Study and characterisation of the prodromal motor phase of Parkinson's Disease

Ph.D. Thesis

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Dedicated to Paco Simonet, my loving grandfather who laid the first stone on this path.

Dedicada a nes meu abuelo Paco, qui va posar sa primera pedra a n'aquest camí que acaba.

Statement of originality

I, Cristina Simonet Hernandez, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

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Details of collaborative work

Each collaboration is indicated below by chapter:

- Chapter 2 – I carried out the study presented in Chapter 2 in conjunction with other members of the research team in the Preventive Neurology Unit at Wolfson Institute of Population Health. My contribution was mainly focused on interpreting the results and writing up the manuscript for publication. Of note, I did not work on the statistical analysis. However, I worked closely with the statistical team, Jonathan Bestwick and Mark Jitlal.
- Chapter 4 – I designed and developed the Distal Finger Tapping (DFT) test in conjunction with a software developer (Alex Howard) and two BSc students (Noreen Akram and Li Haoxuan) who I supervised between January and February 2019. I selected the most appropriate mechanism of the test and designed the interface. The BSc students helped with participant recruitment and testing, as well as helped with the troubleshooting of the test and interface design.
- Chapter 4 – I carried out the assessment of the Slow-Motion Analysis of Repetitive Tapping (SMART) test in collaboration with two separate research groups who helped me with the recruitment and testing of participants. Patients with Parkinson's disease (PD) came from the Quantitative Magnetic Resonance Imaging (MRI) for Anatomical Phenotyping in PD (QMAP-PD) study based at the Institute of Neurology, University College London. Dr Christian Lambert, principal investigator in QMAP-PD study, was the person in charge of recruiting and testing patients with early PD. Individuals with anosmia were recruited from the PREDICT-PD study, after referral from specialist ENT clinics. Dr Richard Rees, a clinical researcher involved in the PREDICT-PD study, was the person in charge of recruiting and testing patients with anosmia. The control group were recruited and tested by two different studies. I recruited and tested part of the control group from the PREDICT-PD study and Dr Christian Lambert recruited and tested the rest of controls from the QMAP-PD study. Dr Miquel A Galmés was commissioned to develop the software able to automatically detect the hand and process video images to analyse the finger tapping task.
- Chapter 5 – Michaela Francis, a medical student, reviewed and counted the number of blinks in the rapid blinking test which is part of the motor battery. Emma Bache, an expert graphologist, reviewed participants' handwriting scripts.

Publications relating to work contained within this thesis

1. Simonet C, Bestwick J, Jitlal M, et al. Assessment of Risk Factors and Early Presentations of Parkinson Disease in Primary Care in a Diverse UK Population. JAMA Neurol. 2022 Apr 1;79(4):359-369. doi: 10.1001/jamaneurol.2022.0003.
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1. Garrido A, Santamaría E, Fernández-Irigoyen J, Soto M, Simonet C, Fernández M, Obiang D, Tolosa E, Martí MJ, Padmanabhan S, Malagelada C, Ezquerra M, Fernández-Santiago R. Differential Phospho-Signatures in Blood Cells Identify *LRRK2* G2019S Carriers in Parkinson's Disease. Mov Disord. 2022 May;37(5):1004-1015. doi: 10.1002/mds.28927.
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Abstract

There is sufficient evidence that a neurodegenerative process in Parkinson's Disease (PD) starts many years before the clinical diagnosis. The progression of PD is generally slow and, because it is diagnosed based on established motor features, it is probable that subtle motor manifestations appear in the pre-diagnostic phase of PD. Isolated rapid eye movement (REM) sleep behaviour disorder (iRBD) is a condition known to be part of the prodromal phase of PD. The PREDICT-PD study is a population-based cohort which aims to identify individuals at risk of PD based on the presence and absence of risk factors. The first project of this thesis investigated the association between first presentation of motor symptoms (tremor, rigidity and balance difficulties) and subsequent PD in a large primary care dataset in East London, including almost 3 decades of clinical information from over a million individuals. People who went on to develop PD reported motor symptoms up to 10 years before PD diagnosis. Tremor had the highest association with future PD followed by balance difficulties and rigidity. The second project aimed to identify the range of motor features in the elderly population participating in the PREDICT-PD cohort study and document their rate of progression over time. People classified as having a higher risk of future PD (using the PREDICT-PD algorithm) were more likely to have early parkinsonian signs than the lower risk group. Six years later, they also showed a bigger motor decline compared with people in the lower risk group. The third project was focused on developing two new objective motor tools, the Distal Finger Tapping test and the Slow-Motion Analysis of Repetitive Tapping. Both tests were able to detect abnormal patterns of movement amongst people with early PD. Finally, a motor battery was created and implemented in a group of patients with iRBD. A higher proportion of patients with iRBD had early parkinsonian signs compared with controls. The motor battery was able to detect early patterns of motor dysfunction not captured by standardised clinical scales. The work presented in this thesis demonstrates that motor features start in the pre-diagnostic phase of PD and describes new motor signatures in the prodromal phase of PD.

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List of abbreviations

AD – Alzheimer's Disease

AT20 – Akinesia Time from the DFT test in Chapter 4

AT-DFT– Akinesia Time from the DFT test in Chapter 5

AT30 – Akinesia Time from the BRAIN test in Chapter 4

AT-BRAIN – Akinesia Time from the BRAIN test Chapter 5

AUC – Area Under Curve

BRAIN test – BRadykinesia Akinesia INcoordination test

BSIT – Brief Smell Identification Test

CCG – Clinical Commissioning Groups

CEG – Clinical Effectiveness Group

CI – Confidence Interval

CNN – Convolutional Neural Network

DAT – Dopamine Transporter

DFT – Distal Finger Tapping

DLB – Dementia with Lewy Bodies

EMIS – Egton Medical Information Systems

FT – Finger Tapping

GBA – Acid β-Glucosidase (also known as Glucocerebrosidase)

GP – General Practitioner

HADS – Hospital Anxiety Depression Scale

HELIAD – Hellenic Longitudinal Investigation of Aging and Diet cohort

HHP/HAAS – Honolulu Heart Program / Honolulu-Asia Aging Study

HR – Higher Risk group

ICC – Intraclass Correlation Coefficient

IMD – Index of Multiple Deprivation

IQR – Interquartile Range

iRBD – Isolated Rapid eye movement sleep Behaviour Disorder

IS20 – Incoordination Score from the DFT test in Chapter 4

IS-DFT– Incoordination Score from the DFT test in Chapter 5

IS30 – Incoordination Score from the BRAIN test in Chapter 4

IS-BRAIN – Incoordination Score from the BRAIN test Chapter 5

KS20 – Kinesia Score from the DFT test in Chapter 4

KS-DFT– Kinesia Score from the DFT test in Chapter 5

KS30 – Kinesia Score from the BRAIN test in Chapter 4

KS-BRAIN – Kinesia Score from the BRAIN test Chapter 5

LR – Lower Risk group

LRRK2 – Leucine Rich Repeat Kinase 2

MDS – Movement Disorders Society

MoCA – Montreal Cognitive Assessment

MPS – Mild Parkinsonian Signs

MRI – Magnetic Resonance Imaging

MSA – Multiple System Atrophy

NHS – National Health System

NPV – Negative Predictive Value

NSAIDs – Non-Steroidal Anti-Inflammatory Drugs

OR – Odds Ratio

PARS – Parkinson's At-Risk Study

PD – Parkinson's Disease

PRIPS – Prospective validation of Risk factors for the development of Parkinson Syndromes

PPV – Positive Predictive Value

PST – Pocket Smell Test

QMAP – Quantitative MRI for Anatomical Phenotyping

RBDSQ – REM sleep Behaviour Disorder Screening Questionnaire

REM – Rapid Eye Movement

ROC – Receiver Operator Characteristic

RR – Relative Risk

RSWA– REM Sleep Without Atonia

SCOPA-AUT – SCales for Outcomes in PArkinson's disease - Autonomic Dysfunction

SD – Standard Deviation

SMART test – Slow-Motion Analysis of Repetitive Tapping test

SN – Substantia Nigra

SP – Subthreshold Parkinsonism

SPECT – Photon Emission Computed Tomography

T2D – Type 2 Diabetes

THIN – The UK Health Improvement Network

TUG – Timed Up-and-Go test

TREND – Tübinger evaluation of Risk factors for the Early detection of NeuroDegeneration

UPDRS – Unified Parkinson's Disease Rating Scale

UPSIT – University of Pennsylvania Smell Identification Test

V-PSG – video-polysomnography

Glossary

Akinesia time: average dwell time (msec) that keys are depressed reflecting akinesia

Higher risk group: PREDICT-PD risk score above 15th centile

Incoordination score: variance (msec2) of travelling time between keystrokes, reflecting rhythm

Intermediate risk group: PREDICT-PD risk score between 15th and 85th centile

Kinesia Score: a measure of rate of movement reflecting speed, the number of keystrokes in 20 (DFT test) or 30 (BRAIN test) seconds

Lower risk: PREDICT-PD risk score below 85th centile

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

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

MDS-UPDRS-III: motor examination

Motor decline: MDRS-UPDRS change [motor score at follow-up] − [motor score at baseline] = ≥ 5-point change

Subthreshold parkinsonism (MDS Task Force criteria): MDS-UPDRS-III (excluding postural and kinetic tremor) >6 points

Subthreshold parkinsonism (Louis et al criteria): binary definition (i.e. present or absent) when any of the following conditions was met: 1) two or more UPDRS ratings = 1, or 2) one UPDRS rating ≥2, or 3) UPDRS rest tremor rating ≥1

Chapter 1

Introduction

1.1 Thesis framework

The phase before Parkinson's Disease (PD) has often been referred to as the 'pre-motor' phase. However, it is difficult to say whether there is a definite 'pre-motor' phase, when objective motor dysfunction has been observed in many prodromal cohorts.[1–4] Although several studies have objectively documented motor markers of neurodegeneration in PD, the truth is that motor features in the pre-diagnostic phase have received surprisingly little attention compared to non-motor features.[5] There is still controversy about when they start, how reliably they can be detected and in which manner they progress.

1.2 The stages of Parkinson's Disease

Important discoveries have been made since the first description of PD by James Parkinson in 1817. These include biochemical and neuropathological findings, and more recently, risk factors, disease biomarkers and new therapeutic approaches.[6,7]

Despite these advances, PD diagnosis still relies on operational clinical diagnostic criteria first proposed in 1992 and based on clinico-pathological correlation.[8] Diagnosis requires the presence of bradykinesia which is defined as a progressive reduction in the speed and amplitude of repetitive self-willed movements, plus at least one further parkinsonian sign from rigidity and/or rest tremor.[9,10]

The International Parkinson and Movement Disorder Society (MDS) Task Force on the definition of PD was created to address the challenges involving the definition of PD. It suggested dividing early PD into three stages: **preclinical,** which is defined by initial neurodegeneration with no clinical evidence; **prodromal,** which occurs as neurodegeneration progresses with emerging motor dysfunction and non-motor symptoms; and the **clinical** stage, where there is strong evidence of classical motor signs fulfilling the clinical diagnostic criteria.[11] This thesis will be focused on the prodromal phase of PD.

There is evidence to support the multi-system nature of PD in which pathology starts many years before the diagnosis.[12–14] Apart from the dopaminergic system, other systems such as the cholinergic, noradrenergic, serotonergic and olfactory system are also affected and are associated with pre-diagnostic manifestations.

Although many prodromal features have been described, their exact onset and progression remain unclear. Three aspects have been and will be crucial to address these issues:

- **firstly**, the pathological definition of PD, including Lewy body pathology,[15] the Braak stages of degeneration in preclinical and clinical PD,[14,16] and alpha-synuclein misfolding with the cell-to-cell (prion-like) spreading theory.[17] This helped to understand the onset and progression of symptoms from early stages of the disease.
- **secondly**, the expanding knowledge about imaging and fluid biomarkers that correlate with early degeneration even in the preclinical stages.[12]
- **thirdly**, the increasing number of population-based longitudinal studies with the common aim to create algorithms to predict phenoconversion by combining different risk factors and prodromal markers of PD.[18] Following large cohorts of people is the final stage to expand our knowledge about the prediagnostic stage of PD.

A large number of longitudinal studies support the link between symptoms such as hyposmia, isolated Rapid Eye Movement (REM)-sleep Behaviour Disorder (iRBD), constipation, depression, with the subsequent emergence of PD.[19] These clinical features do not necessarily occur in synchrony and little is known about their natural course,[18] or whether they progress at all. Somewhat surprisingly, much less attention has been paid to motor dysfunction that precedes overt bradykinesia and a diagnosis of PD.[5] The progression of PD is generally slow and, because it is diagnosed based on established and typical motor features, it is probable that subtle motor manifestations appear years before the diagnosis.[10] The motor domain is intrinsically affected in PD and progression of motor disability is the norm. Hence, early motor signs could provide crucial information about progression in the prodromal stages of PD, as well as being clear indicators for response markers in clinical trials (Figure 1.1).

Despite PD being a relatively slowly progressing disorder it is often diagnosed at a late stage. When individuals fulfil the diagnostic criteria, at least 40% of nigral dopaminergic neurons have already been lost with cardinal symptoms being more noticeable when there is 70% to 80% of striatal dopamine depletion.[13] This reveals a significant shortcoming of the current diagnostic criteria and might partially explain why trials on disease modifying therapies have been largely unsuccessful. Hence, earlier diagnosis is important in identifying individuals in a prodromal stage of PD as the ideal candidates for clinical trials of neuroprotective therapies.[20] Great efforts have gone into earlier detection of PD, including understanding the risk factors, disease biomarkers and prodromal features which involve motor, cognition, mood and autonomic domains.[21]

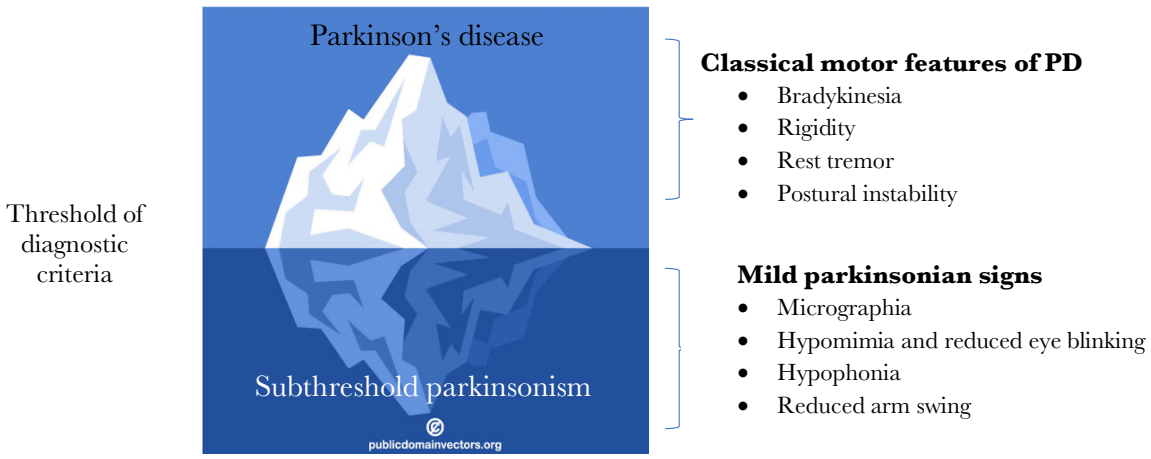

Figure 1.1 The iceberg of the motor domain during the neurodegeneration process. Super-threshold: easily noticeable features on the upper half of the iceberg. Sub-threshold: mild parkinsonian signs hidden on the lower half of the iceberg

1.3 Towards early and timely diagnosis

Early treatment of motor and non-motor symptoms may improve quality of life even if patients do not yet fulfil the clinical diagnostic criteria of PD.[22] To this end, more focus on timely diagnosis rather than simply earlier diagnosis may be useful.[23] On the other hand, several factors may influence a delay in diagnosis even when motor signs are evident. These include available and equitable access to healthcare and health professionals, the clinical experience and expertise of those health professionals, as well as patient comorbidity that may mask or confound the clinical impression. In addition to these challenges, there are patient-related factors that may lead to a delay in recognition of the early motor features of PD. Many patients with PD seem to have a visuo-perceptual agnosia to their motor handicap, or attribute changes to tiredness or ageing.[24] This can result in substantial delays in their initial presentation.[25,26] In general settings raising awareness will help clinicians to consider the possibility of a unifying explanation, such as PD, for the symptoms patients notice but have not received a conclusive diagnosis for.

The notion of earlier diagnosis of PD in the context of a disease-modifying or neuroprotective intervention is easy to promote. However, given the current absence of such interventions, the more relevant issue is what makes a timely diagnosis for patients. Giving a diagnosis before any decline in quality of life would arguably not be in most patients' interest and could instead be detrimental.[23] On the other hand, for individuals who had prominent non-motor prodromes, a timely diagnosis may avoid mismanagement and clinicians should be familiar with these features and their treatment.[27]

Apart from improving quality of life, an early diagnosis might also have long-term prognostic implications. PD is a heterogenous disease. It can be classified in separate clinical subtypes each of them with a different prognosis. For example, the diffuse-malignant subtype, where 'diffuse'

refers to wide range of non-motor symptoms and rapid progression, is known to have a poor prognosis.[28] Clinical heterogeneity in PD is noticeable from early stages of the disease.[29] Similar to established PD, prodromal PD can be classified into different subtypes based on the coexistence of motor and non-motor prodromes. Future research should be towards using the analogy between prodromal and established PD to predict the progression of each prodromal subtype. Thus, the recognition of an early clinical subtype could help to establish a prognosis from the beginning, which will allow patients and clinicians take the appropriate decision for their future.[23]

1.4 Evidence from enriched populations

Many well-recognised prodromes of PD, such as constipation and depression, are nonspecific and frequent in the general population. Whether and how quickly people who have these symptoms will go on to develop PD remains unclear. In contrast, there are other prodromal markers that are more specific for PD. For example, iRBD is both rare and highly specific for alpha-synuclein related diseases.

The term 'enriched population' refers to cohorts with a well-stratified and delineated prodromal marker which denote an *a priori* increased risk of PD. Patients with iRBD, idiopathic hyposmia, and carriers of Leucine Rich Repeat Kinase 2 (*LRRK2*) and Glucocerebrosidase (*GBA*) mutations are some examples of these enriched groups. In contrast to population-based cohorts, enriched groups are expected to have a higher PD conversion rate. Thus, the recognition of motor impairment in individuals belonging to these groups would be seen as a harbinger to PD diagnosis, perhaps far more than motor impairment in an unselected elderly population.

On the other hand, enriched cohorts might not necessarily reflect the global picture of prodromal PD described in the general population. As an example, iRBD only occurs in approximately a third (25% to 58%) of patients with PD.[30] They might have a different pathological substrate which can be manifested with a different clinical phenotype from idiopathic PD known to affect the general population. For that reason, longitudinal studies following enriched cohorts may lack generalisability.

a. Isolated REM-sleep Behaviour Disorder

RBD was first described by Schenck and collaborators in 1986.[31] It is a REM parasomnia characterised by the presence of vivid dreams and 'acting-out' behaviours that occur when there is a loss of physiological muscle atonia during REM-sleep. It is caused by the disruption of the descending neuronal glutamatergic projections that normally induce muscle atonia during the REM phase of sleep. The diagnosis of RBD needs to be confirmed by overnight video-polysomnography (v-PSG), the availability of which is limited. As such the prevalence of RBD in the general population might be underestimated, with most of the studies reporting

1% over age of 60.[32,33,34] A recent systematic review and meta-analysis found the prevalence depends on the diagnostic level of certainty. When based on clinical suspicion, the prevalence was much higher than when it was confirmed by v-PSG (5.7% and 0.7% respectively).[35]

RBD can be categorised into primary (also known as idiopathic) and secondary. In contrast with the latter term, the former occurs in the absence of external factors that can trigger the symptoms such as antidepressants, beta-adrenergic blockers or underlying neurological conditions affecting pontine subcoeruleus/magnocellularis nuclei in the brainstem and their projections. This is the case of a tumour, vascular lesion, autoimmune diseases or demyelination. Throughout this thesis, the term iRBD will be used to refer to the prodromal marker of PD.[30]

Nowadays, isolated RBD (hereafter, iRBD) is the preferred term, instead of idiopathic RBD. Although the underlying cause of iRBD is still unknown, there exists enough evidence suggesting that alpha-synuclein deposits are the pathological substrate of iRBD. Thus, it cannot be considered an exclusive (idiopathic) parasomnia but rather part of the clinical spectrum of alpha-synuclein related pathologies.[36] At least ten prospective longitudinal studies of well-phenotyped cohorts have demonstrated a strong link between iRBD and alpha-synuclein related conditions, with more than 80% of patients with iRBD developing an alpha-synuclein related condition later in life. PD seems to be the most likely diagnosis, followed by Dementia with Lewy Bodies (DLB) and, in rare cases, Multiple System Atrophy (MSA). Annual conversion rates from iRBD an alpha-synuclein condition range between 6.3% [37] and 8% per year.[38]

Motor dysfunction has been found to be the strongest predictive marker of conversion of future parkinsonism or dementia in patients with iRBD. The largest longitudinal study to date included 1280 patients from 24 centres, who were followed up on average 3.6 years and a maximum of 19 years. Out of 21 potential parameters of phenoconversion, motor impairment was associated with the highest hazard ratio compared to other clinical and neuroimaging markers. Motor dysfunction was characterised by using the Purdue Pegboard, alternate-tapping test and Timed Up-and-Go (TUG) test, along with the motor scale of MDS-Unified Parkinson's Disease Rating Scale (UPDRS) (thereafter MDS-UPDRS-III).[37] A smaller sample of iRBD was followed by one of the centres that took part in a large multicentre study.[39] In this single centre longitudinal study, 78 people with v-PSG-confirmed iRBD were followed for 5 years. Twenty people developed parkinsonism or dementia over this period. They used the same motor battery that was used in the multicentre study. Motor dysfunction was detected 6 to 9 years before PD diagnosis, with voice changes and hypomimia being reported as the earliest symptoms (9.8 years), followed by impairment on the Purdue Pegboard and alternating tapping tests (8.6 and 8.2 years respectively).[39]

In 2019, Fereshtehnejad and colleagues published the first longitudinal study to track conversion trajectories to PD using an unbiased approach based on linear mixed effect models.[I] To do that, they followed-up 154 patients with v-PSG confirmed iRBD over an average period of 5 years (range: 2 to 12 years).[3] Apart from non-motor features, they gathered information on motor signs and symptoms using the same set of motor assessments used in the study mentioned above. They accounted for the possible role of age and sex by testing in parallel a group of 102 age- and sex-matched controls. Motor symptoms (e.g. difficulties in handwriting, turning in bed, walking, speech, and salivation) were reported between 7 and 11 years prior to PD diagnosis. Similar to what Postuma and colleagues found in 2012,[39] slow alternate tapping test was the earliest motor objective motor marker to appear (8 to 10 years before PD), followed by the cardinal motor features in PD assessed by MDS-UPDRS-III: bradykinesia, rigidity and tremor, which appeared 6, 3 and 2 years prior to PD diagnosis respectively. Receiver Operator Characteristic (ROC) analysis showed that having a score of at least 4 in the MDS-UPDRS-III and 2 in the MDS-UPDRS-II 2 years before conversion had a discriminatory power of 60% sensitivity for more than 90% specificity. Mixed effects models showed that whereas most prodromal manifestations progressed in a linear fashion, motor markers had an exponential progression 2 years before PD diagnosis.

Subtle gait abnormalities and balance problems have been described in observational studies of patients with iRBD. For example, gait patterns were analysed using a sensor carpet (but no body sensors so arm swing could not been assessed) in 24 polysomnography-confirmed iRBD patients and 14 controls.[40] During dual tasking (counting backwards, naming as many animals as possible, subtracting 7 from 100), patients with iRBD increased the variability in their step width and demonstrated more asymmetry in step length when asked to walk quickly. Alibiglou and collaborators studied gait initiation biomechanics and correlated them with the level of REM sleep without atonia (RSWA) recorded by PSG in four groups of 10 patients each: (1) v-PSG-confirmed iRBD, (2) PD with freezing of gait, (3) PD without freezing of gait and (4) healthy controls. They found that the iRBD group had similar impairments in self-initiating gait to PD patients with freezing of gait. Moreover, RSWA was strongly inversely correlated with the capacity to couple posture and movement during gait initiation.[41] Although they need to be confirmed by longitudinal studies, these findings might suggest that the degree of iRBD severity, based on the level of RWSA recorded by PSG, could predict future freezing of gait.

In contrast, two studies found the MDS-UPDRS-III scores did not differ between iRBD and control groups. Although this could be explained by differences in disease duration,[42,43] it could support the idea that the MDS-UPDRS-III may not be an appropriate tool to test people without established PD. Thus, truly quantitative motor tools are needed to define early motor dysfunction in PD.

[I] In each model: Fixed effect= the effect of time and individual trend. Random effect= intercept and slope. Estimate= the extra annual change in each prodromal outcome that occurred in the group of interest versus the reference group. Note: effects were adjusted for age at baseline.

b. Idiopathic hyposmia

Impaired olfaction is defined by a reduced ability to identify and recognise smells. It ranges from hyposmia to complete smell loss or anosmia. Whereas anosmia is almost always reported by patients, this is not necessarily the case with hyposmia. However, it can be captured by objective smell tests. Although reduced sense of smell is a commonly reported symptoms in the general population (12.4%), the prevalence is higher (22.0%) when it is measured objectively. Almost three quarters (74.2%) of otherwise healthy people with objective impaired olfaction do not have symptoms.[44] The University of Pennsylvania Smell Identification Test (UPSIT) is the most extensively used smell test to screen people with impaired olfaction. It consists of a 40-item 'scratch-and-sniff' smell test.[45] A cut-off ≤15th centile, which corresponds to an UPSIT score of ≤ 27, is normally used to indicate olfactory loss. The 12-item Brief Smell Identification Test (BSIT), the 4-item Pocket Smell Test (PST) and a hypothetical 16-item UPSIT are shorter versions of UPSIT using the same odours. The BSIT and the PST have been validated in the PREDICT-PD cohort[46] and the 16-item UPSIT in the Tracking Parkinson's study and PREDICT-PD study.[47] The three of them showed high discriminatory accuracy compared with UPSIT.

Unlike secondary hyposmia, which occurs in healthy people as a result of an external factor (e.g. infection, trauma), primary (idiopathic) hyposmia is not associated with any external factor. However, idiopathic hyposmia is a frequent symptom in PD and has been described as part of the non-motor clinical picture in patients with established PD. A multicentre study found that up to 90% of patients with PD had an abnormal odour identification and discrimination in smell tests,[48] which is greater to the prevalence of tremor in PD.[49] Although idiopathic hyposmia is not a core feature of PD in the way that tremor is, it is a supportive feature in the updated PD diagnostic criteria.[11]

Idiopathic hyposmia has also been reported as part of the non-motor prodrome in people at risk of future PD.[50] There is some evidence that it might predict an imminent (less than 4 years) conversion to PD.[51] The Health, Aging and Body Composition study found that having a poor sense of smell (the lowest tertile of BSIT) was associated with higher risk of PD with a hazard ratio of 4.8 for PD over 10 years of follow up.[52] Another large population-based study, the PRIPS cohort (Prospective evaluation of Risk factors for Idiopathic Parkinson's Syndrome), reported a relative risk of 6.5 of incident PD in participants with reduced sense of smell (Sniffin' sticks correct identification of less than 75%) after 3 years follow-up.[53] The PARS study (Parkinson Associated Risk Study), in which sense of smell was assessed using a self-administered UPSIT, found a similar proportion of subtle motor symptoms (defined by two or more symptoms on the Symptom Rating Scale) and average UPDRS-III motor scores in the normosmic and hyposmic groups (UPSIT score of ≤ 27).[54] Dopamine transporter (DAT) Single Photon Emission Computed Tomography (SPECT) imaging was used as the main outcome in this study, but subtle motor signs at baseline were not associated with presynaptic dopaminergic degeneration. On the other hand, being male, having constipation and hyposmia, were

associated with a reduced dopaminergic uptake.[1] However, most of the studies rely on an empirical definition of hyposmia based on cut-off points on the odour identification tests mentioned above, without an alternative external explanation for impaired olfaction being ruled out.

