protocols for brain banking and research have been approved by the local ethics committee. The QSBB donation program meet all the necessary quality standards and is part of the Medical Research Council UK Brain Bank Network and BrainNet Europe.

Clinical information

Once potential donors are registered to the donation program they provide written consent for the research use of clinical data and donated tissue. They are asked to provide detailed clinical information about his general health and neurological conditions using self-assessment questionnaires by post and more recently online. After the death of a donor, access to the medical records is provided to the QSBB including GP files, drug records and all hospitals specialists' correspondence including neurologists, geriatricians and other medical specialists or health professionals involved.

Brain removal and tissue processing

After the death of a donor, the body is refrigerated within 4 hours at 4˚C until the body is transferred to the mortuary of the nearest hospital. Brain removal is performed by trained staff of the pathology department in the hospital premises and only donated tissue is transferred to the QSBB. Brain tissue is subsequently processed by the QSBB neuropathology technicians as soon as possible and always within 48 hours from the time of death.

The brain is weighed and hemi-dissected into its two hemispheres. One hemisphere is coronally dissected and flash-frozen to -80˚C for tissue

preservation. The other hemisphere is fixed in 10% buffered formalin for three weeks and representative tissue blocks are sampled following QSBB protocols covering structures included in the diagnosis and staging systems of common neurodegenerative conditions including PD (see table below). Histological sections of 8 µm thickness are cut and deparaffinised, followed by pre-treatment with formic acid and pressure cooking in citrate buffer at pH 6.0. Routine histological staining using H&E and silver Gallyas techniques are followed by immunohistochemistry against α-synuclein, Aβ peptide, hyperphosphorilated tau protein, p62 and TAR DNA binding-protein 43 (TDP43) using a standard avidin-biotin method.

No.	**BLOCK**	**Level at which block was taken**	**Stains requested**
1	Anterior frontal F1-F2	Just rostral to temporal tip	H&E, α-Syn
1A	Anterior frontal F1-F2	Just rostral to temporal tip	
1B	Whole frontal lobe	Anterior to genu of corpus callosum	
1C	Whole frontal lobe	Anterior to genu of corpus callosum	
2	Posterior frontal	Pulvinar	H&E
2A	Posterior frontal	Pulvinar	
3	Temporal T1-T3	Mammillary body	
4	Temporal T1-T3	Mammillary body	H&E, AT8
4A	Whole temporal lobe	Posterior to mammillary body	
4B	Whole temporal lobe	Posterior to 3A	
5	Tip of Temporal		
6	Parietal	Splenium	H&E
6A	Parietal	Mirror of 6	
7	Occipital		α-Syn
7A	Occipital	Mirror of 7	
8	Anterior cingulate	Nucleus accumbens	
8A	Anterior cingulate	Posterior to 8	
9	Hippocampus	Mammillary Body	
9A	Hippocampus	Posterior to 9	
9B	Hippocampus	Posterior to 9A if available	
10	Hippocampus	Lateral geniculate body	H&E, Aβ, α-Syn, AT8, TDP-43, p62
10A	Hippocampus	Lateral geniculate body	
10B	Hippocampus	Posterior to 10A if available	
11	Amygdala		H&E, α-Syn, TDP-43, AT8
11A	Amygdala		
12	Basal ganglia, ant.	Nucleus accumbens	H&E

12A	Basal ganglia, ant.	Posterior to 12	H&E
13	Basal ganglia, mid.	Anterior commissure	H&E
13A	Basal ganglia, mid.	Anterior commissure	H&E,
13B	Basal ganglia, mid	Posterior to 13A	H&E
14	Basal ganglia, post.	Mammillary body	H&E
14A	Basal ganglia, post	Posterior to 14	H&E
15	Thalamus	Subthalamic nucl., massa intermedia	H&E
15A	Thalamus	Subthalamic nucl., massa intermedia	H&E
16	Upper Midbrain	Red nucleus	H&E, AT8, α-Syn
17	Lower Midbrain	Decussation of SCP	
18	Upper pons	Locus coeruleus	H&E
18A	Upper pons	Caudal to 18	
19	Lower pons		
19A	Lower pons	Caudal to 19	
20	Upper Medulla		
21	Lower Medulla	With 10^{th} and 12^{th} nuclei	H&E, α-Syn
21A	Lower medulla	Caudal to 21	
22	Vermis		
23	Cerebellum		H&E
23A	Cerebellum	Include dentate	
24	Cerebellum		
25	Midbrain	RINMLF	
26	Frontoorbital/medial	At the level of the temporal tip	
27	Frontoorbital//lateral	At the level of the temporal tip	
28	F3A – Broca (anterior)	Br 44; ant. lat. fissure	
29	F3B – Broca (posterior	Br 44; ant. lat. fissure	
30	T1 – Wernicke	Br22; posterior T1 with planum temporale	
31	Posterior cingulate	Level - splenium	
32	Precuneus	Level of occipital horn (post)	
33	OB	Olfactory Bulb	
34	Cervical cord		
35	Thoracic cord		
36	Lumbar cord		
37	Sacral cord		
38	Pineal gland		H&E
39	Dura mater		H&E

QSBB protocol for brain tissue block sampling (2017 version)