Based on these findings, there is a clear need for longitudinal studies of people with confirmed idiopathic hyposmia to support the findings described above. These findings highlight the potential value in identifying cases with idiopathic hyposmia to study their motor prodromes and the coexistence of other manifestations that might help to define prodromal clinical phenotypes.

c. Gene mutation/variant carriers

GBA and *LRRK2* mutation carriers have an age-specific risk of PD diagnosis compared with non-carriers (both estimated to be about 30% at the age of 80 years).[55–57]

The prevalence of the *LRRK2* G2019S mutation is approximately 1% and 4% of sporadic and familial forms of PD respectively.[58] *LRRK2*-related parkinsonism and idiopathic PD have similar clinical features and response to dopaminergic therapy.[50] However, subtle differences may exist with lower overall motor UPDRS scores, a higher proportion with postural instability and action tremor observed among *LRRK2* mutation carriers with PD.[59,60]

Motor signs assessed using the UPDRS appear to be greater in non-manifesting carriers of *LRRK2* than in non-carriers, and in first-degree relatives of *LRRK2* PD cases, independent of their mutation status.[61,62] Gait analysis in non-manifesting carriers has received special attention as a comparative tool to differentiate them from non-carriers. Using sensors to detect gait patterns, carriers show greater variability in gait under challenging conditions than non-carriers.[63] Another study showed higher intra-individual walking variability in non-manifesting carriers and *LRRK2*-related PD compared with controls. Unlike non-manifesting carriers, PD patients (idiopathic and *LRRK2*-related) demonstrated greater arm swing asymmetry.[64]

In a longitudinal study, whereas motor UPDRS score increased over a 4-year period in carriers of *LRRK2* mutations, those who 'converted' to PD did not have significantly higher UPDRS motor scores than 'non-converters' at baseline.[65] Another longitudinal study followed a group of carriers of *LRRK2* mutations and healthy controls for 5 years.[66] Most individuals who fulfilled the MDS Research Criteria for Prodromal PD at baseline were carriers of *LRRK2* mutations and, during follow-up, the ten patients who were diagnosed with PD were all carriers.[67]

Disease-associated *GBA* heterozygous variants are common in PD and are found in 5% to 10% of patients, with higher proportions in the Ashkenazi Jewish population.[68] Same *GBA* variants in the homozygous form are recognised for causing Gaucher disease, the most common lysosomal storage disorder.[69] Both homozygous and heterogynous *GBA* variants seem to be a risk factor for PD.[68] In a longitudinal study of *GBA* homozygous and heterozygous carriers, the UPDRS motor score showed greater progression during 6 years of follow up in carriers of *GBA* mutation compared to controls.[70] Similarly, two other studies which compared controls with

Gaucher disease patients and mutation carriers showed that mutation carriers had a higher score in the motor UPDRS than the other groups.[71,72]

1.5 Evidence from general population studies

1.5.1 Healthcare records studies

The pre-diagnostic medical histories of people who go on to develop PD offer a valuable source of information to study the prodromal phase of PD. Primary care is usually the 'front door' for the initial presentations of PD, as patients generally report their symptoms to their General Practitioner (GP) rather than to a neurologist. Thus, primary care plays potentially an essential role in the definition of the prodromal phase of PD.[73]

Studies using healthcare records are retrospective, therefore they are more susceptible to missing data.[74] The diagnosis of PD might have a reduced validity if it is not made by a specialist. Cross-validating PD diagnosis in a subset of patients and applying multiple criteria are solutions to reducing false positive PD diagnosis in health records data. In terms of prodromal marker assessment, most of the studies using healthcare records are based on self-report and clinical interviews, increasing the risk of recall bias. Some symptoms might be underreported due to a deliberate false statement – for example in the case of symptoms that might cause embarrassment like erectile dysfunction and depression – or a lack of awareness such as slowness and loss of smell.

Despite these limitations, the use of routinely collected medical record data offers the advantage of studying large numbers of individuals and obtaining a realistic picture of which manifestations prompt patients to go to their GP years before PD diagnosis. Gonera and colleagues were one of the first groups to use this approach in the context of PD.[75] They reviewed the medical records of Dutch patients in general practice over a decade prior to PD diagnosis. They found that prodromal symptoms began between 4 and 6 years prior to diagnosis. Fibromyalgia, shoulder pain and hypertension were significantly more common in people who went on to develop PD. Another study carried out in the Netherlands by Plouvier and collaborators used data from the Continuous Morbidity Registration database linked with the Dutch healthcare system comprising primary care data of approximately 12000 patients.[76] Functional somatic symptoms, constipation and hyperhidrosis were found to be the most common presentations 2 years prior to diagnosis. Both studies compared the PD group with an age and sex matched control group. Surprisingly, no motor symptoms were found amongst GP records in either of the two studies. In contrast with motor prodromes, non-motor prodromes have been largely studied in primary care. For example, depression,[77,78] constipation,[79–81] erectile dysfunction[82] and sleep disturbances[83,84] were found to be more prevalent in the healthcare records of people with subsequent PD than controls.

The lack of studies exploring the early motor manifestations in primary care changed with a comprehensive population-based study carried out by Schrag and colleagues. They used The UK Health Improvement Network (THIN) database, which had 11 million people registered, and from these they selected 8166 PD patients and 46455 healthy controls.[85] Medical codes from clinical consultations were extracted to identify recognised non-motor prodromes of PD. In addition, they included pre-diagnostic motor presentations, including tremor, rigidity, shoulder pain or stiffness and balance impairment. Tremor was the most common manifestation amongst all pre-diagnostic markers (motor and non-motor), being reported for the first time up to 10 years prior to diagnosis. Although rigidity and balance impairment were more common in people who went on to develop PD, they occurred in the period closer to diagnosis (less than 5 years prior to diagnosis).

1.5.2 General population cohorts

Population-based studies offer a realistic and broad picture of prodromal PD and allow to generalise the results to the whole population. However, due to the low incidence of PD in the general population, large sample sizes and long studies are required.

Table 1.1 summarises the most noteworthy epidemiological studies supporting the existence of motor prodromes. The Rotterdam study is one of the longest running longitudinal studies of PD with 15.8 years of follow-up.[86] Motor assessments came from standardised clinical interviews and assessments carried out by trained nurses. Subjective complaints were extracted from clinical interviews, including stiffness, slowness, tremor, loss of balance and number of falls in the previous year. Bradykinesia and tremor tended to be the earliest motor markers, occurring 7 years and 6 years before the diagnosis respectively. In contrast, postural features and changes in gait were reported later (3.8 years before diagnosis).[86]

The TREND cohort study (Tubinger evaluation of Risk factors for Early detection of NeuroDegeneration) recruited 1046 subjects from the general population. After the first screening, individuals with selected non-motor prodromal markers (depression, anxiety, and suggestive RBD symptoms) were followed-up. Motor assessment was based on the motor score of UPDRS and a list of five potential motor features beyond general bradykinesia and rest tremor: sialorrhoea, hypophonia, micrographia, arm swing reduction, and dysarthria. No clear differences were found in the motor features occurring in the three different non-motor subgroups. However, a positive relationship between the motor score of the UPDRS and the number of non-motor prodromal markers was seen.[87]

In the Bruneck Study, a group of elderly people were assessed at baseline and 5 years later.[88] Subtle motor signs were used as the endpoint of the study. They used Louis et al definition (see Glossary). Objectively reduced sense of smell and hyperechogenicity of the substantia nigra on transcranial sonography were linked to subtle motor signs.

Table 1.1 Summary of remarkable list of epidemiological studies proving the existence of motor prodromes

Study	Design	Follow-up (years)	Sample size	Age (years)	Motor assessment	Findings
Rotterdam [86]	Nested case-control	23	1090 (PD) 109 (AMC)	78 (SD 7)	Clinical interviews Medical record General impression	Motor progression (<8 years before diagnosis): slowness>tremor>rigidity>postural abnormalities>falls
Bruneck [88]	Longitudinal cohort	5	284 (MPS+) 109 (MPS-)	66.5 (SD 7.8)	UPDRS-III	MPS were associated to SN-hyperechogenicity (OR 2.0), hyposmia (OR 1.6), but not with VRF
TREND [87]	Cross-sectional	NA	698	64	UPDRS-III Motor symptoms questionnaire*	Positive relationship between motor score and number of non-motor markers (depression, anxiety and probable RBD)
PREDICT-PD[2]	Longitudinal	NA	74 (HR) 111 (LR)	HR:72.2 (95% CI 69.0- 75.5) LR: 64.9 (95% CI 62.8-66.6)	MDS-UPDRS-III, Global impression**	HR: significant higher motor scores than LR HR: more likely to fulfil MPS criteria Risk estimates predicted motor scores
THIN[85]	Longitudinal Case-control	17	8166 (PD) 46755 (AMC)	75 (95% CI 68–81)	Medical records	Tremor: the most common motor marker (RR 7.6 at 10 years, RR 13.7 at 5 years before diagnosis) Balance impairment and rigidity appeared 2-5 years before diagnosis
PARS[89]	Longitudinal cohort	8	185 (hyposmia)	66.6 (SD 5.7)	UPDRS-III	Higher motor score (2.7 vs 1.3) and rate of phenoconversion to PD in subjects with abnormal dopamine transporter scan

TREND: Tübinger evaluation of Risk factors for Early detection of NeuroDegeneration, THIN: The UK Health Improvement Network, PARS: Parkinson Associated Risk Syndrome, NA: no-applicable, AMC: age-matched controls, MPS: mild parkinsonian signs, HR: higher risk (above the 15th centile of risk estimates), LR: lower risk (below the 85th centile), SD: standard deviation, CI: confidence interval, OR: odds ratio, RR: relative risk, SN: subtantia nigra, VRF: vascular risk factors, RBD: REM-sleep behaviour disorder, * Motor questionnaire: sialorrhea, hypophonia, micrographia, slowing of fine hand movements, arm swing reduction, dysarthria and rest tremor, **Global impression scale: 0—normal, 1—unspecific minor abnormality, 2—subtle signs associated with PD, 3—possible early PD, 4—probable PD

Buchman and colleagues followed-up a large group of people without PD.[90] At post-mortem, nigral neuronal loss and the presence of Lewy body pathology were correlated with the presence of subtle motor signs that had been evaluated with an adapted version of UPDRS. An association was found only with nigral neuronal loss, but not with Lewy body pathology, suggesting that the captured motor signs may be more associated with pathological ageing rather than being PD-specific. Similar results were demonstrated in another post-mortem study in elderly people presenting with mild parkinsonian signs but no diagnosis of PD.[91]

Finally, the PREDICT-PD is a population-based study which investigated evidence for early motor dysfunction in individuals at higher risk of PD. Given that one project from this thesis is directly linked with the PREDICT-PD study, I have described the study design and risk algorithm below.

1.6 PREDICT-PD study cohort

1.6.1 Study design

The PREDICT-PD study is a web-based longitudinal study whose main aim is to identify people at higher risk of PD from the general population. The pilot cohort was established in 2011 with 1323 participants.[92] At the time that this thesis was written, there were more than 8000 people involved.

At baseline, healthy people from the UK population aged between 60 and 80 were recruited via the internet (www.predictpd.com). People with pre-existing PD, other movement disorder, stroke, motor neuron disease, dementia, or taking treatments known to cause pharmacological parkinsonism were excluded from the study. Eligible participants who gave informed consent online were asked to complete a validated, evidence-based questionnaire derived from a systematic review published by Noyce and collaborators in 2012.[93] The survey gathered demographic information and answers to questions about non-motor symptoms and risk factors for PD. It also included validated questionnaires such as the Hospital Anxiety Depression Scale (HADS)[94] and the REM sleep Behaviour Disorder Screening Questionnaire (RBDSQ).[95] Immediately after the questionnaire, participants were invited to perform a remote keyboard tapping test, also called the BRadykinesia Akinesia INcoordination (BRAIN)-tap test. It is a software tool available online at www.braintaptest.com which was validated to test upper limb motor function by Noyce and colleagues in 2014.[96] I describe the validation process of the BRAIN test in a separate section (1.8.3) within this chapter, which covers technology-based tools to assess motor dysfunction. In brief, participants were instructed to strike alternately the 'S' and ';' keys on their keyboard, as fast and accurately as possible. Participants were asked to undertake the task with their right and left hand separately. The number of keys tapped during a 30-second task (Kinesia Score -KS-) and the average dwell time that keys were depressed (Akinesia Time -AT-) were applied in the prediction algorithm described above.

Volunteers also received a smell test via post. It consists of 40-item scratch and sniff smell test known as UPSIT. They were asked to enter their answers on the study website (Figure 1.2).

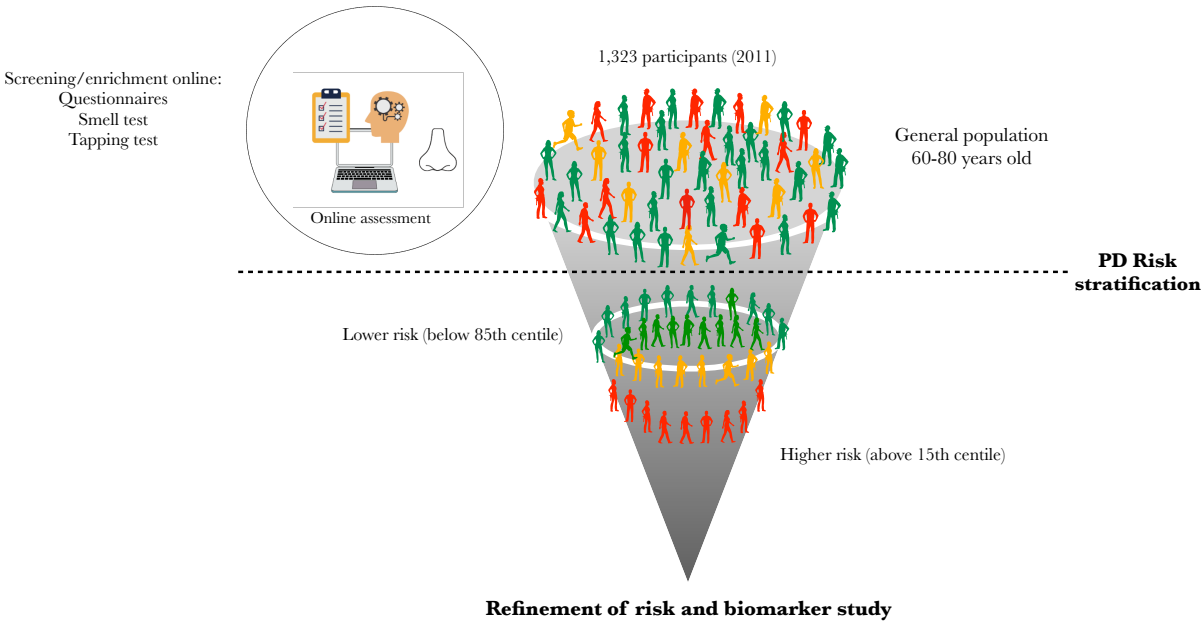

Figure 1.2 Schematic of PREDICT-PD online study indicating how algorithm stratifies people from the general population into higher risk and lower risk groups. Red, orange and green colours represent participant stratification based on risk estimates: higher risk in red (above 15th centile), intermediate risk in orange (between 15th and 85th centile) and lower risk in green (below 85th centile).

1.6.2 Algorithm development

A prediction algorithm was applied to stratify participants into higher and lower risk for subsequent PD using the available information. After 3 years of follow-up, seven participants developed PD. It allowed for the calculation of the relationship between the risk score at baseline and incident PD, obtaining a hazard ratio of 4.39 (95% Confidence Interval -CI- 1.03 to 18.68).[97]

The PREDICT-PD algorithm was refined 10 years later, using the pilot study data. The basic PREDICT-PD algorithm used prodromal features (kinetic parameters from BRAIN test, hyposmia and probable RBD) as "intermediate" markers of prodromal PD until sufficient incident cases of PD were collected. With the increase of incident cases and expanding knowledge from the literature, a new PREDICT-PD algorithm was created to include continuous exposure information from those intermediate markers, with expected improvement in accuracy of risk estimates.[98,99] Another change was to use likelihood ratios instead of odds ratios (ORs) as a method of risk estimation. In contrast with ORs, likelihood ratios allow us to account not only for the presence but also the absence of each exposure. Another measure of improvement was to incorporate intermediate clinical markers of prodromal PD into risk estimates such as continuous objective measures, including finger-tapping speed (KS and AT parameters from the BRAIN test described in the previous section)

and smell test scores, together with self-reported RBD. Throughout this thesis, the initial algorithm is referred to as the 'basic' algorithm and the updated version is referred to as 'enhanced' algorithm.

In brief, for each participant, the *a priori* age-related PD risk (expressed as an odds) was calculated and then adjusted depending on the presence and absence of determinants of risk. Regarding the intermediate clinical markers, continuous scores were used for BRAIN test tapping speed and smell test scores. In contrast, RBD was used as a dichotomous variable (probable or not probable RBD) based on a pre-established cut-off (>5 points) extracted from RBDSQ. More than 5 points in the RBDSQ has a sensitivity of 96% and specificity of 56% for v-PSG-confirmed iRBD.[95]

In the final step, participants were classified in the Higher Risk (HR) and Lower Risk (LR) group based on risk estimates above the 15th centile and below the 85th centile respectively (Figure 1.2).

1.6.3 Comparison with MDS algorithm

In 2015, a Task Force of the MDS published evidence-based, research criteria for prodromal PD to identify people at risk of PD.[67] The algorithm used by the MDS Task Force (hereafter, MDS algorithm) shares several similarities with the PREDICT-PD algorithm (Table 1.2). Both were created for research purposes, are based on risk factors extracted from evidence-based studies and use simple screening tests. In terms of statistical approaches, both algorithms use a Bayesian classifier based on the prior age specific PD probability plus the likelihood ratio related to the presence or absence of risk factors and those continuous biomarkers that surpass the cut offs to be considered pathological. For a marker to be included in the algorithm there must be strong evidence proving its association with PD. Similar to the PREDICT-PD algorithm, the MDS algorithm has been recently updated. It added several new risk factors which are Type 2 Diabetes (T2D), low plasma urate levels for men, physical inactivity, global cognitive deficit, known gene mutations with intermediate penetrance (*GBA*, *LRRK2*) and polygenic risk scores.[100] On the other hand, both algorithms differ in several aspects. The MDS algorithm bases age-related risk on 5-age intervals with the risk of losing information between intervals. In contrast, PREDICT-PD uses the exact age as a continuous function. This discrepancy also applies for smell test and motor impairment. The MDS algorithm includes motor dysfunction as a binary variable based on the MDS-UPDRS-III[II] whereas the PREDICT-PD algorithm uses KS and AT parameters as continuous variables. Moreover, the MDS algorithm does not account for head injury, which was suggested to be a relevant risk factor in a systematic review (head injury OR of 1.58).[93] On the other hand, the MDS algorithm includes in-person assessments such as the MDS-UPDRS-III and imaging markers such as transcranial

[II] Motor impairment: present (1): MDS-UPDRS-III (excluding postural and kinetic tremor) >6, absent (0): MDS-UPDRS-III (excluding postural and kinetic tremor) ≤ 6

sonography and DAT-SPECT. Members of our research team objectively compared the performance of both algorithms.[99] The PREDICT-PD algorithm showed a wider distribution of risk estimates than the MDS algorithm, suggesting that it could have a greater accuracy and discrimination power between those who went on to develop PD and those who did not.

Table 1.2 Comparison of the MDS algorithm with the PREDICT-PD algorithm (basic and enhanced)

	MDS	Basic PREDICT-PD	Enhanced PREDICT-PD
Age	Categorical 5-years age interval	Continuous age-based equation	Continuous age-based equation
Probability measure	LR	OR	LR
Surrogate markers (motor impairment, RBD, hyposmia)	Dichotomised results (presence, absence)	Not used	Quantitative data (total scoring)
Singular factors	T2D Physical inactivity Genetic mutations		T2D Head injury NSAID and Beta Blocker use Alcohol consumption
Higher risk cut-off	>80% of probability	Above 15th centile	Above 15th centile

MDS algorithm: MDS Research Criteria for Prodromal PD; Basic PREDICT-PD: original version; Enhanced PREDICT-PD: updated version; LR: likelihood ratio; OR: odds ratio; RBD: REM sleep Behaviour Disorder; T2D: type 2 diabetes; NSAID: Non-Steroidal Anti-Inflammatory Drugs

The basic PREDICT-PD algorithm was validated in another community-based cohort in Innsbruck with twice as many cases of incident PD as the PREDICT-PD cohort.[101] The Bruneck study is a prospective population-based study on cardiovascular and neurological diseases carried out in Innsbruck and initiated in 1990.[102] Risk scores at baseline (using the PREDICT-PD approach) were associated with incident PD with ORs of 2.09 and of 1.95 after 5 and 10 years of follow-up respectively, meaning that per 1-unit change in log risk scores, the odds of developing PD doubled. In addition, higher risk scores were correlated with surrogate markers such as olfactory dysfunction, probable RBD and motor deficits. Of note, they used UPDRS-III scores as the intermediate motor marker, instead of BRAIN test tapping data.

The MDS algorithm has been tested in five cohorts so far: four population-based cohorts[103,104,105] and one v-PSG-confirmed iRBD cohort.[106] When it was tested in the general population, the sensitivity and positive predictive value were limited (ranging from 4.5% to 66.7%) despite having a high specificity (>80%). In contrast, when it was tested in an enriched cohort, such as patients with iRBD, both sensitivity and specificity were high.

1.6.4 Evidence of motor disturbance in the PREDICT-PD cohort

Previously Noyce (co-principal investigator of the PREDICT-PD study) explored whether people with more risk of developing PD exhibited subclinical parkinsonian signs than those with lower risk.[2] A representative sample of people in the HR and LR groups were sampled and seen in person. All the examinations were recorded on video and rated using the MDS-UPDRS-III (motor score). In addition, two blinded experts rated the videos following the same instructions in the MDS-UPDRS-III and gave their subjective global impression which was based on a semiquantitative clinical scale.[III] Two definitions (Louis et al and MDS Task Force) were applied to calculate the number of participants fulfilling criteria for Subthreshold Parkinsonism (SP)[67,107] (for details about both criteria see Glossary). People stratified in the HR group had on average higher motor scores than the LR group. The HR group was also more likely to fulfil criteria for SP based on both definitions with subtle motor changes being present in 18% (based on Louis criteria) and 31% (based on MDS Task Force criteria) in the HR group compared with the LR group (6% and 10% respectively). These findings were supported by the global impression scale score of the two blinded clinical experts; participants in the HR group were more likely to have suggestive parkinsonism (score ≥3) (2.7% of HR vs 0.9% of LR) and subtle features of parkinsonism (scores ≥1.5 and <3) (23.0% of HR vs 6.3% of LR, p=0.001) than those in the LR group.

1.7 Defining motor prodromes

1.7.1 Historic clinical descriptions

Kinnier Wilson was one of the first clinicians to introduce the concept of symptoms occurring in the pre-diagnostic phase of PD (Kinnier Wilson, Neurology; Volume II, 1940). He described the difficulties in detecting early motor signs in older people, where stiffness and slowness could be misinterpreted as features of senility. He noted, as did James Parkinson in his original description, the insidious nature of these symptoms, such that patients often struggle to recall early clinical feature of PD - "Long before rigidity actually develops, patients have significant difficulty performing ordinary activities […] even a cursory exam demonstrates that their problem relates more to slowness in the execution of movement rather than a real weakness". Separately, William Gowers reported in 1888 that intermittent tremor could occur years before diagnosis.

In 1992 Lees described non-specific and sometimes transient symptoms over a 12-year period before the football player Ray Kennedy was eventually diagnosed with PD.[25] In this case report, a range of videos recorded from football matches were reviewed and it was observed that during

[III] Global impression scale: 0—normal, 1—minor clinical abnormality not necessarily associated with PD, 2—subtle clinical observation associated with PD, 3—suspected/possible early PD/parkinsonism, and 4—probable PD

matches Kennedy's arm was held stiffly and flexed at the elbow. This case report together with the finding that nigral cell loss probably began at least five years before bradykinesia was detectable,[13] coincided with renewed interest in Kinnier Wilson's 'Parkinson prodrome'.

1.7.2 Mild parkinsonian signs

Mild Parkinsonian Signs (MPS) describe the motor spectrum that spans from normal ageing to the early stages of PD.[12] There is a degree of uncertainty around the terminology in MPS. A variety of other terms have been used to describe these features, such as SP, subtle motor/parkinsonian signs and 'soft' basal ganglia (extrapyramidal) signs. These terms are often used interchangeably and without precision. In broad terms, MPS can be defined as parkinsonian signs which do not surpass the threshold established for the diagnosis of PD.[108] In this thesis, the term SP will be used in its broadest sense to refer to all MPS.

Several studies have specifically assessed the relative risk of SP for subsequent diagnosis of PD and, in one example, SP at baseline had a relative risk of 5.5 (2.4 to 12.6) for incident PD over 10 years of follow-up.[109] However, SP has not exclusively been considered part of the prodromal spectrum of PD; SP has also been associated with normal ageing, vascular risk factors and cognitive impairment. Based on the multiple trajectories that SP can have, it seems reasonable to focus our attention on distinguishing which individuals with SP will continue to age normally and which may be in the early stages of PD or dementia.

There is substantial overlap between SP and normal ageing. The prevalence of SP across studies ranges between 15% and 50% in individuals older than 65, without a known neurological condition.[108] Clinical examination may reveal clues to define the boundaries between normal ageing, SP and PD – a non-progressive course, symmetric distribution, and slowness with a lack of decrement – are all motor features observed in natural ageing.[110] Axial signs can predominate in older people with SP and are usually less responsive to L-dopa in patients with PD. It might indicate that axial signs might not be exclusively explained by the nigrostriatal dysfunction seen in PD.[111]

Vascular risk factors and cognitive impairment usually coexist in individuals with SP who will not develop PD. Cerebrovascular disease and the combination of T2D and heart disease have been found to increase the probability of parkinsonian signs by 70%.[112] In a cross-sectional study, which involved individuals older than 50 and carried out in Singapore, MPS and cognitive impairment were evaluated along with a screening of non-motor symptoms of PD. The main purpose was to know how frequent SP was in an elderly community without PD and evaluate if these features were associated with a loss of cognitive abilities or other features known to be part of the early phase of PD including reduced sense of smell, constipation, depression and sleep disturbances.[113] The authors found that one quarter of the group had SP, and this proportion increased with age, with three out of ten people older than 75 years having SP. They also found that cognitive dysfunction and symptoms of RBD were associated with SP

after adjusting for age and sex. From these findings, we could conclude that SP in their cohort were not explained merely by ageing and might be indicative of an underlying neurodegenerative process. On the other hand, SP is associated with an increase in the risk of dementia. Numerous studies summarised in a review published by Louis et al showed that people with SP have a higher incidence of Alzheimer's Disease (AD).[110] In one study, a third of patients with AD were found to have SP, which in turn was associated with the presence of neurofibrillary tangles in the substantia nigra.[114]

The underlying neuropathology of SP remains unclear. The loss of pigmented neurons in the Substantia Nigra (SN) pars compacta together with the presence of Lewy Bodies (LB) are the hallmarks of PD. However, post-mortem studies have shown that Lewy body pathology is not exclusive to PD and have been found incidentally in 2% to 61% of healthy brain donors.[115] Fearnley and Lees found that individuals with incidental LB had an intermediate SN neuronal loss between PD cases and controls and postulated that they might represent a preclinical stage of PD.[13] On the other hand, SP can be found in elderly people with SN neuronal loss and without LB. Ross and collaborators examined the brains of participants in the Honolulu Heart Program/ Honolulu-Asia Aging Study (HHP/HAAS). They estimated the density of neurons in the SN in PD cases, individuals with incidental LB and elderly people without either condition.[91] They found that brains from older individuals without LB but who had SP had lower neuron density in the dorsomedial and dorsolateral quadrants of SN, in contrast to ventrolateral portion of the SN, which is seen in PD and incidental LB.

1.7.3 Bradykinesia

Bradykinesia is the only clinical sign that is required to be present in every patient with PD according to the Queen Square Brain Bank Criteria.[8] It is defined as the 'slowness of movement initiation with progressive reduction in speed and amplitude (sequence effect) of repetitive actions'.[116] It is often described by patients as clumsiness or weakness when carrying out delicate tasks.[25] Therefore, questions should be directed towards loss of dexterity in repetitive manual tasks such as buttoning clothes, shaving, beating eggs, shampooing, stirring, or writing and when possible, close family members should also be asked whether they have noticed any subtle changes) (Box 1.1).[26] The evaluation of bradykinesia stills relies on clinical observation, and it is usually assessed using the MDS-UPDRS part III (motor score). Although the MDS-UPDRS-III is a comprehensive assessment, the integer scale prevents detection of subtle motor changes[117,118] and is subject to high inter- and intra-rater variability.[119] Hence, there is a need for objective and consistent methods of assessing motor dysfunction which would help to stratify bradykinesia into ranges from the prodromal phase to clinically stablished PD.

In terms of pathophysiology, bradykinesia has been correlated with cell loss in the pars compacta of the substantia nigra which is related to a failure in the recruitment of cortical motor neurons during an intended task[120] and also the feature that better reflects the nigrostriatal deficit in PD.[121] A deeper understanding of the pathophysiological mechanisms of

bradykinesia is essential for a guided examination at prodromal PD stages. Due to efficient compensatory mechanisms for striatal dopamine deficiency, the classic motor signs only became noticeable when there is a depletion of 70% to 80% in striatal dopamine. These mechanisms are based on up and down regulation of dopamine level and dopamine transporters, and help to maintain stable dopaminergic transmission and motor function at early stages of the disease.[122] Moreover, there exist external supporting regions which increase cortico-striatal input and help to perform simple motor tasks. However, these can fail when more challenging tasks are performed and can subsequently make motor deficits more noticeable.[5] For these reasons, one approach would be to design challenging motor tasks to breakdown these regulatory strategies and evaluate early subtle motor signs.

Box 1.1 Symptoms first noticed by patients

- Handwriting changes: progressively smaller, cramped, sloping
- Dry eyes due to reduced blinking
- Lack of facial expression: distracted, vacant, blank (often reported by relatives and friends)
- Reduced arm swing (reported by relatives and friends)
- Frozen shoulder
- Impaired manual dexterity in repetitive tasks: beating eggs, shaving, typing, playing an instrument
- Painful abnormal posture in their foot (typically in young-onset PD)
- Scuffing the sole or heel of one foot when walking
- Feeling of imbalance

1.7.4 Tremor

Beyond recognising that tremor may be transient and predate the other features of PD, William Gowers made another key observation - '*In the early stage, only an apparent intermission of contraction is recognised, either in rest or on movement*' (Gowers 1888).[123] This intermission before the emergence of tremor is readily recognisable in clinical practice and helps to distinguish a postural tremor in PD from that seen in essential tremor.

In fact, action and rest tremor were found as early symptoms before PD diagnosis in a longitudinal cohort of individuals with mild parkinsonian signs but no indicative signs of definitive bradykinesia. During their follow-up, an association between the presence of action and/or rest tremor and the development of PD was found by the doubling the risk of PD when they were present (OR 2.8; 95% CI, 1.10 to 7.09; p=0.020).[4]

A monosymptomatic rest tremor without bradykinesia has been associated with dopamine denervation on dopamine transporter imaging in some patients and all these cases went on to

develop PD.[124] Other studies have indicated that longstanding asymmetrical postural tremor could be a predictive factor for development of PD and others have argued that even bilateral essential tremor could be a risk factor for PD.[125,126] Of note, a significant number of patients with 'benign tremulous parkinsonism' have a family history of tremor and/or PD.[127,128]

Neuropathological studies comparing patients with confirmed PD and those with a slowly progressive tremor-dominant parkinsonism suggest that there is less nigral cell loss in the latter.[129] The authors discussed the importance of follow-up for elderly patients with late onset of essential tremor, since parkinsonism may ultimately emerge.

1.7.5 Posture

A stiff arm held flexed at the elbow and the fingers in a flexed-adduction position are 'tell-tale' signs of early parkinsonism. Patients can also appear preternaturally still; maintaining the same position for long periods without the small fidgets and adjustments that healthy people make when sitting or standing (Kinnier Wilson, 1940). In young-onset PD, bradykinesia is not infrequently preceded by foot dystonia and occasionally writer's cramp by several years. A few patients present with a motor restlessness related to difficulty finding a comfortable posture in which to rest.

Although postural instability was considered the fourth cardinal sign in the first published criteria, it was excluded after being reviewed by MDS in 2015;[11] it typically emerges at the advanced stages of the disease and an early presence should make clinicians think about other causes of parkinsonism.

1.7.6 Gait

Walking is an automated, rhythmic motor task that requires both motor and executive skills.[130,131] The gait pattern is defined by arm swing (amplitude and symmetry), stride (length, off-ground elevation), and coordination between four limbs (rhythm and smoothness). In the elderly, musculo-skeletal disorders and the motor signs of diffuse cerebrovascular disease need to be distinguished from true parkinsonism. Step-to-step variability, decreased amplitude of arm swing or arm swing asymmetry and reduction in the 'smoothness' of gait may be early signs of SP.[132–134]

Several approaches to quantification of gait have been used, from the TUG test to more sophisticated analyses using sensors. The TUG test is a simple test which has been used to measure mobility and risk of falls in PD and elderly people.[135] Moreover, it has been demonstrated to have a good test-retest and inter- and intra-rater reliability in PD, with intraclass correlation coefficient (ICC) estimates ranging from 0.87 to 0.99.[136–138] It consists of timing how long it takes for an individual to rise from a chair, walk three metres at a comfortable pace, turn round, walk back, and sit down again. The TUG test was originally designed and

subsequently validated as a fall prediction tool in elderly people.[131] It was also found to be related to executive function which is known to be affected in PD.[135] However, its role as a potential early motor marker in PD is unknown.

In the early stages of PD, compensatory mechanisms may prevent gait disturbances. Dual tasking (e.g. asking the patient to do mental arithmetic while walking) is a useful clinical method to unmask subtle signs.[139,140] This strategy has been used to study people at risk of developing PD, such as non-manifesting carriers of *LRRK2* mutations and people with hyperechogenicity of the SN. The most characteristic gait patterns found in the at-risk groups under challenging conditions were: higher stride time variability, arm swing asymmetry and decrease in the smoothness of trunk rotation.[63,133,140] Reduction in the smoothness in axial swaying could be due to an increase in axial rigidity, such as that which happens with limb rigidity during distraction tasks (i.e. Froment´s manoeuvre).

Since the prevalence of PD increases with age and gait abnormalities are common in the elderly population, it is important to consider the effects of ageing, which involves other co-morbidities with polypharmacy, as a confounding factor and consider multifactorial effects of ageing on gait from those of PD.[141] Mirelman and colleagues studied 60 healthy controls aged between 30 and 77 years old. Whereas arm swing amplitude decreased with age and a dual task, arm swing asymmetry and limb coordination (stepping consistency and rhythm) appeared to be less susceptible to ageing.[142]

1.7.7 Handwriting and typing

Handwriting is a fine motor task that imposes a substantial demand on cognition.[143] The concept of handwriting difficulties as an early motor feature of PD dates back to 1817 when James Parkinson documented handwriting changes preceding impairment in walking. Schwab demonstrated that micrographia could be present 4 years earlier than PD diagnosis by reviewing serial signatures from cheque stubs of patients prior to them developing the classical features of PD.[144] Micrographia is defined by gradual reduction in letter size and has been proposed by some as a relevant clinical biomarker.[145] The script is often 'crabby and cramped', and in right-handers tends to slope upwards.

There is very little correlation between micrographia and bradykinesia severity in PD.[144] In recent years, with the exploration of technologies to objectively measure handwriting abnormalities in parkinsonism, the term dysgraphia has been introduced to embrace not only script size but other kinetic variables such as fluency, velocity and duration. Drótar and colleagues demonstrated that artificial intelligence might be promising to develop predictive models for PD diagnosis based on classifiers algorithms able to detect abnormal handwriting patterns beyond script size. They used digitalised signals which included stroke speed, acceleration, jerk, number of changes in velocity and surface pressure amongst others[146]. This

may offer the possibility of picking up more abnormalities in the script of people with PD in an early stage.[143]

The kinematics of typing have also been tracked remotely in *de novo* patients with PD from the initiation of dopaminergic treatment.[147] Investigators from this study observed that improvement in typing kinematic parameters occurred earlier than in motor scores on the UPDRS after commencing treatment.

1.7.8 Voice

Hypokinetic dysarthria is a characteristic speech pattern in PD, which is defined by hypophonia, lack of voice modulation, poor articulation, hesitations and stoppages.[148] Voice and face expression changes appeared more than 8 years prior to PD diagnosis in people with iRBD.[39] However these findings have not been confirmed in population-based studies.[149] Researchers working on the Oxford Discovery Parkinson's cohort have used a smartphone app and machine learning to distinguish patients with PD, iRBD and controls.[150] Voice analysis was part of the test battery, which also included balance, gait, finger tapping, reaction time and tremor assessment. Along with tremor, voice analysis was found to be the most distinguishing feature between the three groups. The same team has demonstrated how this approach could be used to predict the milestones of progression in PD.[151] Another study focused on abnormal speech patterns in prodromal PD showed that voice frequency variability could be detected up to 5 years prior to the diagnosis.[152]

1.7.9 Blink rate and facial hypomimia

The normal rate of blinking ranges between 14 and 25 blinks per minute. It is influenced by mental tasks (reading, watching a film, speaking) and mental state (anxiety, depression). Several studies have shown a positive association between blinking rate and central levels of dopamine by comparing conditions with inverse dopamine activity (PD and schizophrenia) and testing the effect of dopamine agonists and antagonists on eye-blink rate.[153]

Relatives and friends often notice a frozen facial expression which they describe as a dullness, a 'poker face', 'a mask', a sadness or a coldness. A reduction of blink rate as a feature of PD has long been recognised - *'A valuable early symptom is infrequent blinking of the eyelids' (*Kinnier Wilson, 1940). This may lead to a complaint of dry sore eyes or watering of the eyes. However, Fitzpatrick and colleagues could not find any link between blink rate and disease severity and duration in one study, which led to the conclusion that reduced blink rate is an early and unalterable feature of PD.[154]

In contrast to spontaneous blinking, little attention has been paid to assessing voluntary eye blinking in PD. Agostino and collaborators evaluated the rapid eye blinking in patients and controls with PD cases showing a significantly prolonged pause between the opening and

closing phases of voluntary blinking in PD patients.[155] Moreover, Alarcon and colleagues found a greater deterioration in 'fast blinking' over time in incident cases of PD before they could be diagnosed.[4]

1.8 Methods of identifying subtle motor abnormalities

Motor analysis in the early phases of PD has several limitations. Heterogeneity in prodromal phenotypes may make it difficult to standardise methods of analysis. As mentioned, with clinical rating scales, parkinsonian signs have been described in between 30% and 40% of the elderly population without PD.[141] No consensus exists on the ideal method of measuring motor dysfunction in the early stages of PD, including which scales to use, which kinetic parameter are best to analyse (e.g. amplitude or velocity for bradykinesia), and under what conditions (home monitoring, lab or clinic environment, challenging conditions). Standardised approaches may be required to set the boundaries between prodromal and established PD.[156]

Extranigral structures may play a compensatory role in the progressive dopamine loss in PD.[157] At early stages, such mechanisms are strong enough to mask such motor deficits. Thus, as outlined in some of the aforementioned studies, clinical examination and assessment should aim to challenge compensatory mechanisms (Figure 1.3).[5]

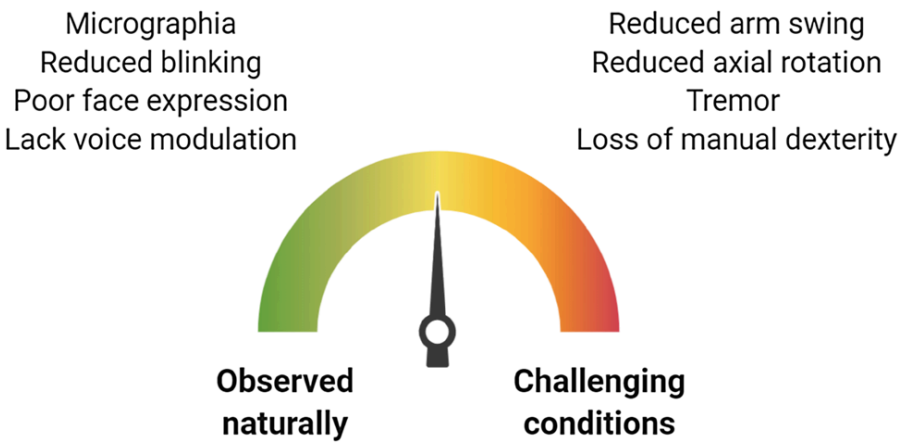

Figure 1.3 On the left, features to explore on normal condition (spontaneous movements). On the right, other signs emerge under challenging conditions (e.g. dual tasking and fast walking).

1.8.1 Questionnaires

Questionnaires asking about subjective motor complaints have been used as a diagnostic tool with apparent high sensitivity and specificity.[158,159] However, the role of bias in reporting subjective symptoms has to be considered.[160]

Telephone questionnaires have been used to study patients' perception of prodromal symptoms.[161] In one study, slowing of fine hand movements, general bradykinesia, dysarthria

and reduced arm swing were noticed between 2.2 and 4.7 years before the patient fulfilled the diagnostic criteria of PD. Although PD patients reported motor difficulties more frequently, 10% of age-matched controls had also noticed some changes in their dexterity and speed of movement.

Maraki and colleagues used a motor screening battery, including a questionnaire focused on gait and postural difficulties in elderly people taking part of the HELIAD study (Hellenic Longitudinal Investigation of Aging and Diet cohort). Having at least one subjective gait/postural difficulty increased the probability of having prodromal PD (based on the MDS Research Criteria for Prodromal PD).[67] Difficulty walking outdoors, poor balance, using a walking aid and the presence of a shuffling gait were reported more commonly (between 25% and 35%) by subjects with probable prodromal PD.[162]

1.8.2 Clinical scales

The standard motor assessment for established PD is the MDS-UPDRS-III.[67] It is a validated semi-quantitative scale universally used in the clinical setting of PD. It encompasses several motor tasks to evaluate motor domains known to be affected in PD such as bradykinesia, rigidity and tremor.[163] However, it was not designed for use in the prodromal stages of PD and may not be sensitive enough to pick up subtle motor features at early stages.[5] This varies depending on the level of experience and the item being rated.[164] In particular, bradykinesia-related items appeared to have a poor interrater reliability.[165] For these reasons, a further in-depth study of the motor signs throughout the disease course, including the prodromal phase, is needed. Despite the limitations described above, in the absence of a widely accepted and validated alternative, the MDS-UPDRS-III remains popular.

Research Criteria for Prodromal PD established a cut-off of 6 (after excluding postural and kinetic tremor) on the MDS-UPDRS-III for defining SP.[67] In one study bradykinesia-related items presented the lowest reliability of all the UPDRS parameters, with a finger tapping interrater reliability kappa coefficient below 0.50.[166]

In the prodromal stages of PD, the scoring used in the MDS-UPDRS is susceptible to a floor effect between scores of 0 and 1 (normal and slight abnormalities). To overcome this limitation, a modified bradykinesia score was created which separately scores three kinetic parameters (frequency, rhythm and amplitude) for each movement.[165] However, even with these modifications, there are additional important features such as manual dexterity, posture and gait under challenging conditions that are not captured in this scale.

1.8.3 Technology

There has been considerable interest in technological solutions to the issue of how to capture subtle motor features of PD. Finger movements have received a lot of interest (Table 1.3).

However, a reliance on technology -including artificial intelligence along with machine and deep learning, and other 'big data' approaches- can lead to oversimplification and loss of appreciation for the particularities and subtleties of the motor features of PD. The clinician's observations and the evaluation of a patient's co-morbidities, including osteoarticular problems, peripheral neuropathy and polypharmacy, are essential to avoid misdiagnosis.

Technological approaches can be classified according to whether the assessments are home or lab-based, the type of device (body sensors, smartphone app, 3D motion capture, computer keyboard) and the kind of movement tested (gait, finger-tapping, handwriting, spiral drawing) (Figure 1.4). One important question is whether, particularly in the PD prodrome, technology should be validated against scales used for established PD, or whether new scoring paradigms should be prioritised[167]. While technology will certainly play a far greater role in the assessment of handicap in PD, it remains hard to envisage how it could wholly replace a detailed neurological examination, with an appreciation of the complex and heterogeneous motor and non-motor manifestations of PD and clinical expertise.

The Purdue Pegboard Test is an example of a simple tool that has been proven to be accurate to distinguish patients from controls and might be also able to predict future PD. It assesses manual dexterity by using a board with four cups across the top and two vertical rows of 25 small holes down the centre. Participants are invited to place as many pins as possible down on the row within 30 seconds. Proud and colleagues found that it had a high test–retest reliability (ICC ≥0.90)[168] and was able to distinguish PD patients from controls with high degree of accuracy (Area Under Curve –AUC=0.80).[169] Finally, a large population study showed that the Purdue Pegboard test could predict future PD diagnosis (hazard ratio 1.35; 95% CI, 1.11 to 1.67).[170]

Similarly, the BRAIN test was designed to measure manual dexterity in patients with PD. It has validated several times in patients with PD and also Multiple Sclerosis.[96,171,172] The BRAIN test is also part of the online assessment of PREDICT-PD study. It consists of an online tapping test where participants are instructed to alternately tap the 'S' and ';' keys on a computer keyboard using one index finger, as quickly and accurately as possible, for 30 seconds.[96] The test captures proximal motor impairment, as movement arises at the level of the elbow and shoulder. Existing literature suggests that proximal and distal movements are governed by two distinct neural pathways.[173] Manual dexterity mandates both proximal and distal muscles of the forearm; for example the 'reaching' action requires proximal muscles and 'grasping' action entails distal muscles.[174] Functional neuroimaging with PET showed that appendicular (distal) muscles and precision grip depend on the primary motor cortex[175] which is interconnected with the putamen, anterior cingulate, supplementary motor area and dorsolateral prefrontal cortex.[176]On the other hand, proximal movements have a widely distributed network from the premotor cortex to the basal ganglia and upper brainstem (pedunculopontine nucleus). [177,178] Furthermore, functional neuroimaging in non-human primates highlights segregated functional neuronal circuits for fine distal movements and for coarse proximal 'reaching'

movements.[179] The lateral ventral premotor cortex and primary motor cortex appear key in distal motor control, and the dorsal premotor cortex and brainstem areas appear prominent in proximal motor control.[179] This possibly explains why, as a diagnostic test, the BRAIN test historically demonstrates a relatively low detection rate (sensitivity) for PD (58% to 65%) given high specificity (81% to 88%).[172] Overall, these findings establish the requirement for new methods to differentiate between proximal and distal bradykinesia.

In summary, the motor prodromes of PD are an important but relatively neglected part of research into PD. In contrast to individual non-motor features, by current definition, all patients with PD will develop early motor signs, and motor dysfunction will progress as the disease advances. Available questionnaires and clinical scales are not suitably adapted for early stages of PD, and electronic measures are not currently sufficiently developed and validated. New ways and measures are still needed to reliably pick up motor dysfunction at the earliest stage.

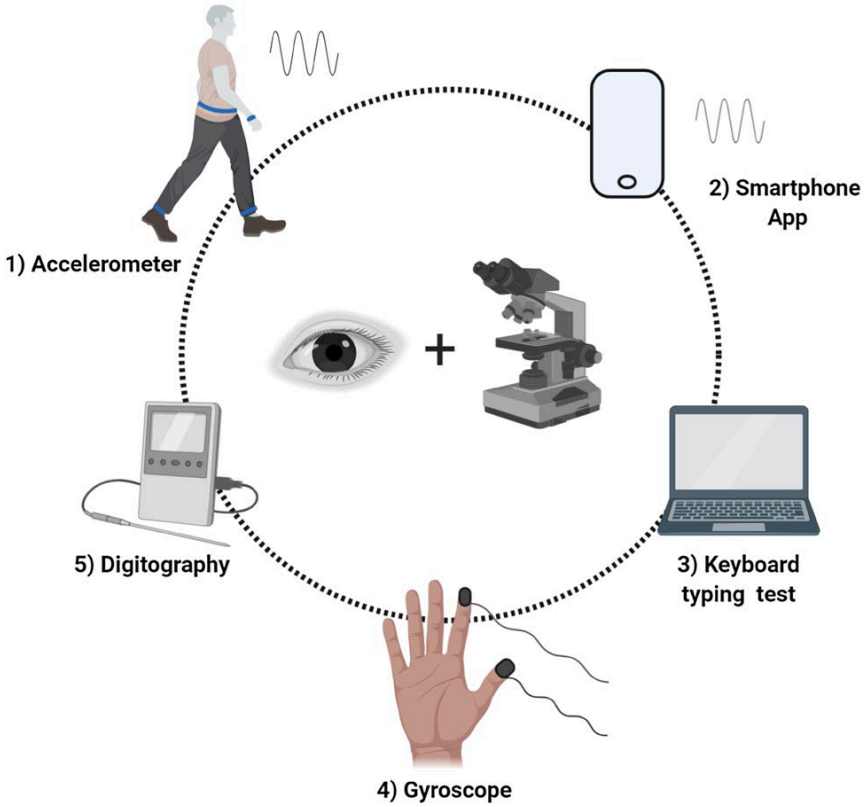

Figure 1.4 New technology era. The importance of combining clinical expertise (naked eye) with more sophisticated quantitative tools (microscope) to capture granular motor dysfunction. 1: Body sensors with incorporated accelerometers able to monitor movement in a home-environment. 2: Smartphone-based tools to assess bradykinesia, tremor and voice modulation. 3: Keyboard typing test to quantify velocity and rhythm during the task. 4: Digital sensors associated with a gyroscope to quantify changes in velocity, amplitude and rhythm during finger tapping. 5: Digital screen and sensory pen for handwriting and spiral drawing assessment.

Table 1.3 Representative literature about quantitative measures of finger movements

Reference	Test	Application	Task	Sample	Parameter studied	Accuracy	Clinical correlation
Noyce et al. 2014[96]	BRAIN test	Remotely	ATT (30sec)	58 PD 93 AMC	KS** AT IS	KS: 56% sensitivity, 80% specificity	KS - UPDRS-III: r = -0.53
Maetzler et al. 2015[180]	Digitomotography	Lab	FT	33 PD 18 HC	IPI TF DEV	NR	IPI – UPDRS-III: $r^2 = 0.02$ TF - UPDRS-III: $r^2 = 0.02$ DEV - UPDRS-III: $r^2 = 0.16$
Lee et al. 2016[181]	Smartphone tapper	Remotely	ATT (10sec)	57 PD 87 HC	MCoT T-Dist** IT- Dist IT- DwT	T-Dist: AUC 0.92 (95% CI 0.88–0.96)	Test - UPDRS-III: $r^2 = 0.25$ Test - UPDRS- FT: $r^2 = 0.32$
Růžička et al. 2016[169]	Contactless 3D motion capture system	Lab	FT (10sec)	22 PD 22 HC	AvgFrq MaxOpV AmpDec	AmpDec: AUC 0.87 MaxOpV: AUC 0.81	MaxOpV – UPDRS-FT: r = -0.48
Mitsi et al. 2017[182]	Tablet based application (iMotor)	Remotely	ATT PS RTT (30sec)	19 PD 17 HC	Number taps Tap interval** Reaction time	Tap interval: AUC 0.90 Combined model (ATT, RTT): AUC 0.98 (95% CI 0.93-1), 94% sensitivity, 93% specificity	PS/number taps – UPDRS-III: r = 0.45 PS/tap interval – UPDRS-III: r = -0.45
Gao et al. 2018[183]	Electromagnetic tracking sensors	Lab	FT (30sec)	107 PD 49 HC 41 ET	EA- dynamical classifiers PD-monitor score	Right hand: AUC 0.976 (94.6% sensitivity, 91.8% specificity) Left hand: AUC 0.959 (85.1% sensitivity, 91.8% specificity)	Right hand – MDS-UPDRS-FT: r = 0.82 Left hand – MDS-UPDRS-FT: r = 0.78
Zhan et al. 2018[184]	Smartphone and ML	Remotely	ATT	129 PD	Voice, FT, gait, balance, reaction time	NR	Overall test – UPDRS-III; r=0.88

Prince et al. 2018[185]	Smartphone	Remotely	ATT	949 PD 866 HC	DNN	AUC 65.7 ± 1.05	NR
Bobic et al. 2019[186]	Wearable sensors and 3D gyroscope Decision support system	Lab	FT	13 PD 17 MSA 14 PSP 12 HC	Individual taps Amplitude Amplitude decrement Hesitations & freezes Speed	Overall test accuracy 82.69% +/- 2.72	NR
Shin et al. 2020[187]	Conventional camera DL tracking algorithm	Lab	FT (10sec)	29 PD 1 HC	Amplitude Mean**, variability Interpeak interval Mean, variability**	NR	Mean amplitude– UPDRS-III: R = -0.60 Interpeak interval variability– UPDRS-III: R = 0.66
Williams et al. 2020[188]	Smartphone camera DL tracking algorithm	Lab	FT (10sec)	39 PD 30 HC	Speed Amplitude CV Rhythm	NR	Speed – Speed MBRS: R=0.74 Amplitude CV – Amplitude MBRS: R=0.66 Rhythm CV – Frequency MBRS: R= -0.65
Alberts et al. 2021[189]	Smartphone	Remotely	ATT	23 PD	Number of taps Errors (double tapping)	NR	Number taps – vMDS-UPDRS-III: R=-0.31 Errors – vMDS-UPDRS-III: R=0.36

Grey: machine learning based analysis, **: best parameter, between brackets: task duration in seconds. **AMC**: age matched controls, **AmpDec**: Amplitude Decrement, **AT**: alternating score, **ATT**: alternating tapping test, **AUC**: Area Under Curve, **AvgFrq**: Average Frequency, **BRAIN**: BRadykinesia Akinesia INcoordination test, **CI**: Confidence Interval, **CV**: coefficient variance, **DEV**: Tap Deviation, **DL**: Deep Learning, **DNN**: Deep Neural Network, **EA**: Evolutionary Algorithms, **ET**: essential tremor, **FT**: finger tapping, **HC**: healthy controls, **IPI**: Interpeak Interval, **IS**: incoordination score, **IT- Dist**: mean InterTap Distance, **IT- DwT**: mean InterTap dwelling time, **KS**: kinesia score, **MaxOpV**: Maximum Opening Velocity, **MBRS**: Modified Bradykinesia Rating Scale, **MCoT**: Mean number of Correct Tapping, **MDS**: Movement Disorder Society, **ML**: Machine Learning, **MSA**: Multi System Atrophy, **NR**: not reported, **PD**: Parkinson's Disease, **PS**: Pronation-Supination test, **PSP**: Progressive Supranuclear Palsy, **r**: Pearson correlation, **R**: Spearman's rank correlation, **r^2**: coefficient of determination for simple regression analysis, **RTT**: Reaction Tapping Test, **T-Dist**: mean Total Distance of finger movement, **Tap interval**: the average time between two consecutive finger screen taps, **TF**: Tap Force, **UPDRS-FT**: finger tapping sub-score, **UPDRS-III**: Unified Parkinson's Disease Rating Scale-motor part

1.9 Thesis aims and objectives

Based on this thesis overview (Figure 1.5), the work contained in the following chapters aims to characterise the motor prodromes of PD by finding an answer for the following questions: **when** do motor prodromes start? **how** do motor prodromes evolve and therefore how can they be predicted? and **which** motor markers do we need to investigate?

- to demonstrate that people from the general population who went on to develop PD reported motor manifestations in primary care years before getting the diagnosis of PD (Chapter 2)
- to determine whether people from the general population estimated as being at higher risk (defined as those above the 15th centile of risk PREDICT-PD estimates) have a more pronounced motor dysfunction than those that are lower risk (combined intermediate and lower risk) (Chapter 3); motor dysfunction is defined as follows:
    - SP proposed by the MDS Task Force on the definition of prodromal PD
    - motor decline based on an increased score in the MDS-UPDRS-III over time
    - novel motor features not captured by standardised clinical rating scales
- to develop two quantitative tools to objectively assess bradykinesia which is the cardinal sign of PD (Chapter 4); the design of both tools involves three phases:
    - pre-clinical phase: design and testing in healthy volunteers
    - proof-of-concept phase: measure of the test accuracy by comparing patients with PD with healthy controls
    - correlation phase: score correlation with the current standard of evaluation, the MDS-UPDRS-III
- to combine standardised clinical rating scales with quantitative tools in a motor battery to be tested on patients with iRBD, which is known to have a strong link with future development of PD, and demonstrate that they have motor prodromes that go beyond having SP (Chapter 5).

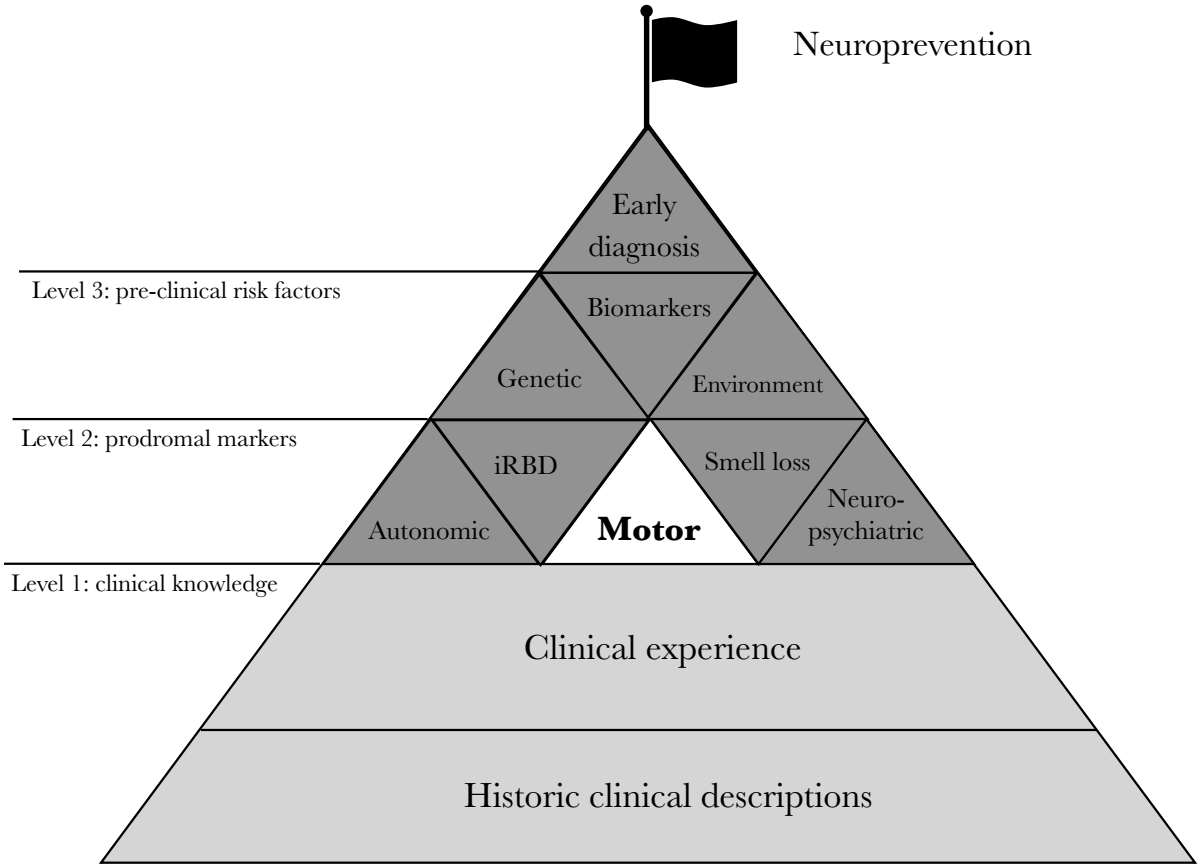

Figure 1.5 Thesis overview illustrating the journey towards neuroprotection (final goal) throughout early diagnosis and risk factor prevention (level 3). Clinical knowledge (historic clinical descriptions and clinical expertise) is the first step (level 1) to guide research on prodromal markers (level 2). Filling the gap of knowledge in motor prodromes (triangle in white) is essential to reach the final goal towards an early diagnosis.

Chapter 2

Early motor presentations of Parkinson's Disease in Primary Care

In the published version of the paper, we replicated some of the novel associations (hearing loss and epilepsy) in another cohort (UK Biobank).[190] I did not include these results, since they were out of the scope of this thesis. Instead, I adapted the results and discussion to the main topic of this thesis, which is the motor prodromes of PD. Although the paper covers a broad range of early presentations of PD (risk factors and early motor and non-motor manifestations), in this chapter I place emphasis on early motor manifestations.

2.1 Introduction

Pre-diagnostic presentations of PD have been identified through large, population-based, observational studies.[75,76,85,191,192] Recognising prodromal symptoms in primary care is essential for timely referral and early intervention.[193,194] General Practitioners (GPs) play a crucial role in this since they are the first contact point of patients before referring them to a neurologist. Knowledge and awareness of the prodromal symptoms of PD are essential for healthcare professionals working in primary care.[73] Most of the studies of prodromal manifestations in PD are population-based cohorts of volunteers who want to take part in research or case-control studies of pre-selected participants. In contrast, studies focused on a truly unselected primary care database offer a good opportunity to detect which might be the early presentations of PD in a 'real-word' setting.

Although there is enough evidence supporting the existence of motor prodromes years prior to PD diagnosis, pre-diagnostic motor manifestations have been little studied in primary care. In one of the most comprehensive observational studies done in this setting, Schrag and colleagues studied early presentations and risk factors of PD in The Health Improvement Network (THIN) large primary care database.[85] They found that patients who develop PD reported a higher incidence of tremor (Relative Risk -RR- 13.70; 95% CI, 7.82 to 24.31) and balance disturbances (RR 2.19; 95% CI, 1.09 to 4.16) 5 years prior to diagnosis, compared with controls. Interestingly, the incidence of tremor (RR 7.59; 95% CI, 1.11 to 44·83) was higher even 10 years before diagnosis. The THIN database covered a mainly White and higher-income population.

In the present project, we wanted to know what the pre-diagnostic manifestations (particularly motor symptoms) of PD that present to primary care are. We used a similar approach to the THIN study, but in an ethnically diverse population from East London, with some of the highest levels of deprivation in the UK.

2.2 Methods

2.2.1 Study design

We performed a nested case-control study in a large primary care dataset in East London. Primary care data were compiled from searches of the EMIS (Egton Medical Information Systems) electronic healthcare records system for the SHARE project (Secure Health Analysis and Research in East London). The database included health records of 1016277 patients from general practices across four Clinical Commissioning Groups (CCGs) in East London: Hackney & City of London, Newham, Tower Hamlets and Waltham Forest. The proportion of practice/population coverage is mentioned in Appendix A. Use of EMIS began in the UK in 1990 and paper records acquired prior to this were manually transcribed into the system. In the UK National Health System (NHS), individuals are identified by a unique NHS number which enables linkage to their medical records. When individuals move between healthcare providers, their records move with them. All non-emergency secondary care referrals originate from primary care, and outcomes are communicated back, thus primary care records represent aggregated medical information about an individual throughout their life. The NHS Health Research Authority waived the need for ethical approval when using anonymised datasets such as these.

2.2.2 Identification of cases and controls

All individuals with a code diagnosis of "Parkinson's disease" were included as cases in the analysis. Patients with PD but missing a date of diagnosis were excluded as well as those with a coded diagnosis of dementia, atypical Parkinson's and other neurodegenerative conditions including multiple sclerosis and motor neurone disease (Table A2.1). Controls were those without a code of "Parkinson's disease" or other chronic neurological conditions including dementia, multiple sclerosis, atypical Parkinson's and motor neurone disease (Table A2.2). Controls were assigned a "dummy date of PD diagnosis", which was calculated as follows: the median age of PD diagnosis (69.0 years) was added to the year of birth of each control to create a dummy date of 'diagnosis' in controls. Then the earliest date between the "dummy date of PD diagnosis" and 6th February 2018, which was when the database was locked, was used as the time point to categorise the selected exposures as pre-diagnostic risk factors. The minimum required age in both groups was 18.

2.2.3 Exposure selection and extraction

Exposures were selected based on a comprehensive meta-analysis of pre-diagnostic features and risk factors for PD, carried out by some members of our research group in 2012[93] and three

other large studies of the pre-diagnostic phase of PD [85,192,195]. Epilepsy and hearing loss were included, given preliminary evidence that these might be pre-diagnostic features of PD.[191,192,196]

Overall, 24 exposure variables were selected and subdivided into three categories: 1) comorbidities and risk factors, 2) pre-diagnostic motor manifestations and 3) pre-diagnostic non-motor manifestations (metabolic, sensory, autonomic and neuropsychiatric). Individual patient information was extracted by the Clinical Effectiveness Group (CEG) at Queen Mary University of London on 6th February 2018. All exposures were recorded up to twice on our database (earliest ever record and the most recent record). Where there were repeat observations, the earliest date was used for the analysis.

Given the cross-sectional nature of data extraction, incidence rates could not be calculated. However, we wished to examine temporal relationships and so three periods of time were established to evaluate exposure-outcome associations (<2 years, 2 to <5 years, and 5 to <10 years before PD diagnosis/dummy diagnosis)[IV]. We selected the same periods used in the THIN primary care analysis to make findings comparable and see whether there were differences between the two different populations with divergent socio-economic backgrounds and ethnicities.[85] Exposure variables with less than 1% prevalence among PD cases across all time periods were excluded from the analysis (e.g. smell loss and subjective RBD).

2.2.4 Definition of Exposures

The variables extracted were based on identified data comprising diagnoses, laboratory results, and demographic details coded using the Read coding system (https://digital.nhs.uk/services/terminology-and-classifications/read-codes). Variables were defined so that as much data as possible could be used in the modelling. For this reason, missing data were categorised as "unknown" in the models rather than excluded.

Age. Age was taken as the age at data extraction (6th February 2018).

Ethnicity. Ethnicity was defined by the self-reported UK census categories, grouped here into major ethnic groups in the East London population: White (British, Irish, Other White), Black (African, Caribbean, Other Black), South Asian (Bangladeshi, Indian, Pakistani), other (Chinese, other and mixed groups) and unknown.

Index of Multiple Deprivation (IMD). IMD is a global measure based on socio-economic terms, including income, employment, education, health, crime, housing and environment. Raw IMD scores were assigned to the national deciles derived from national data and converted into quintiles. Quintile 1 (IMD 1 to 2) represented the most deprived 20% and quintile 5 (IMD 9 to 10) the least deprived 20%. IMD group 1 to 2 was used as the reference category in the analyses.

[IV] Hereafter: <2 years, 2 to 5 years, 5 to 10 years

Vascular risk factors, smoking, body mass index. For detailed information of how all the factors were defined and extracted, see Appendix A.

Other comorbidities. Hearing loss and epilepsy were defined by a coded diagnosis. In the case of hearing loss, a referral for assessment due to reported hearing difficulty was also included.

Motor and non-motor pre-diagnostic manifestations. Motor features included rigidity, tremor and balance difficulties. Clinical symptoms of PD included coded non-motor features such as memory problems, depression, anxiety, fatigue, erectile dysfunction, shoulder pain, neck pain and constipation.

2.2.5 Statistical modelling

For the periods from <2 years, 2 to 5 years and 5 to 10 years before the index date (date of diagnosis), the overall occurrence of pre-diagnostic symptoms was calculated as the absolute number and percentage. A matched case-control analysis was run by matching 10 controls for each case according to age (calendar year) and sex. This was used to estimate OR for PD and 95% CI for each variable of interest, in each period and in all three periods combined. The matched analysis was then re-run, adjusting for ethnicity and IMD. A separate multivariable logistic regression model, which included PD cases and all controls, was run and estimates for each exposure of interest were adjusted for age and sex. To examine an association with PD as a trend across IMD quintiles, conditional logistic regression was undertaken, treating IMD quintiles as continuous. To examine a difference in the odds of PD by ethnicity, a comparison of the logistic regression models with and without ethnicity was undertaken using a likelihood ratio test. In a sub-analysis, we examined associations between pre-diagnostic symptoms and subsequent PD, stratified by ethnic group. For this analysis, we used the unmatched analysis and the full pre-diagnostic period, excluding the 2 years closest to diagnosis (i.e. 2 to 10 years) and adjusting for age and sex. The analyses were performed using R, version 4.0.2; Stata, v.13 (StataCorp, College Station, TX).

2.2.6 Ethical and governance approvals

The data used was based on routinely collected data in GP electronic health records which is de-identified and published using aggregate counts and did not require ethics committee approval. The CEG is the data processor, and the General Practices in the four CCGs are the data controllers. CEG has the written consent of all practices in the study area to use pseudonymised patient data for audit and research for patient benefit. The researchers adhere to the data protection principles of the Data Protection Act 2018, and all data were managed according to UK NHS information governance requirements. All outputs were in the form of aggregate patient data. The NHS Health Research Authority toolkit (http://www.hra-decisiontools.org.uk/ethics/) identified that Research Ethics Approval was not required for this

project as all data are pseudonymised and presented in aggregate form. This was confirmed by the Chair of the North East London Strategic Information Governance Network. All necessary patient/participant consent has been obtained and the appropriate institutional forms have been archived.

2.3 Results

2.3.1 Demographic information

This case-control study included 1055 patients with PD and 1009523 controls. Demographic information for cases and controls is summarised in Table 2.1. Patients with PD were more likely to be older (mean age for cases 72.9 years; controls 40.3 years) and male (cases 59.9% male; controls 51.2% male). No association with PD was found when examining an association across IMD (p=0.710), but most participants (>75%) resided in the most deprived IMD quintile. However, the prevalence of PD was higher in the most affluent areas compared with most deprived areas (0.23 vs 0.1). The ethnicity of participants reflected the diversity of the local population in East London and was similar among PD cases and controls (likelihood ratio test: p=0.180).

Table 2.1 Demographic information on PD cases and unmatched controls in East London primary care data

	PD n=1055	Controls n=1,009,523	Prevalence (% PD cases per group)
Age (years): Mean (SD)	72.9 (11.3)	40.3 (15.2)	NA
Female, n (%)	423 (40.1)	492,647 (48.8)	0.09
Male, n (%)	632 (59.9)	516,862(51.2)	0.12
Ethnicity*			
Black	166 (15.7)	134,629 (13.3)	0.12
South Asian	208 (19.7)	216,763 (21.5)	0.10
White	537 (50.9)	441,522 (43.7)	0.12
Other	88 (8.3)	114,503 (11.3)	0.08
Unknown	56 (5.3)	102,106 (10.1)	0.05
IMD			
1-2 (most deprived)	472 (44.7)	453,747 (44.9)	0.10
3-4	419 (39.7)	424,944 (42.1)	0.10
5-6	68 (6.4)	76,773 (7.6)	0.09
7-8	25 (2.4)	19,258 (1.9)	0.13
9-10(least deprived)	16 (1.5)	6,895 (0.7)	0.23
Unknown	55 (5.2)	27,906 (2.8)	0.20

* Ethnic groups: White (British, Irish, Other White), Black (African, Caribbean, Other Black), South Asian (Bangladeshi, Indian, Pakistani), other (Chinese and mixed groups). Unknown: missing information

Association with midlife risk factors and comorbidities

Table A2.3 in Appendix A summarises associations of comorbidities or risk factors with PD over three time periods. The main matched analysis (matching 10 controls for each case according to age and sex) showed that the strongest association was for epilepsy across each of the three time periods analysed (<2 years: OR 10.00; 95% CI, 1.41 to 70.99; 2 to 5 years: OR 5.00; 95% CI, 1.25 to 19.99; 5 to 10 years: OR 5.46; 95% CI, 2.02 to 14.76). When we analysed all three periods together, the association was weaker (OR 2.50) but more precise (95% CI, 1.63 to 3.83).

Vascular risk factors such as hypertension and T2D were associated with subsequent PD long (5 to 10 years) in advance of diagnosis. In contrast, there were no associations with cholesterol. Being underweight, but not overweight, in the period closest (<2 years) to diagnosis was also associated with higher odds of PD.

Although ethnicity and IMD were not associated with PD, we adjusted for both co-variates in the matched analysis. There was no suggestion of confounding (<10% relative difference between unadjusted and adjusted OR) for associations of midlife comorbidities (Table A2.3 in Appendix A).

2.3.2 Pre-diagnostic manifestations of Parkinson's Disease

Table 2.2 summarises motor manifestations in the three periods of time from the matched case-control analysis. Table A2.4 in Appendix A gives information about non-motor exposures including neuropsychiatric, autonomic, sensory and metabolic domains. Missing data of motor exposures was less than 1.5% in controls and between 1% and 3% in PD patients. To be able to do a logistic regression analysis, we included 'missing data' as a third category and only used the OR for the presence or absence of each exposure.

Motor manifestations

Tremor was most strongly associated with subsequent PD across the three time periods. Most presentations of tremor were within 2 years of PD diagnosis (25% cases and <1% of controls; OR, 151.24; 95% CI, 93.74 to 244.02). The prevalence of tremor was also higher in the PD group compared with controls in the two earlier periods (2 to 5 years: OR, 14.51; 95% CI, 9.02 to 23.30; 5 to 10 years: OR, 11.40; 95% CI, 6.43 to 20.22). Individuals who went on to receive a diagnosis of PD had a higher prevalence of balance difficulties across the three periods (4%) compared with controls (2%). Although rigidity was more frequently reported in the PD group than controls, it was not as common as other motor features, with a prevalence of 1.2% during the first period (<2 years) and <1% within the other two periods.

There was no suggestion of confounding for ethnicity and IMD the association of pre-diagnostic features with the future risk of PD (Table 2.2).

Non-motor manifestations

Cognitive symptoms were the most frequently reported non-motor manifestations. Almost 5% of patients who were subsequently diagnosed with PD presented with 'memory symptoms', compared with <1% controls. Within 2 years of diagnosis, people with 'memory symptoms' had an approximate 9-fold increased odds of PD (OR 8.73; 95% CI, 6.0 to 12.70). This association remained up to 5 years prior to diagnosis but not further (2 to 5 years: OR 3.09; 95% CI, 1.81 to 5.26; 5 to 10 years: OR 2.01; 95% CI, 0.93 to 4.31).

Overall, patients in East London presenting to primary care with psychiatric symptoms including depression, anxiety, fatigue and insomnia within 2 years prior of diagnosis had about 2 to 4-fold increased odds of receiving a PD diagnosis (depression: OR 4.61; 95% CI, 2.82 to 7.52, anxiety: OR 3.01; 95% CI, 2.02 to 4.50; fatigue: OR 1.86; 95% CI, 1.21 to 2.85; insomnia: OR 2.17; 95% CI, 1.35 to 3.48) compared to those without.

Associations between pain and subsequent PD varied according to the location of pain. Shoulder pain was associated with a doubling of the odds of PD diagnosis up to 5 years prior to diagnosis (<2 years: OR 2.25; 95% CI, 1.52 to 3.33; 2 to 5 years: OR 1.89; 95% CI, 1.33 to 2.68), whereas neck pain did not show an association with PD across the three periods.

Autonomic symptoms such as constipation and hypotension, together with hearing loss, were also more common amongst people who went on to develop PD in the future.

2.3.3 Unmatched analysis

Table A2.5 and A2.6 in Appendix A summarise the same midlife comorbidities and pre-diagnostic manifestations using an unmatched analysis, adjusted for age and sex. In general, the results from the unmatched analysis were similar to those from the matched analysis. However, in contrast with matched analysis, when increasing the sample size by including all controls, the association between memory symptoms and future risk of PD remained positive up to 10 years prior to diagnosis (OR 2.78; 95% CI, 1.38 to 5.61). Additionally, in the unmatched analysis, neck pain showed an association with subsequent PD, although it was weaker than the association with shoulder pain (ORs ranging between 1.50 and 1.71) (Table 2.3).

Table 2.2 Matched case-control analysis for direct and indirect motor markers according to time of presentation

		Time period								
		<2 years			2-<5 years			5-<10 years		
Exposure	**Category**	**% (PD: Control)**	**Unadjusted OR**	**Adjusted OR**	**% (PD: Control)**	**Unadjusted OR**	**Adjusted OR**	**% (PD: Control)**	**Unadjusted OR**	**Adjusted OR**
Shoulder pain	Sensory/ indirect motor	31 (2.9%): 142 (1.3%)	2.23 (1.5 to 3.3)	**2.25 (1.52 to 3.33)**	39 (3.7%): 212 (2%)	1.88 (1.33 to 2.66)	**1.89 (1.33 to 2.68)**	45 (4.3%): 348 (3.3%)	1.32 (0.96 to 1.81)	1.31 (0.95 to 1.80)
Neck pain		26 (2.5%): 188 (1.8%)	1.39 (0.92 to 2.11)	1.39 (0.92 to 2.11)	32 (3%):235 (2.2%)	1.38 (0.95 to 2.01)	1.37 (0.94 to 1.99)	36 (3.4%): 300 (2.8%)	1.21 (0.85 to 1.72)	1.21 (0.85 to 1.72)
Tremor	Motor	267 (25.3%): 26 (0.2%)	145.96 (90.55 to 235.28)	**151.24 (93.74 to 244.02)**	42 (4%): 29 (0.3%)	14.48 (9.02 to 23.25)	**14.51 (9.02 to 23.3)**	26 (2.5%): 24 (0.2%)	11.66 (6.59 to 20.64)	**11.4 (6.43 to 20.22)**
Rigidity		13 (1.2%): 1 (0%)	129.99 (17.01 to 993.63)	**124.84 (16.3 to 956.36)**	1 (0.1%): 4 (0%)	2.5 (.28 to 22.37)	2.48 (0.28 to 22.26)	2 (0.2%): 2 (0%)	10 (1.41 to 71.0)	**8.27 (1.14 to 59.8)**
Balance difficulties		44 (4.2%): 187 (1.8%)	2.42 (1.73 to 3.39)	**2.4 (1.71 to 3.36)**	42 (4%): 202 (1.9%)	2.14 (1.52 to 3.01)	**2.1 (1.49 to 2.95)**	33 (3.1%): 223 (2.1%)	1.51 (1.04 to 2.19)	**1.49 (1.02 to 2.17)**

Matched case-control analysis: matching 10 controls for each case according to age and sex (unadjusted) and adjusted for ethnicity and IMD. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date (PD diagnosis). OR: Odds Ratio. CI: Confidence Interval. PD: Parkinson's disease patients (n=1055), controls (n=10,550). In bold: significant association (CI without including 1)

Table 2.3 Unmatched analysis (adjusted for age and sex) for direct and indirect motor markers according to time of presentation

Exposure	Category	<2 years		2-<5 years		5-<10 years	
		% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95%CI)
Neck pain	Sensory /Indirect motor	26 (2.5%): 15077 (1.5%)	**1.71 (1.16 to 2.53)**	32 (3%): 19585 (1.9%)	**1.67 (1.17 to 2.39)**	36 (3.4%): 25424 (2.5%)	**1.5 (1.07 to 2.09)**
Shoulder pain		31 (2.9%): 5594 (0.6%)	**2.54 (1.77 to 3.65)**	39 (3.7%): 8003 (0.8%)	**2.2 (1.59 to 3.04)**	45 (4.3%): 15537 (1.5%)	**1.68 (1.24 to 2.27)**
Rigidity		13 (1.2%): 110 (0%)	**89.35 (46.88 to 170.31)**	18 (1.7%): 125 (0%)	5.3 (0.72 to 38.95)	2 (0.2%): 158 (0%)	**7.73 (1.86 to 32.14)**
Balance difficulties	Motor	44 (4.2%): 11999 (1.2%)	**3.54 (2.61 to 4.81)**	42 (4%): 15608 (1.5%)	**2.74 (2 to 3.74)**	33 (3.1%): 15916 (1.6%)	**2.07 (1.46 to 2.94)**
Tremor		267 (25.3%): 1094 (0.1%)	**181.69 (151.91 to 217.31)**	42 (4%): 1334 (0.1%)	**21.32 (15.3 to 29.71**	26 (2.5%): 1331 (0.1%)	**14.61 (9.71 to 21.98)**

Standard: multivariable logistic model for PD with OR and 95% CI adjusted for age and sex. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date. OR: odds ratio. CI: Confidence Interval. In bold: significant association (CI without including 1)

2.3.4 Ethnicity sub-analysis

In a sub-analysis, stratifying exposure variables by ethnic group, many consistent associations were observed for midlife comorbidities (Figure A2.1 in Appendix A), non-motor manifestations (Figure A2.2 in Appendix A) and early motor markers (Table 2.4 and Figure 2.1). Some differences were observed. In contrast to tremor and balance impairment, rigidity was not reported in Black patients prior to diagnosis (sparse data) and was weakly associated in South Asian patients.

Table 2.4 Prevalence* of motor symptoms (tremor, rigidity and/or balance difficulties) amongst PD patients according to ethnicity together with their association with future PD

Ethnicity	Absent	Present	OR (95% CI)
Black	93 (56%)	73 (44%)	1.18 (0.83 to 1.68)
S Asian	108 (52%)	100 (48%)	1.40 (1.01 to 1.93)
White	323 (60%)	214 (40%)	1
Other	51 (58%)	37 (42%)	1.10 (0.69 to 1.73)
Unknown	43 (77%)	13 (23%)	0.46 (0.24 to 0.87)

*The proportion covered the whole 10-year period studied. Ethnic groups: White (British, Irish, Other White), Black (African, Caribbean, Other Black), South Asian (Bangladeshi, Indian, Pakistani), other (Chinese and mixed groups)

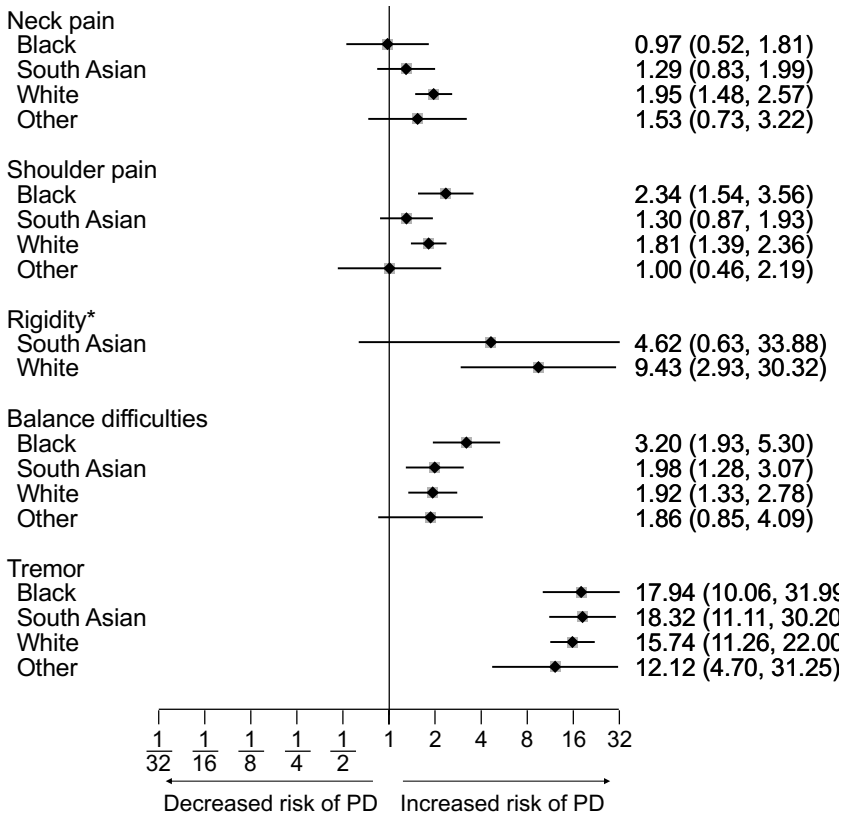

Figure 2.1 Forest plot depicting motor pre-diagnostic manifestations of PD across ethnic groups in the East London population. Data points represent odds ratios +/− 95% confidence intervals. *There was no reported rigidity in the Black group.

2.4 Discussion

We used a large primary care dataset to explore risk factors and early clinical manifestations of PD in a highly diverse and generally deprived population. Based on the 2011 UK Census, London had the greatest ethnic diversity of anywhere in the UK, with the highest proportion of Black, South Asian, and mixed/other ethnic groups, which comprise ~45% of residents in East London compared with 14% in the rest of the UK. East London has one of the highest unemployment rates in the UK (6.7%) and 75% of patients included in this analysis resided in the lowest quintile of national wealth. There was no association between ethnic group or index of multiple deprivation and odds of PD in our data, suggesting that ethnicity and deprivation may not play a major role in PD risk, in contrast to what has been reported for dementia.[197] In line with existing literature[198], the prevalence of PD in East London remained higher in the more affluent population sub-groups. It is known that lower economic status is associated with increased mortality in the general population; the 'protective' association between low socioeconomic status and risk of PD could be explained by a reverse causality due to an increased mortality rate[199]. However, more research is needed to explore the role of environmental and social factors in PD aetiology.

We demonstrated a constellation of symptoms that present to GPs up to a decade before diagnosis of PD. Similar findings have been reported in more homogenous populations.[75,85,161] In the main (matched) analysis, motor and cognitive symptoms were strongly associated with PD up to 10 and 5 years before the diagnosis respectively. When we repeated the analysis, using an unmatched but age and sex adjusted approach (with a larger sample size), there was an association with both factors up to 10 years before diagnosis.

This is the first study focusing on the pre-diagnostic phase of PD in such a diverse population with universal access to healthcare. Plouvier and colleagues carried out a nested case-control study using primary care data in a small sample size and a time period up to 2 years before diagnosis.[76] They found that PD patients presented more often with functional complaints, autonomic symptoms and sleep problems than controls. Another study conducted in the Netherlands used primary care data to compare a group of 60 PD patients and 58 controls.[75] They identified a pre-diagnostic period of 4 to 6 years, comprising a wide range of non-motor manifestations. Neither of these studies reported on motor manifestations prior to diagnosis. A UK study using THIN primary care data shared a similar approach to our present study.[85] Both compared the medical records of a large sample of people with PD to healthy controls using the same time periods (<2 years, 2 to 5 years, and 5 to 10 years) and had similar age and sex distribution. The THIN data differs substantially from East London data in terms of wealth and ethnicity. Tremor was the strongest marker of subsequent PD in both studies. Prescribing data were not available in our study, whereas it was for the THIN analysis, and it was used to help define exposures. This may explain the higher prevalence of certain symptoms in the

THIN analysis (e.g. depression, erectile dysfunction and constipation). However, there may be other reasons for under-ascertainment of symptoms in East London.

Of the pre-diagnostic clinical manifestations, tremor showed the strongest association with subsequent PD, which was present up to 10 years prior to diagnosis. Unsurprisingly, various studies have demonstrated that tremor may be an early feature of PD.[4,85,200,201] In people reporting tremor years before diagnosis, one must consider the possibility that diagnosis is simply delayed in primary care. That is, individuals who in expert hands would have been considered to have PD may not be referred early enough to a movement disorders specialist. Rigidity is a sign rather than a symptom, which might explain why it is rarely reported by patients. Indirect symptoms of rigidity (i.e. shoulder pain but not neck pain) were more common in people with subsequent PD than controls, with an association observed up to 5 years prior to diagnosis. The association between shoulder pain and future PD has already been reported in two other cohorts.[75,85] What stands out in our database is the lack of symptoms related to the other cardinal features of PD one of which is bradykinesia. One reason why bradykinesia might have been underreported is the lack of awareness which has been classically described amongst patients[24]. It is not uncommon that patients with PD have a perceptual agnosia to their motor impairment. As mentioned in Chapter 1 (Box 1.1), asking pro-actively to patients and relatives about problems related to slowness and lack of dexterity might be a way to uncover symptoms derived from bradykinesia. Gaenslen and collaborators took this approach and retrospectively evaluated the presence of several early motor signs, among which there were indicative symptoms of bradykinesia – general slowing and slowing of fine movements. They also asked for other motor manifestations such as hypophonia, dysarthria, sialorrhea and unilateral reduced arm swing.[161] Of note, tremor was not included in the questionnaire. All the motor signs, except for dysarthria, were more common in people who went on to develop PD in the future. Sialorrhea seemed to be the earliest sign (5 years prior to diagnosis), whereas bradykinesia and reduced arm swing were reported closer (less than 3 years) to diagnosis.

Compared to THIN primary care data, the association between cognitive symptoms and subsequent PD was stronger in the East London population and may be a population-specific observation.[85] Black patients with PD have been reported as being more likely to have cognitive impairment.[202,203] In a study comparing Black and Asian patients to White patients with PD, it was found that Black people with PD had a greater cognitive impairment assessed using the MMSE than White patients.[203] However, the method of assessment used may have produced artificially lower scores in certain groups due to bias induced by language, cultural and social determinants. It is notable that both ethnicity and deprivation appear to be determinants of dementia risk (albeit apparently not PD risk), which may explain the greater association between cognitive symptoms and PD in this setting.[197] Depression and anxiety were associated with subsequent PD, but the associations were smaller compared to other previous studies.[75,76,85] It would be interesting to study whether there exists an interactive effect when two exposures, such as cognitive complaints and depression, coexist in the same individual.

This might help to identify clusters of pre-diagnostic manifestations in PD, which can differ depending on clinical phenotypes (for example, PD and PD with dementia).

We believe our findings might raise awareness amongst primary care physicians about pre-diagnostic manifestations. This will potentially have important practical considerations for and the opportunity to address patient concerns at an earlier stage. It is not a case of 'screening' for asymptomatic disease but correctly identifying the underlying cause in patients who are presenting with symptoms and may seek timely onward referral. Patients might otherwise wait for up to 10 years for an explanation of their symptoms. Early treatment of symptoms (motor and non-motor) may improve quality of life even if patients do not fulfil the clinical diagnostic criteria of PD yet.[22] To this end, we should focus more on timely diagnosis rather than simply earlier diagnosis.[23] Table 2.5 outlines practical advice and observations that might serve a purpose in primary care.

Table 2.5 Key findings and implications for primary care practitioners

Findings	Interpretations/Implications
A broad range of motor and non-motor symptoms cause patients to consult primary care practitioners up to a decade before PD diagnosis	Practitioners in primary care should be aware of the range of these presentations and consider PD as a possible cause. It is important to weigh the merits of an early diagnosis compared with a timely diagnosis. Where patients are concerned and seeking an explanation, then onward referral to a specialist movement disorders service may be warranted.
Tremor is reported up to 10 years prior to eventual diagnosis of PD	Patients presenting in primary care with a tremor should be referred to a specialist movement disorders service.
Some motor symptoms (slowness and stiffness) may be under-reported	Practitioners in primary care should consider asking direct questions about motor symptoms to patients in whom early PD is suspected and checking for parkinsonian signs as part of routine neurological examination.
Shoulder pain, and to a lesser extent neck pain, might be an indirect/early sign of rigidity	Consider checking for parkinsonian signs in patients with treatment-resistant shoulder pain, who lack signs of an underlying osteoarticular condition.

There are several limitations to this study. The main limitation is that these data are derived from routinely collected primary care data with under-ascertainment of factors of interest and high missingness. Although the year of recording for each variable was available, data were extracted in a cross-sectional manner, meaning that incidence rates could not be calculated. Another caveat to our study is that, although the NHS provides free care at the point of access to all patients, there may still be under ascertainment of PD cases. For example, there is preliminary evidence for atypical presentations in ethnic minority groups[204] and a higher likelihood of being (mis)labelled with vascular mimics of neurodegenerative disease.[202]

However, we did not observe significant differences in PD prevalence by ethnic group. Another limitation is the lack of information regarding prescriptions of medication which meant that it was not possible to create a more robust definition of PD, or include additional cases not recorded as PD but who were prescribed anti-parkinsonian medication, or exclude cases with drug-induced parkinsonism. Ascertainment of exposure variables and risk factors may also be incomplete, with mild or transient symptoms not being reported or recorded. Furthermore, some symptoms lack context. For example, 'memory problems' recorded in primary care often lack supportive neurological examination or standardised neuropsychological testing to support a formal diagnosis of cognitive impairment. Finally, we excluded patients with PD and associated formal diagnosis of dementia. This fact prevented us from studying whether people with Lewy Body dementia have a different prodromal phenotype of PD, which could have important prognostic implications.

In summary, this study provides evidence that motor symptoms are reported by patients in a primary care setting up to 10 years before diagnosis. Tremor was the pre-diagnostic manifestation with the highest association with future PD.

In the next chapter, I aimed to determine whether early clinical markers of motor deterioration exist in a population estimated to be at risk of developing PD. Considering the rather contradictory results concerning the prevalence of symptoms derived from bradykinesia across studies, I focused my attention on trying to capture prodromal signs of bradykinesia to be applied for the design of objective motor tools adapted to the prodromal phase of PD.

Chapter 3

Evolution of subthreshold parkinsonism in the PREDICT-PD cohort over time

3.1 Introduction

The PREDICT-PD cohort is a longitudinal population-based study which aims to stratify people from the general population aged between 60 and 80 at risk of developing PD. The study was established in 2011 with 1323 participants enrolled. Chapter 1 provides more details about the study. At baseline, a representative sample of people in the higher and the lower/intermediate risk group were selected to be examined in person, looking for the presence of Subthreshold Parkinsonism (SP). Participants classified at risk for subsequent PD were found to be more likely to have SP than individuals in the lower risk group.[2]

This chapter presents the follow-up of those participants examined at baseline after approximately 6 years. The main aim was to explore whether SP remained more prevalent in the Higher Risk (HR) group compared with those in the Lower Risk (LR) group. Moreover, having two consecutive observations allowed for the study of the evolution of SP over time.

3.2 Methods

Before participants were seen in person, they enrolled in the study online and carried out a series of assessments through the PREDICT-PD platform. A selected sample of participants were seen in 2012 (baseline) and 2018 (follow-up). The baseline assessment was done by Dr Alastair Noyce; therefore, it will be not included in this current thesis. The cross-sectional aspect illustrates the clinical situation in 2018. The longitudinal study is focused on clinical changes across this 6-year period (2012-2018).

The PREDICT-PD study was approved by Central London Research Committee 3 (reference number 10/H0716/85) and carried out in accordance with the Declaration of Helsinki.

3.2.1 Online assessment

Chapter 1 describes the online assessment of PREDICT-PD study in detail and the updated ('enhanced') PREDICT-PD algorithm. Here, I will focus on describing the assessment I carried out in person. The enhanced PREDICT-PD risk score was used to classify individual risk using the first assessment corresponding to 2012, as it was close to the date of in-person assessments at baseline. Since risk score changes over time, I used the risk score at baseline to ensure a pairwise comparison between baseline and follow-up.

3.2.2 In-person assessment

A representative sample of participants were selected to be seen at baseline. Six years later, they were contacted again to be followed up in person. I was blind to the risk scores and both examinations were recorded at home to minimise observer bias. Baseline and follow-up exams shared most of the assessments which will be used in the cross-sectional and longitudinal analysis. There were few exams that were added at follow-up, therefore I only included them in the cross-sectional analysis.

To make results comparable to the longitudinal analysis, I used the same motor scale as for the baseline and the follow-up assessment. Participants were recorded at both time points following the same instructions from the motor part (III) of MDS-UPDRS.[163] Given that it is a semi-quantitative scale, high inter-observer variability is expected. For that reason, I scored both clinical examinations, except for rigidity for which I used Noyce's scores. To ensure one assessment was not influencing the score of the other, videos recorded at baseline were scored 6 months after completing rating of the whole batch of follow-up examinations.

In addition to the MDS-UPDRS-III, the follow-up assessment included two new motor tasks measuring upper and lower extremity function: a handwriting task and the TUG test respectively. In the former, participants were timed while copying the sentence '*Mary had a little lamb, its fleece was white as snow*' three times using a pen and white paper. The TUG is described in Chapter 1 (section 1.7.6). In brief, it consisted of timing how long it took for an individual to rise from a chair, walk three metres at a comfortable pace, turn round, walk back, and sit down again. I asked participants to perform the task three times without assistance including walking aid.

Finally, participants completed the Montreal Cognitive Assessment (MoCA). It is a widely used screening instrument to detect cognitive impairment in PD and PD-dementia.[205] It assesses a series of cognitive domains which included visuo-constructional and executive skills, short term memory, attention skills, and orientation. Participants also completed a series of questionnaires asking for non-motor aspects of experiences of daily living (MDS-UPDRS part I), motor symptoms (MDS-UPDRS part II) and symptoms of autonomic dysfunction (SCales for Outcomes in PArkinson's disease-Autonomic Dysfunction -SCOPA-AUT). Except for the MoCA test, participants at baseline did not complete any of the questionnaires mentioned above (MDS-UPDRS-I, II and SCOPA-AUT), therefore they will only be included in the cross-sectional section.

3.2.3 Motor progression outcomes

New diagnoses of PD are the main outcome for the PREDICT-PD study. However, given the low rate of incident PD, studies require long follow-up and a large sample size. In light of the low incidence of PD, I took three possible surrogate markers of PD to be used as a binary outcome for our prediction model: **outcome 1**) development of SP; **outcome 2**) motor decline

(≥5-point change in the MDS-UPDRS-III); and **outcome 3**) presence of single motor domains in the MDS-UPDRS-III. Finally, I explored the relationship of continuous risk estimates with the MDS-UPDRS-III total scores (**outcome 4**). The association between the enhanced PREDICT-PD algorithm with incident SP after 6 years of follow-up was tested in this cohort and in another well-characterised cohort, the Bruneck study.[101]

Although the MDS-UPDRS-III is normally used in patients with established PD, the MDS Task Force established a cut-off >6 (excluding action -postural and kinetic- tremor from the total score) to define people with SP (**outcome 1**). Louis and colleagues published another definition of SP.[206] Although appendicular bradykinesia was not included in that definition because it was considered a mandatory feature in the diagnostic criteria for PD, I decided to include it as an additional outcome to avoid underestimating the proportion of SP in our cohort. It was defined as being present when combined scores from the bradykinesia items (finger tapping, hand movements, hand pronation/supination, toe tapping and leg agility) were ≥ 1.[163] I used both criteria to compare how many participants have SP in each PD risk group. I took a detailed clinical history of those participants with SP and bradykinesia ('clear decrement in amplitude and/or frequency') to make sure none of them had possible PD. Clinical interview was targeted to check symptoms of parkinsonism and their duration.

For **outcome 2**, I examined whether the combination of SP at baseline and higher risk scores might predict a big motor decline (≥5-points change in the MDS-UPDRS-III)[207] over time. To do that, I divided participants into four groups (based on the presence or absence of SP and being classified into the HR and LR group) and compared their motor trajectories.

Finally, individual items in the MDS-UPDRS-III were studied separately as single motor outcomes (**outcome 3**). Bradykinesia was considered present when the combination of all the tasks designed to assess bradykinesia (finger tapping, hand movements, hand pronation/supination, toe tapping and leg agility) scored >1. The same criteria were applied for action tremor (postural tremor and kinetic tremor) and rigidity. The other motor domains (rest tremor and abnormal gait) were dichotomised based on their presence or absence. A score of 1 point was considered positive.

3.2.4 Statistical analysis

Data normality was assessed using the D'Agostino test. I obtained summary statistics and checked for data outliers and skew with histograms and boxplots. I calculated the mean and Standard Deviation (SD) for normally distributed data and median and Interquartile Ranges (IQR) for non-normal distributed data. To see if the sample selected for the in-person analysis was representative of the original cohort, I compared demographic and clinical data from the questionnaires and remote tests with those not seen in-person. I compared the group of participants seen at baseline (excluding those who were not seen in person 6 years later) with

the original PREDICT-PD cohort, risk scores were used at baseline to classify them into higher and lower risk.

I divided the statistical analysis plan in two sections: cross-sectional study and follow-up study. The former aimed to test the null hypothesis of there being no difference in the motor scores between individuals in the HR and the LR group. The latter aimed to test the null hypothesis of there being no differences in change over time between current and baseline motor scores. For both cross-sectional and follow-up analysis, I presented categorical variables by absolute frequency and percentage and compared them with Fisher's exact test. I compared quantitative data for motor outcomes using the two-sample *t* test and Mann-Whitney U test, for normally and non-normal distributed data respectively.

I applied three separate logistic regression models to test the HR group as a predictor of SP (**outcome 1**), motor decline (**outcome 2**), and separate motor domains in the MDS-UPDRS-III (**outcome 3**). For each model, I calculated the ORs and 95% CIs. A replication analysis of outcome 1 was performed using data from the Bruneck Study cohort using the 2005 assessment as baseline and the 2010 assessment as follow-up, as previously published.[88] I excluded any intermediate motor marker from our algorithm as per the BRAIN test, in our cohort, and UPDRS-III at baseline, in the Bruneck cohort. I meta-analysed the data using fixed-effect model from both studies to calculate a single pooled estimate.

I used linear regression models to analyse the relationship of continuous likelihood ratio estimates of PD with the MDS-UPDRS-III (**outcome 4**). I performed a logarithmic transformation of risk scores to transform skewed data to approximately conform to a normal distribution. I used multivariate linear regression to examine the influence of potential confounding factors such as cognitive impairment, which was based on cognitive status using MoCA test scores, and risk factors for cerebrovascular disease, which included hypertension, hypercholesterolaemia and history of ischaemic heart disease. We did not include T2D because it is part of the PREDICT-PD algorithm. Although age can be expected to account for motor scores variation, it also has a strong weighting in the enhanced PREDICT-PD algorithm, rendering adjustment inappropriate. For this reason, I used another regression model without the contribution of age and sex[V] to the likelihood ratios and then adjusted for age and sex. Similarly, the enhanced algorithm included a motor marker (tapping speed scores from the BRAIN test). Since including another motor parameter (MDS-UPDRS-III) in the regression analysis could lead to multicollinearity, I repeated the analysis without tapping scores from the risk estimates.

All statistical tests were two-tailed. Since I present several analyses, I selected a more stringent cut-off for the level of significance ($p<0.010$; Bonferroni corrected for 5 independent hypothesis

[V] Risk scores without the contribution of age were obtained by applying the same formula. Instead of including an 'age-specific' prior risk, I used the same prior risk for everyone which was based on the average age of our cohort (60-80 years).

tests). This adjustment was done to ensure robustness of results and avoid false positives (i.e. type I error). I carried out data analysis using STATA v.13 (StataCorp, College Station, TX).

3.3 Results

From the baseline cohort (n=181), two participants died (both were in the LR group) and one participant, who also was in the LR group at baseline, withdrew from the study because of advanced dementia. By the time I carried out the in-person assessment, he had been admitted to a private residential home. He did not have any close family who I could contact or his NHS number, so it was not possible to find out more clinical details about his condition. Two participants from the HR group left the study due to medical conditions (macular degeneration and prostate cancer) and 12 participants dropped out from the study for personal reasons (HR: 2, LR: 10). Thirty-two participants were missing (HR: 8 -16.7%-, LR: 24 -18%-) and it was not possible to get in contact with them. In the end, 132 participants (HR: 36, LR: 96) were reviewed in person. Four participants were excluded in line with the exclusion criteria of the study: two were diagnosed with PD between baseline and follow-up assessments, and two had probable pharmacological parkinsonism (one was on Aripiprazole and the other one was on Quetiapine and Lithium due to long standing bipolar disorder). Finally, 128 participants (HR: 33, LR: 95) were included in the analysis (Figure 3.1). Separately, I followed up four participants who already had PD at baseline. Six years later, all of them remained stable on oral levodopa.

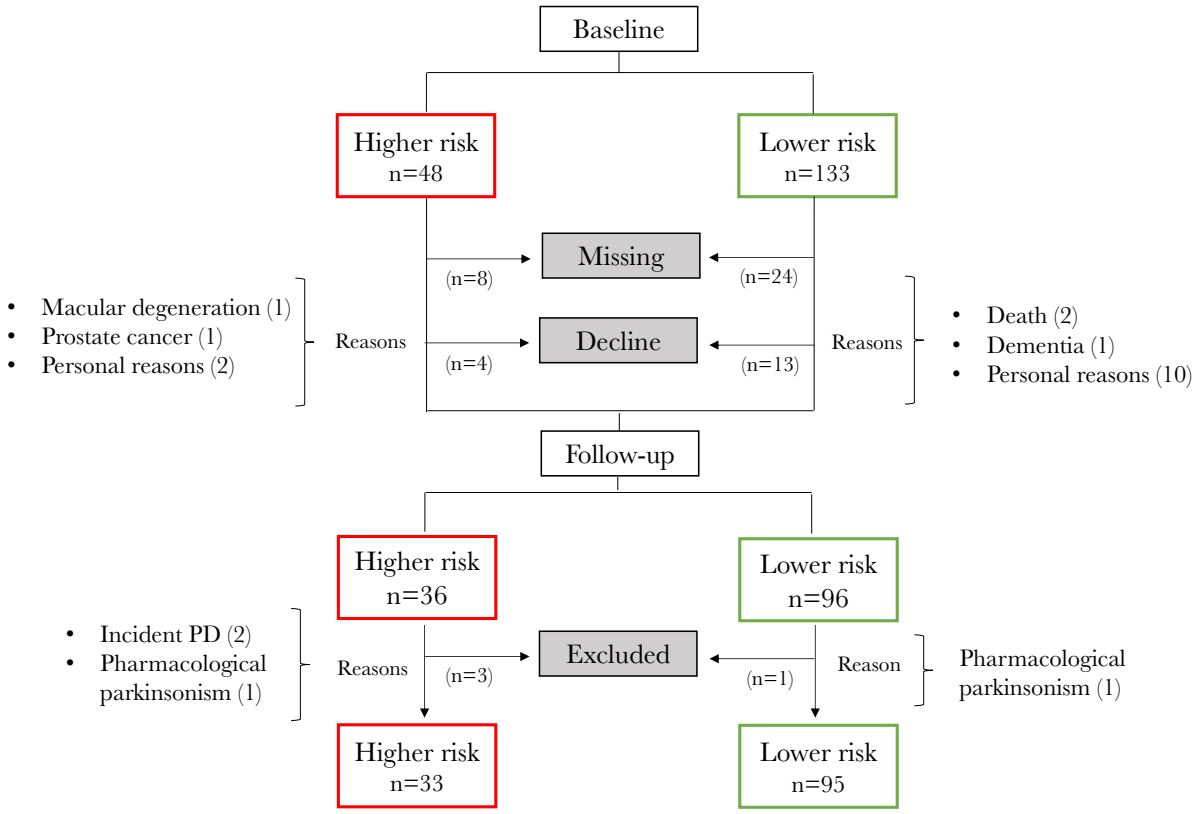

Figure 3.1 Flow chart showing dropouts from the baseline study. Between brackets (number of participants). The higher and lower risk groups were classified using the enhanced risk algorithm at baseline.

The HR participants selected for in person assessment were comparable in terms of demographic and clinical aspects with those HR individuals that were not seen in person in the pilot cohort (Table 3.1). It is important to note that despite the differences in the proportion of people with hyposmia among the HR group seen in-person compared to those not seen in-person, the available data in both groups were limited; 17 people out of 33 were seen in person and 62 people out of 133 were not seen in person. In the LR group, participants selected to be seen in-person were comparable to those not seen in-person except for having a greater proportion of current smokers in those seen in person compared with those not seen in person (8.4% vs 2.2%, p=0.004).

Table 3.1 Baseline demographic, proxy marker and risk factors on higher and lower risk participants seen in person compared with those that were not seen

	Higher risk			**Lower risk**		
	Seen (n=33)	Not seen (n=133)	p-value	Seen (n= 95)	Not seen (n=851)	p-value
Age (SD)	71.10 (4.60)	69.79 (5.25)	0.160	67.21 (5.07)	68.09 (4.56)	0.110
Male sex (%)	26 (78.79)	73 (54.89)	0.017	40 (42.11)	296 (34.78)	0.175
First-degree relative (%)	16 (48.49)	39 (29.32)	0.041	29 (30.53)	176 (20.68)	0.035
Current smoker (%)	1 (3)	4 (3)	1.000	8 (8.42)	19 (2.23)	0.004
Drink coffee (%)	29 (87.88)	118 (88.72)	1.000	89 (93.68)	768 (90.25)	0.355
Drink alcohol (%)	31 (93.94)	111 (83.46)	0.169	88 (92.63)	727 (85.43)	0.059
Constipation (%)	7 (21.21)	38 (28.57)	0.513	13 (13.68)	104 (12.22)	0.625
Use of pesticide (%)	3 (9.38)	5 (5.38)	0.421	1 (1.06)	16 (2.19)	0.709
Anxiety/Depression (%) *	5 (15.15)	22 (16.54)	1.000	12 (12.63)	103 (12.10)	0.869
Head injury (%)	13 (40.62)	55 (42.31)	1.000	22 (23.40)	198 (23.66)	1.000
Hyposmia ≤27/40 on UPSIT cases/available (%)	5/17 (29.41)	42/62 (67.74)	0.006	8/83 (9.64)	65/640 (10.16)	1.000
Subjective RBD ≥5 on RBDSQ cases/available (%)	12/30 (40)	29/119 (24.37)	0.109	9/90 (10)	77/798 (9.65)	0.852
Slow finger tapping KS ≤44 taps in 30s cases/available (%)	13/32 (40.62)	49/117 (41.88)	1.000	13/91 (14.29)	82/806 (10.17)	0.213
Baseline risk estimate, median (IQR)	16.70 (2.44-26.04)	10.28 (2.94-27.32)	0.449	1076.10 (291.18-6517.6)	861.96 (172.16-4145.0)	0.341

Information from online assessment at baseline and smell test at baseline. UPSIT, University of Pennsylvania Smell Identification Test; RBDSQ, RBD screening questionnaire; KS, kinesia score. * Defined as moderate in Hospital Anxiety and Depression Scale with score between 10 and 14, cases: participants with abnormal assessment, available: number of participants tested

3.3.1 Cross-sectional analysis

Six years after the baseline study, the remaining 33 HR participants seen in person were older than the 95 LR participants (mean (SD), 77.4 years (4.6) vs 73.6 years (5.0); p<0.001). There was a bigger proportion of males in the HR group compared with the LR (75.8% vs 43.2%; p=0.001). However, both groups had a similar proportion of vascular risk factors including T2D, hypertension, and high cholesterol (Table 3.2). Individuals at HR of PD had scores that were 2-fold higher on the non-motor aspects of experiences of daily living (UPDRS-I median score (IQR): 9 (5 to 12)) compared with the LR group (UPDRS-I median score (IQR): 4.5 (3.0 to 7.5); p=0.001). Moreover, the HR group scored higher in the SCOPA-AUT, with a median of 11 points compared with 8 points in the LR group (p=0.007). The median MoCA score in the HR and LR groups was 27 and 28 (IQR 25 to 28 and 26 to 29 respectively), and the sum of the ranks was larger in the HR group (p<0.001). Both groups had a similar education level (mean (SD), HR: 22.1 years (5.8); LR: 21.5 (7.1); p=0.603).

Table 3.2 Demographic information, risk factors and non-motor manifestations of participants

Follow-up information	Higher risk (n = 33)	Lower risk (n=95)	p-value
Age, mean; SD	77.4; 4.6	73.6; 5.0	<0.001[1]
Male (%)	25 (75.8)	41 (43.2)	0.001
T2D, n (%)	8 (24.2)	10 (10.5)	0.078
Hypertension, n (%)	17 (51.5)	42 (44.2)	0.545
High cholesterol, n (%)	12 (36.4)	25 (26.3)	0.275
MDS-UPDRS-I, median (IQR)	9 (5 to 12)	4.5 (3 to 7.5)	0.001[2]
SCOPA-AUT, median (IQR)	11 (7 to 15)	8 (4 to 11)	0.007[2]
MoCA, median (IQR)	27 (25 to 28)	28 (26 to 9)	0.001[1]

Higher and lower risk group: based on baseline risk scores, T2D: type 2 diabetes; MDS-UPDRS-I: non-motor experiences of daily living; MDS-UPDRS-II: motor experiences of daily living ; SCOPA-AUT: SCales for Outcomes in PArkinson's disease - Autonomic Dysfunction; MoCA, Montreal Cognitive Assessment. IQR: interquartile range. 1) Two-sample t test with equal variances, 2) Two-sample Wilcoxon rank-sum (Mann-Whitney) test

At follow-up, the HR group still had higher motor scores in the MDS-UPDRS-III than the LR group. The median score in the HR was 7 (IQR 3 to 9) and in the LR group it was 3 (IQR 1 to 5; p=0.001). Moreover, the HR group reported a higher number of motor symptoms based on the MDS-UPDRS-II. The median MDS-UPDRS-II score was 2 in the HR (IQR 0 to 6) compared with 1 in the LR group (IQR 0 to 3; p=0.038). The MDS-UPDRS-III motor scale was not the only clinical assessment that showed differences between HR and LR participants. At follow-up, the HR group performed the handwriting test, on average, 10 seconds slower than LR participants (HR: 71.90 secs; 95% CI, 67.55 to 76.28 vs LR: 61.23 secs; 95%CI, 58.61 to 63.86; p<0.001). However, only six people had micrographia (based on my clinical

impression): one (3%) was classified in the HR group and five (5.2%) in the LR group. The TUG test and BRAIN test performance were comparable between the HR and the LR group (Table 3.3).

Table 3.3 Motor manifestations in the higher and lower risk groups

Follow-up motor markers	Higher risk (n = 33)	Lower risk (n=95)	p-value
UPDRS-II, median (IQR)	2 (0-6)	1 (0-3)	0.038
UPDRS-III, median (IQR)	7 (3-9)	3 (1-5)	0.001
BRAIN test-KS (taps/30sec), mean (SD)	55.72 (12.41)	49.74 (11.37)	0.010
TUG (sec), mean (SD)	6.82 (1.44)	6.46 (1.44)	0.310
Handwriting speed (sec), mean (SD)	71.91 (12.11)	61.23 (12.65)	<0.001
SP-Louis, n (%)	26 (78.8)	53 (55.8)	0.022
Incident SP-Louis, n (%)	7 (21.2)	21 (22.1)	1.000
SP-MDS, n (%)	10 (30.3)	9 (9.5)	0.008
Incident SP-MDS, n (%)	6 (18.2)	7 (7.4)	0.096

Both groups were classified based on risk scores at baseline; SD: standard deviation, IQR: interquartile range, UPDRS-II: motor symptoms, UPDRS-III: motor examination, TUG: Timed Up-and-go test, KS: kinesia score, SP: subthreshold parkinsonism, SP-MDS: MDS Task Force SP criteria, SP-Louis: Louis et al SP definition (for more details see Glossary)

Individuals stratified in the HR group at baseline had a greater probability of SP 6 years later compared with those in the LR group. According to the MDS Task Force definition for SP, the proportion of individuals fulfilling criteria for SP was found to be 3-fold greater than the LR group (30.3% vs 9.4%; p=0.008). In contrast, I did not find significant differences between the HR and LR groups (78.8% vs 55.8%; p=0.022) when applying Louis and colleagues' criteria (for criteria details see Glossary). This might be explained by their cut-off being less stringent. In fact, the criteria used by Louis et al might not be biologically plausible. For example, a person with mild rigidity in two arms without any other parkinsonian sign would be considered to have SP based on their criteria. However, we know that rigidity is quite common in old people too. For that reason, I used the MDS Task Force criteria in the longitudinal analysis.

3.3.2 Longitudinal analysis

Two participants from the pilot cohort were diagnosed with PD during 6 years of follow-up. Of note, they were classified in the HR group at baseline and fulfilled criteria for SP 2 and 5 years before receiving a formal PD diagnosis. In this section our prediction model was tested using four separate proxy measures, three binomial outcomes (incident SP, motor decline, and individuals motor domains) and one numerical outcome (MDS-UPDRS-III total score).

Outcome 1: Subthreshold Parkinsonism

According to the MDS Task Force definition of SP, participants stratified in the HR group at baseline were more likely to have SP 6 years later than the LR group (18.2% vs 4.2%; p=0.018). There was some evidence that the HR group also had a higher proportion of new cases of SP (18.2% vs 7.4%), although that difference was not statistically significant (p=0.096). Using incident SP as an outcome showed some evidence that people in the HR group had almost 2-fold increased odds of developing SP 6 years later (OR 1.70; 95%CI, 0.99 to 2.94; p=0.053). A replication of this analysis was carried out in the Bruneck cohort. The same estimation risk algorithm was applied, excluding motor performance (in their case it was the MDS-UPDRS-III). They found a higher association between risk scores and incident SP (OR 2.46; 95% CI 1.77 to 3.41; p<0.001), which remained significant after adjusting for age (p=0.005). Of note, there was a greater proportion of incident SP cases (29.4%) than in the PREDICT-PD cohort (10.1%) (Appendix B). The meta-analysis of both cohorts showed an overall association (OR) of 2.19 (95% CI, 1.56 to 3.06); the PREDICT-PD cohort contributed 32% and the Bruneck cohort 68% (Figure 3.2).

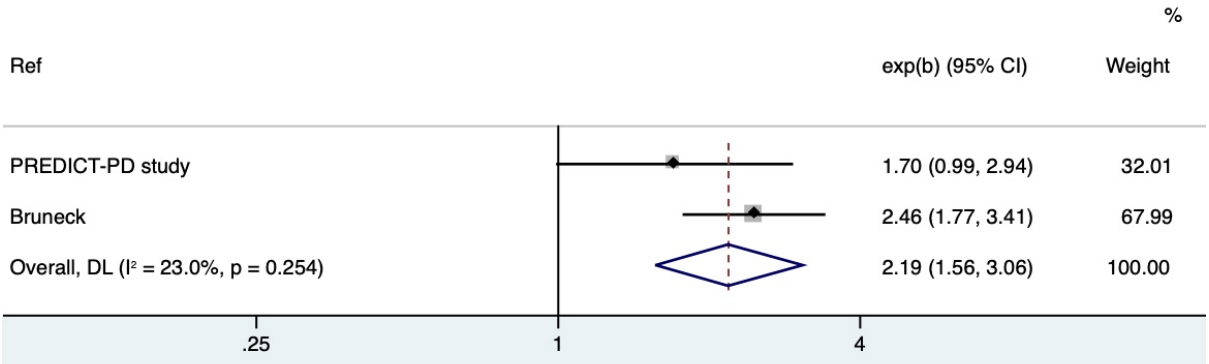

Figure 3.2 Meta-analysis of the PREDICT-PD and Bruneck studies. Mixed effect model using the Odds Ratio (exp(b)) of developing incident SP in higher and lower risk groups.

Outcome 2: Motor Decline

At baseline, the median MDS-UPDRS-III score in the HR group was 5 (IQR 2 to 6) and 2 in the LR group (IQR 0 to 4; p<0.001). Six years later, people in the HR group were still found to have higher motor scores than the LR group; the median MDS-UPDRS-III scores in the HR and LR groups were 7 and 3 (IQR 3 to 9 and 1 to 5 respectively; p=0.001). Not only that, but a greater percentage of people in the HR group (30%) also had a more pronounced motor decline over time (≥ 5-point change in the motor scale) compared with the LR group (12.6%; p=0.031) (Figure 3.3). The motor trajectories of the people with and without SP differed according to whether they were classified in the HR or LR at baseline. People who fulfilled criteria for SP were more likely (57.1%) to have a motor decline if they were classified in the HR group at baseline than if they were in the LR (33.3%). In contrast, people without SP, irrespective of inclusion in the HR or LR group, remained stable over time (89.0% in the LR group without SP, 77.8% in the HR group without SP) (Table 3.4).

Table 3.4 Motor trajectories depending on risk estimates and the presence SP at baseline

MDS-UPDRS-III change	HR-SP	HR-nSP	LR-SP	LR-nSP
≥ 5 points, n (%)	4 (57.14)	6 (22.2)	1 (33.3)	10 (11.0)
< 5 points, n (%)	3 (42.86)	21 (77.8)	2 (66.7)	81 (89.0)

Chi-square test p=0.008, HR-SP: Higher Risk and Subthreshold Parkinsonism, HR-nSP: Higher Risk without Subthreshold Parkinsonism, LR-SP: Lower Risk with Subthreshold Parkinsonism, LR-nSP: Lower Risk without Subthreshold Parkinsonism, MDS-UPDRS-III change: [follow-up score] − [baseline score]

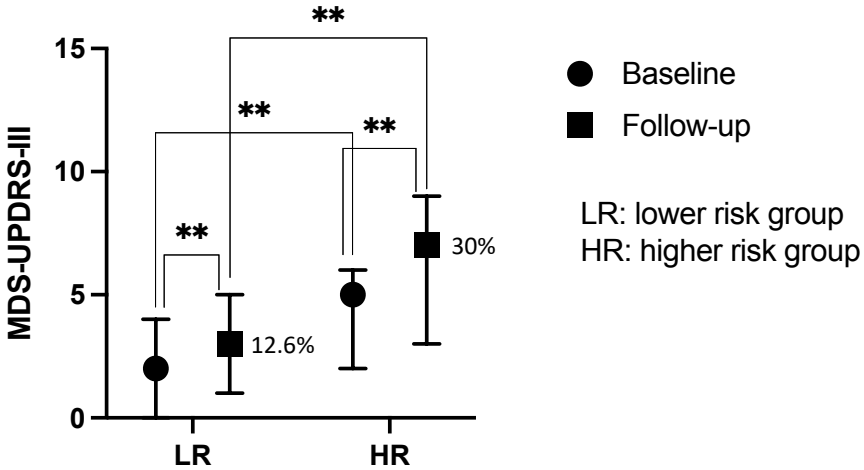

Figure 3.3 Boxplot of the MDS-UPDRS-III performance in the HR (higher risk) and the LR (lower risk) groups at baseline and follow-up. %: ≥ 5-point change (MDS-UPDRS-III). ** p-value < 0.010

In five participants motor scores improved over time: one LR participant regressed 6 points, and four (two HR and two LR) participants regressed 4 points in the MDS-UPDRS-III. Interestingly, one HR participant's risk score also decreased together with the motor score and 6 years later he was classified as LR. In addition, 101 participants remained stable over time[VI]. A higher proportion of people with a 'stable' motor status belonged to the LR risk group (LR: 80 (79.2%), HR: 21(20.8%); p<0.001) and did not fulfil criteria for SP at baseline. Considering 'motor decline' as a final condition (outcome 2), PREDICT-PD risk scores had a high Negative Predictive Value (NPV) (84.2%) and specificity (79.2%) but low Positive Predictive Value (PPV) (36.4%) and sensitivity (44.4%), suggesting that they had a good performance detecting which participants would remain stable over time. In contrast, fulfilling criteria for SP at baseline showed high PPV (90%), NPV (84.7%) and specificity (99%) but low sensitivity (33.3%) for detecting which participants would experience a more pronounced motor decline.

There was some evidence, although it was not significant, that people in the HR group had 3-fold greater odds of experiencing motor decline (≥ 5-point change) than those in the LR (OR 3.01; 95% CI, 1.15 to 7.84; p=0.024). When excluding age and sex from the algorithm and

[VI] MDS-UPDRS-III at follow-up minus MDS-UPDRS-III at baseline: from -3-point-change to 4-point-change)

adjusting for both factors, the association decreased (OR 1.78; 95% CI, 0.65 to 4.86; p=0.259), suggesting a confounding effect.

Outcome 3: Single Motor Domains

Individual motor markers in the MDS-UPDRS-III were also used as surrogate markers of PD.[VII] They included bradykinesia, rigidity, rest tremor, action tremor, and abnormal gait sub-scores. At follow-up, people with HR were more likely to have bradykinesia ('prevalent' bradykinesia) than the LR group (19/33 (57.6%) vs 27/95 (28.4%); p=0.003). Of note, none of the participants had a score of 3 (moderate) or 4 (severe) in any of the repetitive tasks used to assess bradykinesia. Although differences were not as noteworthy as in 'prevalent' bradykinesia, a higher proportion of participants in the HR group developed new onset bradykinesia ('incident' bradykinesia) than LR participants (11/33 (33.3%) vs 15/95 (15.8%); p=0.044). Although action tremor was significantly more prevalent amongst people with HR in developing PD (25/33 (75.7%) vs 44/95 (46.3%); p=0.004), the incidence of new onset action tremor did not differ substantially from the LR group (8/33 (24.2%) vs 17/95 (17.9%); p=0.450). No differences were found between HR and LR in terms of prevalence and incidence of rest tremor, rigidity and abnormal gait.

In a logistic regression analysis using 'incident' bradykinesia as a motor outcome, I observed that HR people had more than twice the odds of developing bradykinesia over time (unadjusted OR 2.67; 95% CI, 1.07 to 6.62; p=0.035). The association between 'incident' bradykinesia and HR of PD remained significant after excluding age and sex from the algorithm and adjusting for both factors (OR 4.51; 95% CI,1.41 to 14.37; p=0.011). People at HR were three more times likely to exhibit action tremor (OR 3.60; 95% CI, 1.60 to 8.21; p=0.002). In contrast with bradykinesia, the association between being HR and having action tremor decreased after excluding from the algorithm and then adjusting for age and sex (OR 2.40; 95% CI, 0.95 to 6.37; p=0.063), suggesting a confounding effect. Participants with action tremor (n=69) were older (75.8 years (SD, 5.3) vs 73.2 (SD, 4.7); p=0.002) and more likely to be male (63.8% vs 41.7%, p=0.004) than those without action tremor. Intriguingly, a larger proportion of participants with action tremor had vascular risk factors (68.1% vs 31.9%; p=0.002) compared with those without action tremor. Finally, an association between HR and rigidity, rest tremor and abnormal gait could not be demonstrated.

There were 32 participants with bradykinesia at baseline (HR: 10, LR: 22). Participants in the HR group with bradykinesia at baseline were more likely to have incident SP (n=6). Moreover, two HR with bradykinesia at baseline were diagnosed with PD (n=2) 6 years later. Most participants without bradykinesia at baseline (HR: 23, LR: 73) remained stable, without

[VII] Bradykinesia sub-score >1, action (postural and kinetic) tremor >1, rigidity >1, rest tremor ≥ 1, abnormal gait ≥ 1 (for more detail go to Methods/Statistical analysis)

reaching the MDS-UPDRS-III cut-off for SP (73.9% (17/23) of those in the HR and 94.6% (69/73) in the LR group).

Outcome 4: MDS-UPDRS-III total scores

Linear regression analysis showed that risk scores at baseline were associated with higher motor scores at both time points (baseline and follow-up). However, the association increased over time: 1) Baseline time-point: per doubling of the risk estimate at baseline, the MDS-UPDRS-III increased 1.05 points (95% CI, 0.64-1.47); 2) Follow-up time point: per doubling of the risk estimate at baseline, the MDS-UPDRS-III increased 1.66 points (95% CI, 1.04 to 2.27; $p<0.001$). Since action tremor is not included in the definition of SP, I repeated the analysis excluding action tremor (69 people in total) from the MDS-UPDRS-III, obtaining a slightly lower regression coefficient (beta score) association between the motor scale and risk estimates (beta 1.36; 95% CI, 0.81 to 1.9 vs 1.66; 95% CI, 1.04 to 2.27; R-squared 0.16 vs 019) (Table 3.5). Excluding tapping speed scores from the algorithm did not make any difference, suggesting that the BRAIN test might not have an important role in the algorithm in this subgroup analysis.

Although differences were found in cognitive tests between the HR and LR group, adjusting the regression models for MoCA scores did not show any major change in the effect estimates. In contrast, the regression coefficient from regression models changed to a greater extent after adjusting for vascular risk factors (hypertension and high cholesterol). This finding suggests a possible confounding role of vascular risk factors in the association between PD risk and motor scores (Table 3.5). A similar trend was seen when adjusting for vascular risk factors after having excluded age and sex from the algorithm (unadjusted beta 1.73; 95% CI, 1.08 to 2.39; adjusted beta 1.44; 95% CI, 0.80 to 2.09), suggesting that the confounding role of vascular risk factors was not explained by age or sex. Finally, regression models were accounted for age and sex after both being excluded from the algorithm. Although there was some evidence of age and sex influencing risk estimates and motor score (unadjusted beta 1.73; 95% CI, 1.08 to 2.39; adjusted beta 1.58; 95% CI, 0.88 to 2.29), the discrepancies between unadjusted and adjusted regression models were not big enough for them to be considered confounding factors (Table 3.5).

Table 3.5 Linear regression analysis between baseline risk estimates and follow-up MDS-UPDRS-III scores

Baseline risk score	Increase in MDS-UPDRS-III per doubling of odds (Beta)	95% CI	p-value
Crude	-1.66	-2.27 to -1.04	<0.001
Adjusted for MoCA	-1.82	-2.45 to -1.19	<0.001
Adjusted for VRF	-1.38	-1.99 to -0.77	<0.001
Adjusted for MoCA and VRF	-1.56	-2.17 to -0.94	<0.001
Baseline risk score (excluding age and sex)	**Increase in MDS-UPDRS-III per doubling of odds (Beta)**	**95% CI**	**p-value**
Crude	-1.73	-2.39 to -1.08	<0.001
Adjusted for age	-1.58	-2.29 to -0.88	<0.001
Adjusted for sex	-1.66	-2.35 to -0.97	<0.001
Adjusted for MoCa	-1.88	-2.56 to -1.21	<0.001
Adjusted for VRF	-1.44	-2.09 to -0.80	<0.001
Adjusted for all co-variates	-1.48	-2.18 to -0.78	<0.001

Simple (crude) and multivariate (adjusted) regression model for the association between baseline risk estimates (independent variable) and follow-up MDS-UPDRS-III scores (dependent variable). VRF: vascular risk factors (hypertension and high cholesterol), MDS-UPDRS-III: motor examination, MoCA: Montreal Cognitive Assessment

3.4 Discussion

In this chapter, I prospectively investigated the course of mild parkinsonian signs in older individuals stratified for future risk of PD. Previously, members of the research team found that individuals stratified for future PD risk using the basic PREDICT-PD algorithm exhibited an increased severity of motor disturbances and a greater proportion fulfilled clinical criteria for SP.[2] Six years later, I found that participants classified in an HR group at baseline had greater progression in their motor scores compared to those with LR score. Furthermore, individuals classified in the HR group were twice as likely to develop SP as the LR group and were four times more likely to have the cardinal sign of PD diagnosis (bradykinesia) in the future (Figure 3.2).

The data suggests that being classified in the HR group and fulfilling criteria for SP increases the chances of developing motor dysfunction in the future, including a more pronounced motor decline[VIII] and new onset bradykinesia. Unlike having SP at baseline, the PREDICT-PD algorithm alone appeared to be suboptimal in predicting a greater motor change in the future. However, it showed a higher specificity and NPV to classify those who will remain stable over time. The main limitation of using SP as a motor prediction marker in large population-based studies is that it requires an in-person assessment, making large-scale application difficult.

[VIII] ≥ 5-points change in the MDS-UPDRS-III

Likewise, the MDS Research Criteria for Prodromal PD score has been proved to have a limited sensitivity. Validation studies from four separate population-based cohorts (HELIAD[103], TREND[104], PRIPS[104] and Bruneck[105] studies) showed that although MDS prodromal PD score had a high specificity (>80%), it had a limited sensitivity (4.5% to 66.7%). Based on this, further research is needed to develop accurate remote motor tools to improve enrichment after population-based risk algorithms.

Having SP at baseline appeared to identify who will suffer a bigger motor decline in the future. The definition of SP is based on the MDS-UPDRS-III scores which were found to be associated with the PREDICT-PD risk score in a linear fashion. Moreover, being classified in the HR risk group almost doubled the odds of developing incident SP in the future. The Bruneck study used the same approach applying the PREDICT-PD algorithm. They found a stronger association between risk score at baseline and future risk of SP. However, the MDS-UPDRS-III has several limitations that might have underestimated the relationship between risk scores and motor dysfunction. It has not been designed to assess people without PD, even if they have early motor features. For example, the disproportionate representation of rest tremor (33% of the total MDS-UPDRS-III items) over other motor signs that are not commonly present at early stages (e.g. freezing, postural instability) might have diluted the association between risk estimates and motor impairment.

To overcome these limitations, I focused on each motor domain in the MDS-UPDRS-III separately and found an association between 'incident' bradykinesia and being classified in the HR group: people in the HR were more than four times more likely to develop bradykinesia in the future after adjusting for age and sex. In contrast, the association of action tremor and abnormal gait with HR appeared to be influenced by age and sex. This is in line with what has been classically described about bradykinesia being a genuine sign of PD from the early stages of the disease, in contrast to abnormal gait and action tremor which are expected to be commonly present in elderly people.[110] Handwriting difficulties, which could be considered a surrogate maker of bradykinesia, are typically reported as an early motor symptom of PD. In our cohort, people in the HR group had slower handwriting than those in the LR group. Based on these findings, it could be argued that bradykinesia might be one of the earliest motor signs in PD. However, the definition of bradykinesia still relies on a scale that has not been designed for early stages of PD. There is a need to validate adapted tools to define the concept of 'prodromal bradykinesia'.

Motor dysfunction is not exclusive to PD and can occur in other contexts such as ageing, cerebrovascular damage and dementia. All of them have a common denominator of nigrostriatal dysfunction and can act as potential confounding factors. The prevalence of SP in population-based studies ranges from 30% to 40% in elderly people, which is much higher than the prevalence of PD.[107] For example, in one study in a community setting, SP was found in more than one third of individuals over the age of 65 years.[141] Minn Aye and collaborators evaluated the presence of SP in an elderly community.[113] They found that one quarter of their

cohort exhibited subtle parkinsonism with this proportion increasing with age, with three out of ten people older than 75 showing some degree of motor dysfunction. After adjusting for age and sex, cognitive dysfunction together with symptoms of RBD were found to be related to mild parkinsonian signs, suggesting that an underlying neurodegenerative process might be present in a proportion of them. The relationship between risk estimates and MDS-UPDRS in our study did not differ considerably after dropping age and sex from the algorithm. That suggests motor dysfunction was not entirely explained by age, but it could be related to the combination of other multiple risk factors included in the algorithm.

SP has also been found to be associated with future dementia, particularly in the elderly population[108] with cerebrovascular disease.[110] I adjusted for both factors in our analysis. Unlike MoCA scores, when the model was adjusted for vascular risk factors the strength of association decreased significantly. This pattern was maintained even after removing age and sex from the algorithm, suggesting that their confounding effect could not be entirely explained by age or sex. In any case, even after adjusting for vascular risk factors, the association between MDS-UPDRS and risk scores remained significant. The contribution of cerebrovascular disease to the presence of mild parkinsonian signs has been studied in the ageing population. This is the case with 418 brain autopsies examined in a cohort that had been evaluated during life for parkinsonism.[208] They found that people with macroscopic infarcts were more prone to having higher global parkinsonian scores. This study, together with our findings, supports the idea of vascular risk factors being involved in PD risk. Considering that vascular risk factors are treatable, it will have relevant implications in terms of primary prevention.

There are several limitations to this study. First, considering that the proportion of participants with a family history of PD in our cohort was higher than in the unselected population-based cohorts (20% vs 4%),[209] selection bias cannot be discounted. However, the incidence of PD in our cohort is not higher than that expected in the general population. Second, two consecutive assessments with 6 years in between makes it difficult to establish accurate motor trends. Changes could be explained by other external factors, such as low mood or concomitant medication, which might have affected the motor performance of participants. I accounted for that possibility and tried to minimise the interference of external factors by examining participants in the same environment (home). I also checked for other external factors such as concurrent medication and diagnosed depression to account for external interactions. With the aim of trying to mitigate observational bias, I scored baseline videos instead of using those scored in person by Dr Noyce. By doing this I might have missed important information not collected properly in the video such as subtle tremor as well as clinical details that can only be appreciated in person. Third, a noticeable proportion of participants (17% of the baseline cohort) were missing and 9% dropped out of the study for unknown reasons. The fact that we do not have access to their medical records makes it difficult to rule out whether some of them went on to develop PD. Finally, the PREDICT-PD algorithm dichotomises a continuous variable (risk score) based on an arbitrary cut-off (15th centile) without accounting for the "dose effect" of risk estimates. In terms of risk ranges, the LR group is expected to be a heterogeneous

group including those participants at middle risk. Thus, it is expected that some participants close to the 15th centile were classified in the LR group (false negative), leading to an ascertainment bias. As we gather more longitudinal data, 15th centile cut-off should be adjusted based on incident PD cases.

Future work in these participants will seek to define the course of motor prodromes and their relationship with other markers in the prodromal phase. Adding new incident PD cases will help to understand which pre-diagnostic features best predict future diagnosis of PD.

To conclude, this study helps to identify early motor signs and their progression over time. The PREDICT-PD risk algorithm seems to be accurate in excluding those individuals whose motor progression will be unlikely. There is some evidence suggesting that our algorithm might also be useful in predicting SP and bradykinesia in the future, which in some HR individuals will be a harbinger of future PD (Figure 3.4). The next chapters are focused on creating quantitative tools to measure bradykinesia at early stages and replicate handwriting speed results in other at-risk groups.

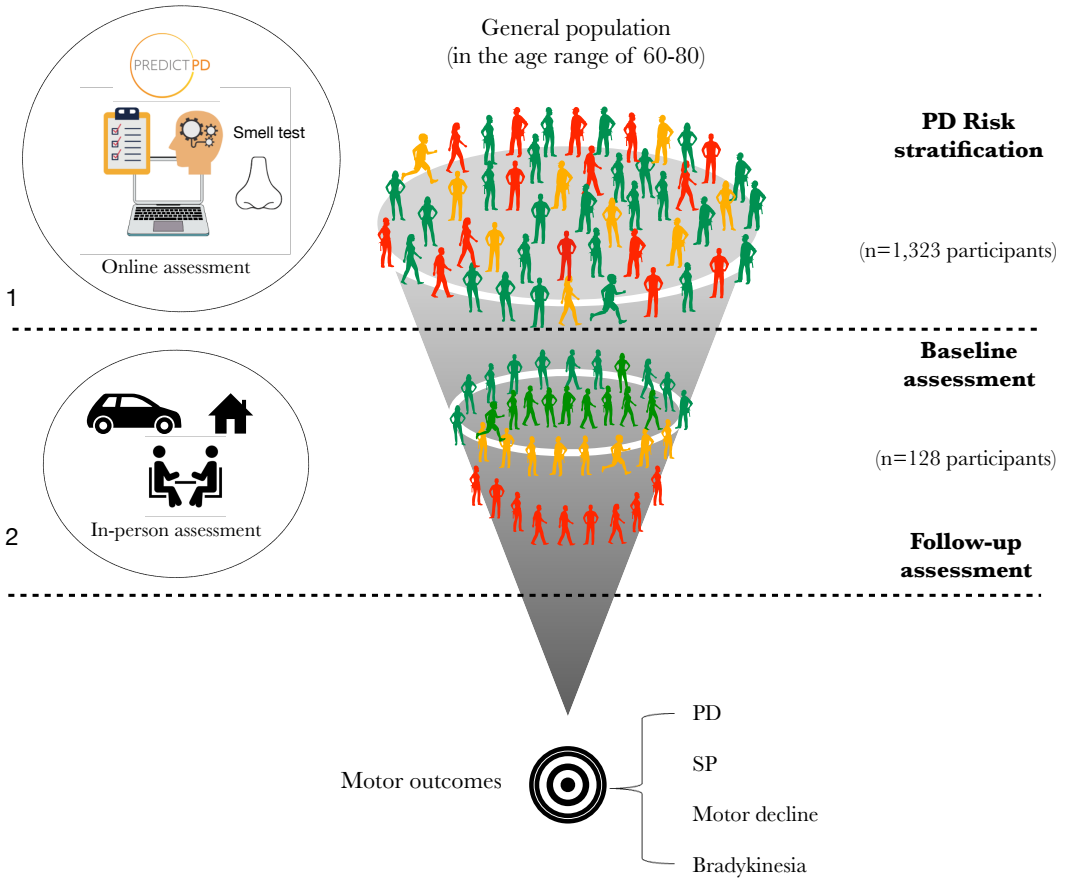

Figure 3.4 Schematic of PREDICT-PD study. (**1**) Online population-based risk stratification. Red, orange and green colours represent participant stratification based on risk estimates: higher risk in red, intermediate risk in orange and lower risk in green (for more details, see Figure 1.2). (**2**) Two time-point in-person assessment of a representative group of participants (n= 128) to check for motor impairment. Motor outcomes: PD (Parkinson's Disease), SP (Subthreshold Parkinsonism), motor decline (MDS-UPDRS-III ≥5) and bradykinesia (bradykinesia MDS-UPDRS-III sub-score >1).

Chapter 4

Design and development of objective measures of distal motor dysfunction: the DFT test and SMART test

4.1 Introduction

Bradykinesia relates to the slowness of movement, and it is the core clinical sign of PD.[210] Existing literature suggests that bradykinesia might appear years before PD diagnosis. However, it is not always reported by patients as we saw in Chapter 2. Thus, there is a need to develop accurate tools to assess bradykinesia in fine motor skills. I summarised the reasons from the existing literature in Chapter 1. I also proved the relevance of bradykinesia as an early feature of PD in Chapter 3, with bradykinesia being the clinical feature with the strongest association with individuals at risk of developing PD in the future. Not only that, but people in the higher risk and bradykinesia at baseline were more likely to fulfil criteria for Subthreshold Parkinsonism (SP) and developing PD in the future. In line with these findings, handwriting speed was also found to be reduced in people at risk of PD and this could be considered an indirect sign of bradykinesia ('slowness of movement').

I focused on developing fine motor tasks because they offer several advantages that other methods using wearables and assessing other motor domains do not have. For example, finger tapping is a single motor task that assesses a pure movement and less likely to be influenced by other confounding factors (osteoarticular problems, cognitive impairment, peripheral vascular issues, etc) which commonly influence other motor domains such as gait. Finger tapping tests have been widely utilised to assess upper extremity bradykinesia (see Table 1.3 in Chapter 1). The standard assessment of finger tapping is the 5-point rating subscale in the MDS-UPDRS-III (Table 4.1). Although the MDS-UPDRS-III is a comprehensive assessment, the integer scale prevents detection of subtle motor changes[117,118] and inter-rater agreement is moderate at best.[119] Hence, a clear need for objective and consistent methods of assessing motor dysfunction exists.

In this chapter I present two novel objective tools, the Distal Finger Tapping (DFT) test and the Slow-Motion Analysis of Repetitive Tapping (SMART) test. I designed both tools as proof-of-concept studies to explore whether they would be capable to detect measurable differences in the finger tapping of people with PD compared with controls. I designed the DFT test to demonstrate that a simple keyboard finger tapping test can be used to remotely monitor distal finger movements and quantify separate components of distal movement such as speed, akinesia and rhythm. I combined the DFT test with the validated BRAIN test which has been

described in detail in Chapter 1. I designed the SMART test to provide proof of concept that motion capture using a smart phone could assess different elements of bradykinesia which may be sensitive to changes in early PD. It is important to note that I did not design either of the tests to be used as a diagnostic tool in isolation. The kinetic parameters analysed in both tests were correlated with the current standard for measuring disease severity in PD, which is the MDS-UPDRS-III and particularly the Finger Tapping (FT)-sub-score.

Table 4.1 MDS-UPDRS-III Finger Tapping sub-score

Score	Description
0 -Normal	No problems
1 -Slight	Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps
2 -Mild	Any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence
3 -Moderate	Any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st tap
4 -Severe	Cannot or can only barely perform the task because of slowing, interruptions, or decrements

Instructions: 'tap the index finger on the thumb as fast and wide as you can for 10 seconds'

4.2 Methods

I divided the development of the DFT and SMART tests into three stages:

i. **Design:** software development and testing in healthy volunteers

ii. **Comparison**: to measure the accuracy of the test by comparing cases and controls

iii. **Correlation**: to correlate their scores with the current standard of evaluation, the finger tapping sub-score in the MDS-UPDRS-III

4.2.1 Distal Finger Tapping test

The DFT test is a web-based tapping test available on the same online platform as the BRAIN test (https://predictpd.com/en/braintest. Both tests are compatible with regular laptops and computers with a keyboard and can run on all standard internet browsers. All participants gave informed consent to take part in the study.

I designed and developed the DFT test in conjunction with a software developer who was commissioned to produce a 20-second test that would record single repetitive key taps and calculate quantitative parameters to measure speed and accuracy of tapping. I selected the most

appropriate mechanism of the test and carried out the troubleshooting of the test and designed the interface.

The DFT was specifically designed to replicate the FT-sub-score in the MDS-UPDRS-III where patients are asked to tap their index finger and thumb repeatedly. To do that, we needed to isolate the index finger and metacarpal joint. In the initial design, volunteers were asked to keep both side keys depressed with the thumb and middle finger whilst tapping the up-narrow key with their index finger. Following preliminary testing, we found that volunteers struggled to perform the task due to the unnatural anatomic position of the hand which needed to be maintained during the task. Keyboards also failed to register the up-key stroke with this mechanism. The key failure was identified as a hardware issue, as integrated laptop keyboards do not allow certain keys to be depressed at the same time. To overcome these difficulties, only the middle finger was selected to fix the hand while the index finger performed the tapping task. By doing this, participants could easily maintain the hand position required to perform the test.

We designed a simple and user-friendly interface so it would be easy to follow for elderly participants (see Figure 4.1). We recorded a video with all the instructions to ensure patients could conduct the test correctly without additional aid, especially in a home environment.

Similar to the BRAIN test, the DFT test involved a 5-second trial test before the real test on both hands. If a key was pressed or unpressed for more than 3 seconds, the test was considered null, and the participant was invited to start the test again. Results generated were connected to a central server, and ready instantaneously to download.

Raw data were generated from key presses as follows:

- Time and date
- Hand tested (right/left)
- Key pressed (the American Standard Code for Information Interchange reference of the key)
- Time down (time at which the key was pressed)
- Time up (time at which the key was released)

Three kinetic parameters were generated by the DFT test:

1. Kinesia score (KS) - a measure of rate of movement reflecting speed, the number of keystrokes in 20 seconds
2. Akinesia time (AT) - average dwell time (msec) that keys are depressed reflecting akinesia
3. Incoordination score (IS) - variance ($msec^2$) of travelling time between keystrokes, reflecting rhythm

Similar kinetic parameters are calculated for the BRAIN test (for details go to Chapter 1).

The second stage of this study was focused on monitoring motor fluctuations in a sub-group of patients with PD. Since PD motor fluctuations are out of the scope of this thesis, I included this analysis in the Appendix C.

Participants

Patients fulfilling the Queen Square Brain Bank criteria for PD were consecutively recruited from the Movement Disorder Clinic at the Royal London Hospital between February and August 2019. They were frequency-matched with healthy controls who were from relatives of patients from the Royal London Hospital and lower risk participants from the PREDICT-PD study.

We invited participants to sit in front of a computer/laptop and read on-screen instructions and independently complete the DFT tests. I selected only the first successful test for each patient. They were instructed to repeatedly tap the down arrow key with their left index finger as fast as possible for 20 seconds, whilst simultaneously depressing the left arrow key with their left middle finger. These instructions were then repeated for the right hand. By asking them to stabilise the wrist and forearm, the index finger movement from the metacarpal joint was isolated, giving a true measurement of distal finger movements (see Figure 4.1). After completing the DFT test, participants were invited to perform the BRAIN test (instructions details given in Chapter 1, Technology-based tools section).

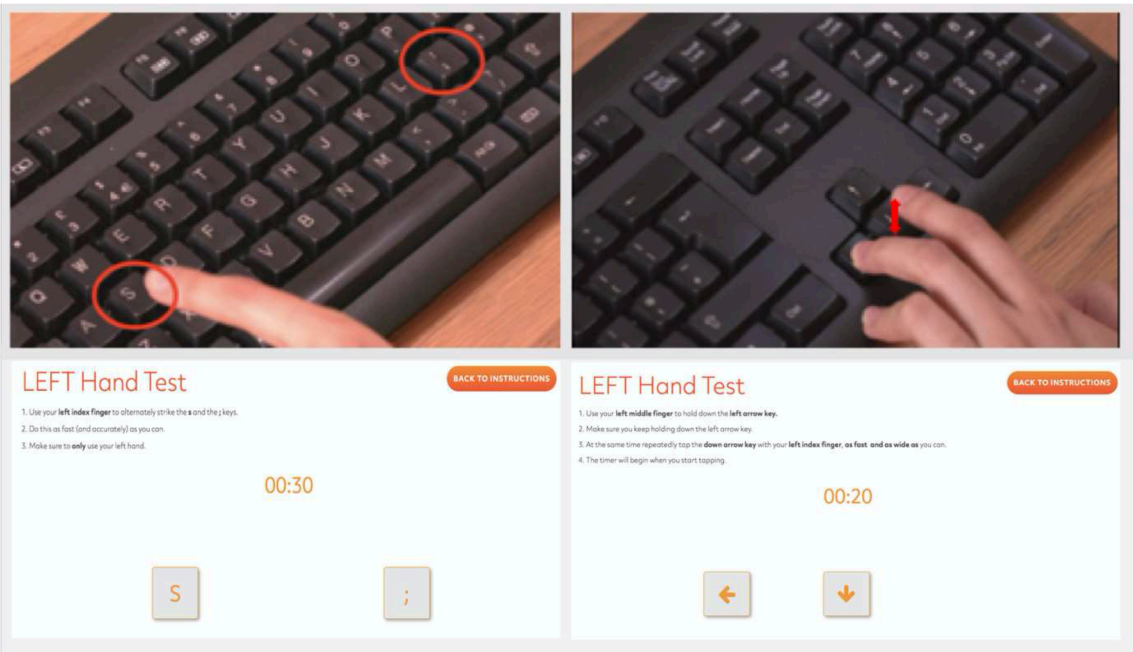

Figure 4.1 Instructions and online interface of the BRAIN and DFT tests. Left: BRAIN test, alternate tapping of the 's' and ';' keys with the index finger and online interface below. Right: DFT test, repeated tapping of down arrow key with left index finger whilst depressing the left arrow key with left middle finger. Below both tests: online interface

I examined patients with PD and controls according to the instructions of the MDS-UPDRS-III before they performed both digital tests. The patients' subjective clinical state was recorded, with 'On' defined as a functional state when there is a good response to medication, and 'Off' defined as a poor functional state despite taking medication or after the symptomatic effect of medication had passed. To avoid patients coming to the hospital on another day, recruited patients participated in the study on the same day as their clinic appointments, hence, it was not logistically possible to control time between levodopa administration and testing.

To investigate the presence of a learning effect, seven of the healthy controls completed the DFT test five times within a 3-hour period.

Statistical analysis

We tested for normality using the D'Agostino test. We calculated the descriptive statistics of all three parameters (KS20, AT20, IS20), reporting mean and SD for normally distributed data and median and IQR for not normally distributed data. We explored whether age and sex might influence KS20, AT20 and IS20 in controls. To do that, we compared the average kinetic performance in men and women using the unpaired t-test and Mann-Whitney for normally and not normally distributed data respectively. In terms of correlation with age, we calculated the Pearson's correlation coefficient and Spearman's rank correlation coefficient depending on whether the data were normally or not normally distributed. We compared the DFT test scores in patients and controls using the unpaired t-test and Mann-Whitney U test for normally and not normally distributed data respectively. We used the Wilson/Brown method to generate ROC curves and determine the accuracy (sensitivity and specificity of parameters) of the test in distinguishing patient from controls. Logistic regression and ROC curves defined AUC values for combination analysis of DFT and BRAIN test variables. Pearson's correlation and Spearman's rank correlation assessed the relationship between test parameters and MDS-UPDRS-III.

Since several main analyses are presented, a type 1 error could be expected. For that reason, we applied the Bonferroni calculation to adjust the cut-off for evidence of association. The significance level derived from Bonferroni calculation was set as $p<0.002$ This cut-off was applied for all calculations. All data were analysed using GraphPad Prism version 8.0.2, IBM SPSS version 27 and STATA v.13 (StataCorp, College Station, TX).

4.2.2 Slow-Motion Analysis of Repetitive Tapping (SMART) test

The SMART test is a video-based tool focused on tracking repetitive finger tapping movements using a slow-motion camera. The test was designed and developed with the collaboration of Dr Miquel A Galmés (Department of Physical and Analytical Chemistry at Universitat Jaume I) who has a background in computational bioscience. The final goal of the test was to see whether a video camera might be able to capture early signatures of repetitive distal movements.

An ordinary smartphone (iPhone X) with slow-motion video recorded a repetitive finger tapping task for 20 seconds. Both hands were assessed consecutively. We assessed the participants in a consistent way, the dominant hand followed by the non-dominant hand. Parameters derived from the SMART test were correlated with the FT-sub-scores of the MDS-UPDRS-III.

Participants

We assessed three separate groups of participants: 1) patients with early PD (defined by a disease duration of less than two years since the diagnosis), 2) individuals with idiopathic anosmia and 3) healthy controls. Since the PD group were on average younger than the anosmia group, we selected two separate groups of controls of a similar age to the group they would be compared with, one group for early PD patients and another group for individuals with idiopathic anosmia. The recruitment criteria of QMAP-PD study included people between 35 and 75 years old, with less than 2 years of PD duration from motor diagnosis (based on the UK Queen Square Brain Bank criteria[8]), and able to undergo MRI. The rationale for restricting the age slightly was the longitudinal design (more than 5 years), and that they wanted to try and minimise other major comorbidities that may confound the clinical picture (a feature that is not considered in community studies). Patients were recruited from a Movement Disorder clinic and were assessed by a Neurology consultant specialist. Particular exclusion criteria for this current study included having any comorbidities that could interfere with task performance, such as arthritis, previous stroke and dementia. Patients with anosmia had a nasal endoscopy and imaging done by an ENT specialist, to rule out any identifiable cause of smell loss. Neither patients with anosmia nor controls had any major neurological conditions (e.g. stroke, dementia, motoneuron disease), and/or any comorbidities that could interfere with performance of the task. Healthy controls were excluded if they had bradykinesia and fulfilled criteria for SP proposed by the MDS Task Force on the definition of prodromal PD (for more details see Glossary).

Patients with PD were recruited from the Quantitative Magnetic Resonance Imaging (MRI) for Anatomical Phenotyping in PD (QMAP-PD) study based at the Institute of Neurology, University College London. Individuals with anosmia were recruited from the PREDICT-PD study, after referral from specialist ENT clinics. Controls came from two separate sites: the PREDICT-PD and QMAP-PD studies. The assessments took place between October 2018 and December 2019. All patients gave informed written consent to the study. The Central London Research Committee 3 (reference number 10/H0716/85) gave ethics approval. The QMAP-PD study has full NHS ethical approval (Fulham Research Ethics Committee, 18/LO/1229).

Assessment

For each participant, finger tapping was recorded for 20 seconds using a smartphone at 240 frames per second (slow-motion capture). To facilitate finger recognition by the software, we

asked participants to make a fist with their third, fourth and fifth fingers and tap the index finger on the thumb. We instructed patients not to rotate and move the arm during the task with the purpose of capturing the real angle at the metacarpal-phalangeal joints between index finger and thumb. Finally, we invited patients to tap their index finger on the thumb as fast and as widely as they could. This task followed the same standardised instructions as the MDS-UPDRS-III (FT-sub-score) (Table 4.1). Patients stopped taking any dopaminergic medication at least 12 hours before the assessment.

Video analysis

We developed a software able to automatically detect the hand and process video images to analyse the finger tapping task. Due to the variability in the video acquisition, hand images were resized, rotated, and flipped to have a homogeneous sample. The videos were processed and analysed together with the collaboration of a bioinformatic with experience using Python programming language and OpenCV[211] library. A 2D Convolutional Neural Network (CNN) was trained to detect eight key landmarks of the first and the second fingers (Figure 4.2). A total of 3934 randomly extracted frames among all the videos were manually labelled and were used as a dataset for the CNN training. The complete dataset was divided into a train and a test dataset using a ratio of 0.8:0.2 respectively. The CNN was implemented in PyTorch.[212] Videos were then processed, and eight key hand landmarks were detected in every frame along the video using the trained CNN. In order to study the fine movement of the tapping task, the distance between the distal part of the first and the second fingers was computed through the video (first and last key landmarks). The computed distances are not real-distances, and they were normalised to be comparable between samples. This step limits the power of the technique since the absolute opening of the hand cannot be seen due to the normalisation process. To overcome this limitation, the angle formed between the distal part of the first and second finger and the key landmark corresponding to the metacarpal joint was also computed (Figure 4.2). This value can give information about the absolute opening of the hand during the tapping task and gives a useful variable to be compared among participants.

Maximum amplitude peaks were detected at each tap and linear regression models were fitted to those signal peaks. Frequencies were measured as number of taps per second. Velocities were calculated as the first derivative of the signal, and a similar process was applied to obtain the peaks of maximum velocities over time. The integrals of the signals were also computed. This value gives a measure of the freezing of the hand during the tapping task. All the signal processing was done using SciPy[213] and NumPy[214] libraries.

I analysed three kinetic parameters

1. Amplitude: angle formed between index finger and thumb
2. Frequency: number of taps per second
3. Velocity: distance travelled per second extracted from the derivative of the amplitude.

For each parameter, I calculated the mean, SD and Coefficient Variation (CV) (SD/mean). I determined the trend of movement over time using linear regression models which were extracted from the intercept and slope of finger tapping over time.

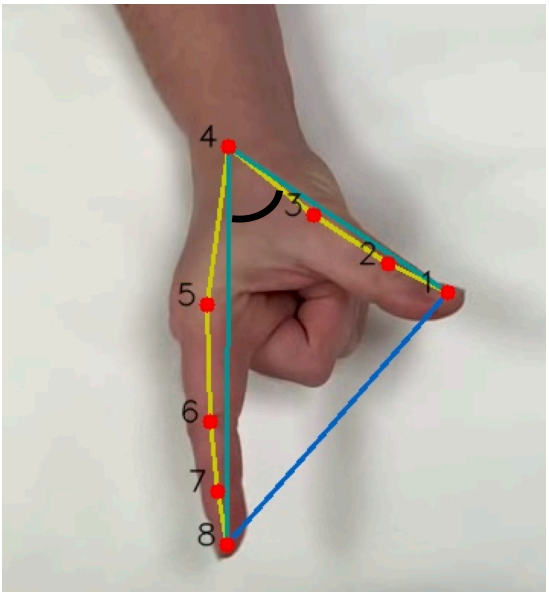

Figure 4.2 SMART test hand detection: 8 key landmarks across the first and the second finger (red). Angle between 1-4-8 key landmarks (black). Extrapolated amplitude between point 1 and 8 (blue)

Statistical analysis

I used the D'Agostino test to assess the normality of the data. Quantitative data were presented as the mean and SD for normally distributed data and median and IQR for not normally distributed. Mann Whitney U tests, t-tests, and Welch's t-tests (two-tailed) were used to compare test parameters between patients and controls, as appropriate. I used linear regression models to determine whether movement parameters derived from finger tapping (dependent variables) were influenced by age. On the other hand, I used logistic regression analysis to explore whether sex and handedness might have influenced kinetic parameters. I compared the motor performance using different combinations:

a. Dominant hand of controls with the most affected side in the PD group
b. Non-dominant hand of controls with the most affected side in the PD group
c. Dominant hand of anosmia with dominant hand of controls
d. Dominant hand of anosmia with asymptomatic side of patients with unilateral PD.

ROC curves were drawn to find the optimal cut-off value which maximises the combination of sensitivity and specificity (Youden's J index) for SMART test parameters separately and in combination. I used Spearman's correlation coefficient to correlate SMART test parameters (continuous) with FT-sub-scores from the MDS-UPDRS-III (ordinal). Since I ran multiple hypothesis tests – one for each component of the test parameters (mean, CV, and slope) – I selected a more stringent cut-off for the level of significance ($p<0.005$, Bonferroni corrected for

nine hypothesis tests). This adjustment was done to ensure robustness of results and avoid false positives (i.e. type I error). I carried out all the analysis of the data throughout STATA v.13 (StataCorp, College Station, TX).

4.3 Results

4.3.1 Distal Finger Tapping test

We tested 55 patients with PD and 65 frequency-matched controls. Table 4.2 summarises the demographic information of patients with PD and controls. Individuals with PD and controls had similar age and sex (mean age (SD), 66.8 years (9.6) vs 71.2 years (9.5); p>0.050). Patients with PD had a mean disease duration of 6.3 years (SD 4.9). Most of them, apart from five patients, were on treatment with levodopa. Patients took levodopa approximately 2.5 hours (median (IQR),150 min (60 to 210)) before assessment. For patients taking levodopa, 48 considered themselves to be 'On' and two 'Off' whilst completing the test. On/Off refers to the question in the MDS-UPDRS-III, which asks whether patients taking levodopa could notice the effect of medication at the time of examination.

Although the PD group and the control group have similar age and sex, we wanted to explore whether age and sex could have influenced the kinetic parameters of the DFT test. Correlation analysis between each factor and kinetic parameters found that neither age nor sex were associated with the test parameters. We found that the correlation coefficients of age with each kinetic parameter ranged between −0.03 and 0.10. Similarly, we did not find any significant difference between the performance of the test in males compared with females (Table 4.3). We also compared the average performance between the dominant and non-dominant hand. We found that it was different in the control group. For that reason, we decided to use the average of both hands for analysis in the control group. Since PD has an asymmetric pattern, we selected the most affected side for the analysis in the PD group. The identification of the most affected side was based on the side with higher MDS-UPDRS-III score.

Table 4.2 Demographic and clinical information from the DFT test study

	PD (n=55)	Controls (n=65)
Mean Age (SD)	66.8 (9.6)	71.2 (9.5)
Sex		
- Female	24 (44%)	36 (55%)
- Male	31 (56%)	29 (45 %)
Education		
- Primary	3 (5%)	-
- Secondary	24 (44%)	-
- Higher	10 (18%)	-
- Further	18 (33%)	-
Occupation		
- Professional	25 (46%)	-
- Non-professional skilled	9 (16%)	-
- Non-professional/non-skilled	21 (38%)	-
Mean years since PD diagnosis (SD)	6.3 (4.9)	-
Most affected side		
- Right hand, n (%)	17 (31)	-
- Left hand, n (%)	36 (65)	-
-Equally, (%)	2 (3)	
Levodopa		
- Yes, n (%)	50 (91)	-
- No, n (%)	5 (9)	-
Median minutes since levodopa dose (IQR)	150 (60 – 210)	-
On/Off*		
- On, n (%)	48 (87.3)	-
- Off, n (%)	2 (7.3)	-
Mean MDS-UPDRS-III total (SD)		
-On (n=48)	38.2 (16.4)	
-Off (n=2)	67.5 (6.4)	
Mean MDS-UPDRS Finger Tapping Sub-score (SD)		
-Off (n=48)	3.8 (1.6)	
-On (n=2)	5.5 (0.7)	

On/off refers to the question in the MDS-UPDRS, which asks whether patients taking levodopa could notice the effect of medication at the time of examination, 5 patients were not taking any levodopa. SD: standard deviation, IQR: interquartile range

Comparison between PD patients and controls

We found that all three DFT parameters discriminated between patients and controls. KS was the best discriminator, with 79% sensitivity for 85% specificity and an AUC of 0.90 (95% CI, 0.85 to 0.96). Patients with PD tapped on average 35 keys fewer than controls (PD: 55 taps; 95% CI, 48.9 to 61.1; controls: 89.3 taps; 95% CI, 85.6 to 93; p=0.001). On average, patients

with PD had a higher akinesia score (PD: 195.6 msec; 95% CI, 168.7 to 222.5; controls: 105.5 msec, 95% CI, 97.5 to 113.4; p=0.001) and performed the task more erratically (PD: 4589 $msec^2$; 95% CI, 1137 to 13464; controls: 779.5 $msec^2$; 95% CI 357.7 to 779.5; p=0.001). The corresponding sensitivity for 85% specificity in AT was 68% with respective AUC's of 0.87 (95% CI, 0.81 to 0.93). IS was found to have a 57% sensitivity for 85% specificity with respective AUC of 0.82 (95% CI, 0.74 to 0.89) (Table 4.4 and Figure 4.3*)*. The KS, AT, IS derived from the BRAIN test achieved AUCs of 0.89, 0.88, and 0.71 respectively. The combination of all three DFT test parameters improved discrimination up to AUC of 0.92 (80% sensitivity with 84% specificity, at 0.5 probability cut-off). When combining both DFT and BRAIN test parameters, we achieved an AUC of 0.95 (80% sensitivity and 94.5% specificity, at 0.6 probability cut-off) (Table 4.5). Moreover, we found that KS and AT had moderate correlation with MDS-UPDRS-III FT-sub-scores (Pearson's r=-0.40; p=0.002; and r=0.36; p=0.006) (Figure 4.4).

Table 4.3 Analysis of characteristics that influence KS20, AT20 and IS20 in controls

		Mean KS20	**p-value**	**Mean AT20**	**p-value**	**Median IS20**	**p-value**
Age, mean years	71.2	r=-0.05	0.67[a]	r=-0.03	0.80[a]	r=0.10	0.42[b]
Sex							
- Female, n	36	84.2	0.49[c]	121.4	0.06[c]	1217	0.52[d]
- Male, n	29	86.6		104.5		1186	

KS20, kinesia score; AT20, akinesia time; IS20, incoordination score Mean and medians given except for associations with age where correlation coefficient (r) is given. [a]Pearson; [b]Spearman; [c]Unpaired t-test; [d]Mann-Whitney, [e]Paired t-test, [f]Wilcoxon test

Table 4.4 Comparison of DFT parameters in PD patients and controls, and corresponding ROC analysis

		Mean KS20 (95% CI)	Mean AT20 (95% CI)	Median IS20 (IQR)
PD (n=55)		55.0	195.6	4589
		(48.9 to 61.1)	(168.7 to 222.5)	(1137 to 13464)
Controls (n=65)		89.3	105.5	779.5
		(85.6 to 93.0)	(97.5 to 113.4)	(357.7 to 779.5)
p-value		<0.001[a]	<0.001[a]	<0.001[b]
		KS20 Sensitivity	**AT20 Sensitivity**	**IS20 Sensitivity**
Specificity	90%	66.2%	58.5%	46.2%
(cut-off)		(82.5)	(108.2)	(717.6)
Specificity	85%	78.5%	67.7%	56.9%
(cut-off)		(80.5)	(116.4)	(853.4)
Specificity	80%	78.5%	76.9%	61.5%
(cut-off)		(78.5)	(127.9)	(957.3)
AUC		0.91	0.87	0.82
(95% CI)		(0.85 to 0.96)	(0.81 to 0.93)	(0.74 to 0.89)

ROC: Receiver Operating Characteristic, AUC: Area Under the Curve, KS20: kinesia score, AT20: akinesia time, IS20: incoordination score, CI: Confidence Interval, IQR: Interquartile Range; SD: Standard Deviation. [a]Unpaired t-test; [b]Mann-Whitney test. ROC analysis plotted in Figure 4.3

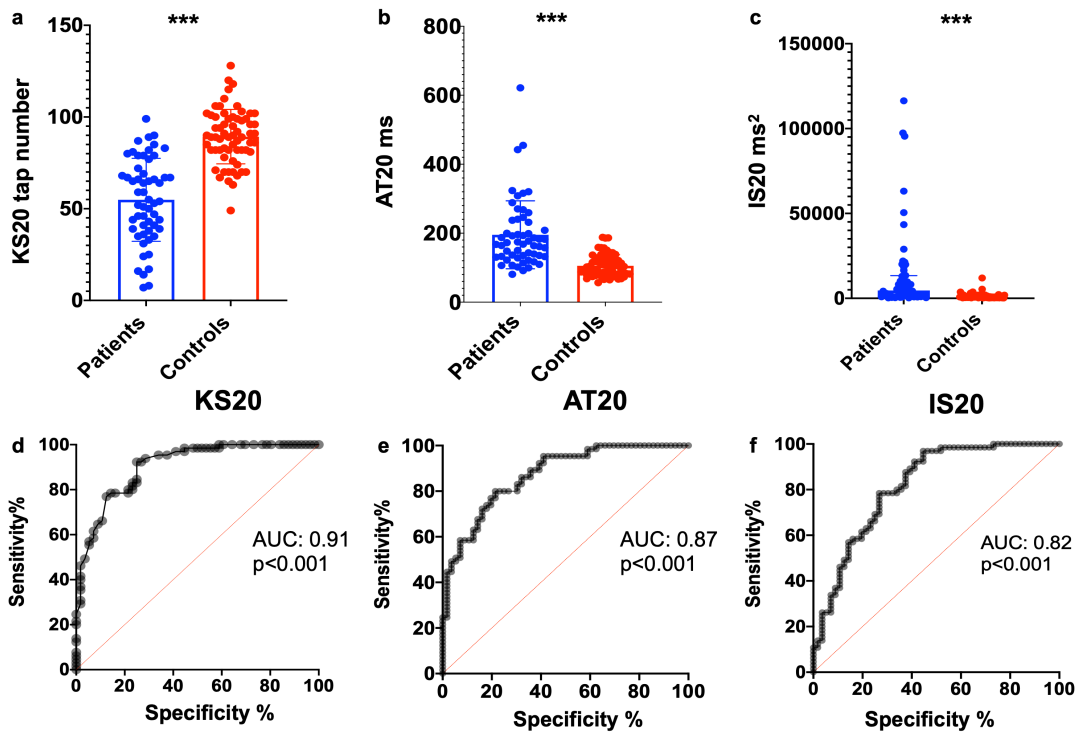

Figure 4.3 Comparison of KS20, AT20 and IS20 in PD patients and controls. Spread of (a) KS20, (b) AT20 (mean and SD) and (c) IS20 (median and IQR) for patients and controls. ROC curves for (d) KS20, (e) AT20 and (f) IS20

Table 4.5 Sensitivity and specificity of combined DFT and BRAIN test parameters

	DFT test parameter combination		DFT and BRAIN test combination	
Probability cut-off	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
0.4	81.8	81.8	89.1	85.5
0.5	80.0	83.6	85.5	87.3
0.6	70.9	90.9	80.0	94.5
0.7	61.8	92.7	76.4	98.2
0.8	58.2	98.2	65.5	98.2
0.9	52.7	98.2	60.0	98.2

ROC analysis combining DFT parameters (KS20, AT20, and IS20) and DFT (KS20, AT20 and IS20) with BRAIN test (KS30, AT30 and IS30)

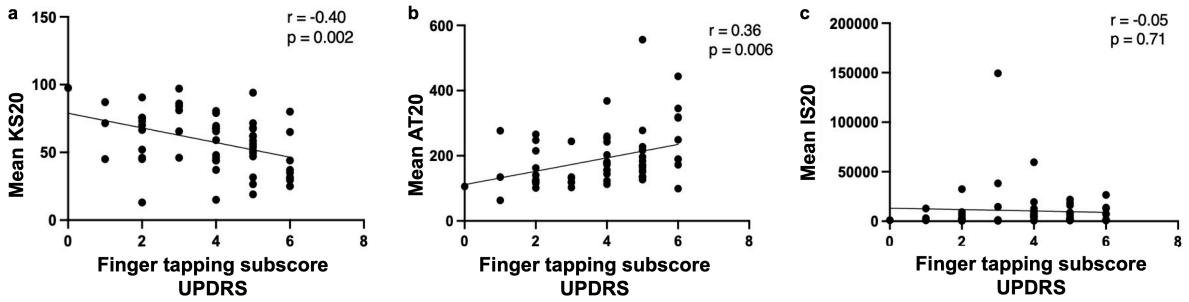

Figure 4.4 Correlation between DFT parameters and MDS-UPDRS-III finger tapping sub-scores. (a) Moderate negative correlation with KS20 and UPDRS. (b) Moderate positive correlation seen with AT20 and finger tapping sub-score. (c) No correlation seen with IS20 and finger tapping sub-score

4.3.2 Slow-Motion Analysis of Repetitive Tapping test

I analysed 198 videos (99 recordings for the right and left hand of all participants). I compared the finger tapping performance of the dominant hand with the non-dominant in both control groups. I did not find any differences. For that reason, I mainly focused the results on the dominant hand in the control and anosmic groups. Even so, I carried out an additional comparison between the non-dominant hand of controls and the PD group. I used the most affected side in PD for comparison since PD is associated with asymmetric onset of motor signs and the patients were all in an early disease stage. The identification of the most affected side was based on the side with the worst FT-sub-score in the MDS-UPDRS-III.

a. Early PD

Clinical and demographic information

I included 26 patients with early PD and 30 controls. The other 34 controls were on average much older than the PD patients and were excluded to make both groups comparable in terms of age (mean (SD), PD: 59.6 years (10.9) vs control: 63.8 years (7.2); p=0.060). Patients with PD

were more likely to be male than the control group (65.4% vs 36.7%; p=0.030). All patients had a disease duration of less than two years (median 0.8 years, IQR 0.5 to 1.2) and were taking levodopa. The median MDS-UPDRS-III score was 20 (IQR 15 to 26). Most of the patients exhibited abnormal finger tapping from a slight to a mild degree (12 patients scored 1 and 12 patients scored 2 in the MDS-UPDRS-III FT-sub-score). One patient was found to have normal finger tapping and another one had moderately abnormal finger tapping performance (score = 3) (Table 4.6).

Associations between kinetic parameters with age, sex and handedness were assessed in control subjects. Neither age nor sex nor handedness overtly affected the test parameters (Table C4.3 in Appendix C) in the 'younger' control group (n=30, mean age: 63.8 years (SD: 7.2)). In contrast, age showed an effect on kinetic parameters (frequency and velocity) when using the complete control group (n=64, mean age: 70 years SD: 9.4) (Table C4.3 in Appendix C). For that reason, I used the two separate control groups – the 'younger' group (control[1]) to be compared with the PD group and the 'older' group (control[2]) to be compared with the anosmia group.

Table 4.6 Demographic and clinical information from the SMART test study

	PD (n=26)	Control[1] (n=30)	Anosmia (n=9)	Control[2] (n=64)
Age, years (SD)	59.60 (10.88)	63.81 (7.21)	70.94 (8.17)	69.19 (7.68)
Sex, male: female	17:9	11:19	7:2	26:38
Median years since PD diagnosis (IQR)	0.75 (0.5 to 1.2)	NA	NA	NA
Last dose of LD, median hours (IQR)	16.6 (15 to 21)	NA	NA	NA
Median MDS-UPDRS-III score (IQR)	20 (15 to 26)	1 (0 to 2)	1 (1 to 3)	1.5 (0 to 3)
Visible tremor during task, n	11	0	0	0
FT sub-score (MDS-UPDRS-III), n				
0	1	30	6	63
1	12	0	2	1
2	12	0	1	0
3	1	0	0	0
4	0	0	0	0

*Finger tapping (FT) sub-score in the MDS-UPDRS-III: 0-normal, 1- slight, 2-mild, 3-moderate, 4-severe. IQR: interquartile range, SD: standard deviation, NA: not applicable. Overall, 64 controls were included. Group (1): 30 out of 64 were extracted to compare with PD. Group (2): overall control group used for comparison with anosmia

SMART scores

When comparing the most affected side in patients with PD to the dominant side of controls, patients with PD performed repetitive finger tapping with slower mean velocity (PD: 1.20 degrees/s; 95% CI, 1.02 to 1.38 vs control: 1.63 degrees/s; 95% CI, 1.44 to 1.81; p<0.001) but

similar mean amplitude to controls with wider CI and overlap between both groups (PD: 27.08 degrees; 95% CI, 22.49 to 31.67 vs control: 31.10 degrees; 95% CI, 26.91 to 35.28; p=0.189). There was some evidence that patients with PD displayed greater variability in frequency (CV frequency) (PD: 0.18; 95% CI, 0.13 to 0.22 vs control: 0.11; 95% CI, 0.08 to 0.14; p=0.007) and more so in velocity (CV velocity) compared with controls (PD: 0.31; 95% CI, 0.27 to 0.34 vs control: 0.20; 95% CI, 0.15 to 0.25; $p < 0.001$). There was also more noticeable decrement (slope) of frequency in patients than controls (PD: −0.02 taps/sec^2; 95% CI, −0.03 to 0.01 vs control: −0.002 taps/sec^2; 95% CI, −0.01 to 0.007; p=0.003) (Table 4.7).

I carried out an additional comparison between the nondominant hand in controls and the most affected side in the PD group. Again, the mean velocity parameter was found to show the greatest difference between groups (PD: 1.20 degrees/s; 95% CI, 1.02 to 1.38 vs control: 1.56 degrees/s; 95% 1.30 to 1.67; p = 0.004). Mean amplitude in PD cases did not differ from controls, with wider CI (PD: 27.08 degrees; 95% CI, 22.49 to 31.67 vs control: 29.72 degrees; 95% CI, 25.77 to 33.66; p = 0.375). The CV of velocity was found to be higher in PD cases than controls (PD: 0.31; 95% CI, 0.27 to 0.34 vs control: 0.21; 95% CI 0.17 to 0.25; p<0.001). However, in contrast to the results using the dominant hand, the CV and slope of frequency were similar between the non-dominant hand of controls and the PD group (all p-values > 0.005 as our pre-established cut-off). When looking at the distribution between non-dominant and dominant hand of controls, the CV and slope of frequency had wider ranges in the non-dominant hand, which might be explained by different degrees of hand dominance amongst controls (Figure C4.2 in Appendix C).

Table 4.7 Comparison of SMART test parameters in PD cases and controls

	PD	Controls	p-value
Amplitude			
Mean (degrees)	27.08 (22.49 to 31.67)	31.10 (26.91 to 35.28)	0.189
CV	0.21 (0.17 to 0.25)	0.18 (0.14 to 0.23)	0.447
Slope	-0.39 (-0.62 to -0.17)	-0.42 (-0.58 to 0.27)	0.817
Frequency			
Mean (tap/sec)	2.63 (2.29 to 2.98)	3.18 (2.84 to 3.53)	0.017
CV	0.18 (0.13 to 0.22)	0.11 (0.08 to 0.14)	0.007
Slope	-0.021 (-0.03 to 0.01)	-.002 (-0.01 to 0.007)	0.003
Velocity			
Mean (degrees/sec)	1.20 (1.02 to 1.38)	1.63 (1.44 to 1.81)	<0.001
CV	0.31 (0.27 to 0.34)	0.20 (0.15 to 0.25)	<0.001
Slope	-0.07 (-0.08 to -0.05)	-0.06 (-0.08 to -0.04)	0.662

The dominant hand from controls and the most affected side from PD cases was used for comparison. All parameters presented with 95% coefficient interval (CI). CV: coefficient variation. P-value: Welch's t-tests (two-tailed), except for frequency, where two-sample Wilcoxon rank-sum (Mann-Whitney) test was used.

Eleven patients exhibited action tremor. To prevent over estimation of an inflated frequency parameter caused by tremor, when two consecutive peaks of amplitude were found without reaching the baseline amplitude of 0 (meaning that both fingers were close together), it was interpreted as a finger tremor instead of a finger tap. I selected the highest peak to avoid under-estimation of the amplitude.

In three patients a re-emergent action tremor was seen with the tremor occurring after a finite period (latency) from the time the patient started the finger tapping task (illustrated in Figure 4.5).

When using the dominant hand of controls for comparison, velocity offered the best discriminatory power with 84.6% sensitivity for 73.3% specificity and an AUC of 0.81 (95% CI, 0.69 to 0.93) (cut-off=3.72 degrees/sec). The CV of frequency also showed reasonable discrimination with 80.8% sensitivity for 70% specificity and an AUC of 0.75 (95% CI, 0.62 to 0.88). Combining both parameters (velocity mean and the CV of frequency) meant that the specificity improved to 86.7% for 80% sensitivity (AUC 0.83; 95% CI, 0.72 to 0.95). The slope of frequency was able to distinguish between groups with moderate accuracy (AUC 0.72; 95% CI 0.59 to 0.86), but when it was combined with velocity the discriminatory power improved, yielding a sensitivity of 80.8% for 83.3% specificity (AUC 0.88; 95% CI, 0.78 to 0.97).

In the same way, when I combined the slope of frequency with CV velocity, both parameters also reached high accuracy (AUC 0.85; 95% CI, 0.74 to 0.95) with 80.8% sensitivity for 85% specificity (Table 4.8 and Figure 4.6).

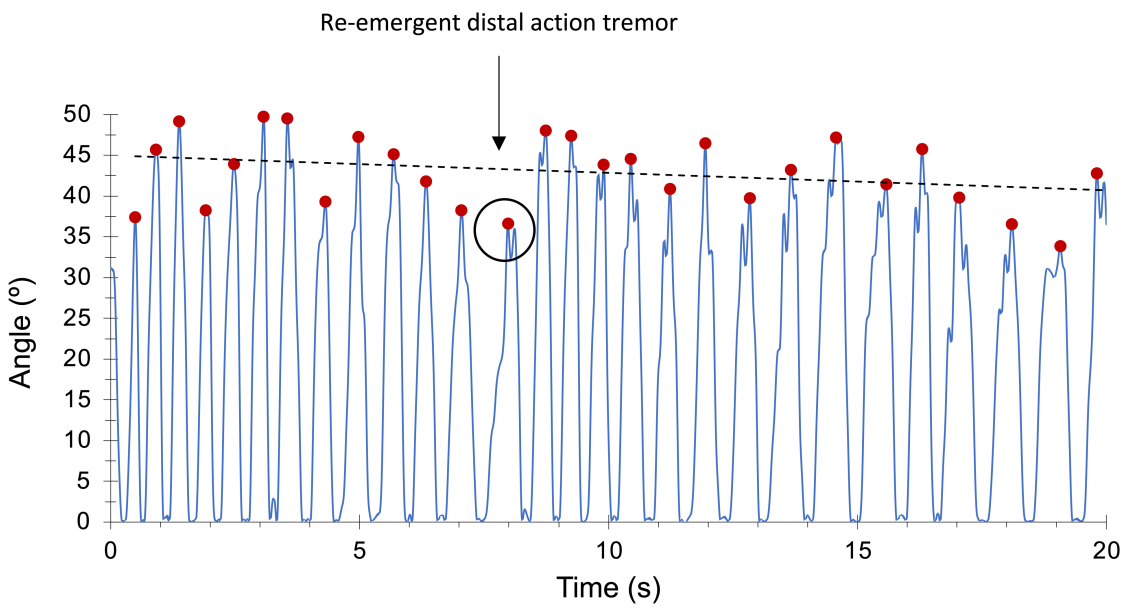

Figure 4.5 Example of re-emergent action tremor. Patient with PD with index finger action tremor appearing after 8 seconds of latency (re-emergence phenomena). Only the highest peak of amplitude was selected.

Table 4.8 SMART test ROC analysis between PD cases and controls

	CV velocity + Slope frequency	Velocity + Slope frequency	Velocity + CV frequency
	Sensitivity	Sensitivity	Sensitivity
Specificity 85%	80.77%	73.08%	73.08%
(cut-off)	(>=0.49)	(>=0.51)	(>=0.53)
Specificity 75%	80.77%	84.62%	80.77%
(cut-off)	(>=0.53)	(>=0.46)	(>=0.39)
AUC (95% CI)	0.85 (0.74 to 0.95)	0.88 (0.78 to 0.97)	0.83 (0.72 to 0.95)

The dominant hand from controls and the most affected side from PD cases was used for the Receiver Operating Characteristic (ROC) analysis, AUC: Area Under the Curve. ROC curves plotted in Figure 4.6

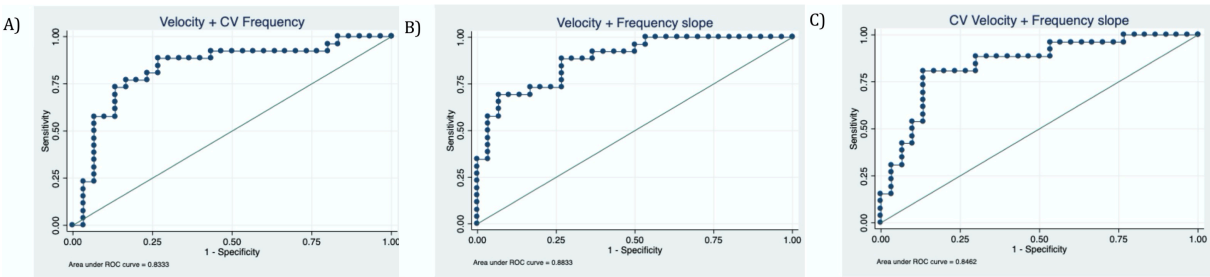

Figure 4.6 ROC curves for the best parameter combination to distinguish patients with PD and controls. A) Velocity and CV frequency, B) Velocity and frequency slope and C) CV velocity and frequency slope

Clinical correlation with MDS-UPDRS-III FT-sub-scores

I correlated the SMART kinetic parameters with the FT-sub-scores of the MDS-UPDRS-III in patients with PD. The FT-sub-score classification is defined Table 4.1. All PD patients apart from two scored between 1 (slight degree) and 2 (mild degree) in the MDSUPDRS- III sub-score. To avoid the two patients scoring 0 (normal degree) and 3 (moderate degree) and influencing the correlation curves (Figure C4.3 in Appendix C), I excluded them from the main correlation analysis. The mean amplitude was found to have the highest correlation with FT-sub-score (r=–0.49; p=0.003) followed by velocity (r=–0.43; p=0.016), whereas there was no correlation with mean frequency. For more detailed information about the correlations explored, see Table C4.4 and Figure C4.3 in Appendix C.

b. Idiopathic anosmia group

Clinical and demographic information

We tested nine patients with idiopathic anosmia. I compared their finger tapping performance with the performance of 64 controls of a similar age (mean (SD), Anosmia: 70.9 years (8.2) vs control: 69.2 years (7.7); p=0.581). There was a higher proportion of males in the anosmia group than in the control group (77.8% vs 40.6%; p=0.040). The mean duration of anosmia was 5.3 years (SD 4.3 years). In the anosmia group, the median motor score on the MDS-

UPDRS-III was 1 (IQR 1 to 3). Of note there were no patients with anosmia meeting PD diagnostic criteria. However, one individual who scored 10 on the MDSUPDRS-III was classified as having SP based on MDS Task Force (for more details see Glossary). The remaining patients with anosmia scored between 0 and 4 in the total MDS-UPDRS-III. Most of the individuals with anosmia (seven out of nine) scored 0 in the FT-sub-scores in the MDS-UPDRS-III, two people exhibited slight bradykinesia (score=1) and one participant was found to have mild bradykinesia (score=2). Table 4.6 summarises the clinical and demographic information of both groups.

SMART scores

Although MDS-UPDRS-FT-sub-scores were normal in most individuals with anosmia (seven out of nine), the SMART test detected motor impairment in their finger tapping performance compared with the control group. The pattern of movement in participants with anosmia shared similarities with PD patients. Individuals with anosmia performed the task with a reduced mean amplitude. Despite broad ranges, there was no overlap between groups (Anosmia: 13.94 degrees; 95% CI, 9.19 to 18.69 vs control: 29.38 degrees; 95% CI, 26.87 to 31.89; p<0.001) (Table 4.9). Compared with controls, the anosmia group showed a slower mean velocity (Anosmia: 0.96 degrees/s; 95% CI, 0.64 to 1.27 vs control: 1.48 degrees/s; 95% CI, 1.37 to 1.60; p<0.001). Although mean frequency was similar between anosmia and controls, there was weak evidence that individuals with anosmia exhibited slightly greater decrement over time compared with controls (p=0.059). In contrast to PD, CV of velocity was similar between groups (p=0.054).

Table 4.9 Comparison of SMART test parameters in anosmia cases and controls

	Anosmia	Controls	p-value
Amplitude			
Mean	13.94 (9.19 to 18.69)	29.38 (26.87 to 31.89)	<0.001
CV	0.30 (0.20 to 0.40)	0.19 (0.16 to 0.22)	0.009
Slope	-0.23 (-0.49 to -0.03)	-0.39 (-0.49 to -0.29)	0.243
Frequency			
Mean	3.26 (2.62 to 3.90)	3.05 (2.82 to 3.28)	0.515
CV	0.15 (0 .05 to 0.26)	0.13 (0.10 to 0.16)	0.560
Slope	-0.020 (-0.04 to -0.003)	-.002 (-0.01 to 0.005)	0.059
Velocity			
Mean	0.96 (0.64 to 1.27)	1.48 (1.37 to 1.60)	0.001
CV	0.28 (0.16 to 0.40)	0.21 (0.18 to 0.23)	0.054
Slope	0.01 (-0.004 to 0.03)	0.02 (0.02 to 0.03)	0.369

All parameters presented with 95% coefficient interval (CI). CV: coefficient variation, p-value: Welch's t-tests (two-tailed)

I then compared the anosmic group with the unaffected side of patients with unilateral PD (n=13). The dominant hand of the anosmia groups exhibited a similar pattern of variation to the unaffected side of PD; the CV of amplitude, frequency and velocity, together with the mean of frequency, were comparable between groups (all $p>0.05$). Of note, the finger tapping in the anosmia group was on average smaller (mean amplitude: 13.95 degrees; 95% CI, 9.18 to 18.69 vs 36.18 degrees; 95% CI, 27.89 to 44.36; $p<0.001$) and slower (mean velocity: 0.96 degrees/s; 95% CI, 0.64 to 1.27 vs 1.89 degrees/s; 95% CI, 1.46 to 2.32; $p<0.001$) than the unaffected side of people with early PD. Of note, the anosmic group was significantly older than the PD group with unilateral signs (mean (SD), Anosmia: 70.9 years (8.2) vs PD: 59.6 years (10.9); $p=0.004$).

4.4 Discussion

Here I present two objective motor tools to quantify motor dysfunction in early PD, the DFT and the SMART test. Both tests analysed finger tapping movement. The former appeared to work synergistically with the BRAIN test to accurately distinguish patients with PD from controls. Their remote nature and easy applicability may offer a promising advantage to be applied in large longitudinal studies. The latter introduced potential new signatures of motor dysfunction in early PD, suggesting that new measures of early motor patterns may be needed beyond the MDS-UPDRS-III.

4.4.1 Distal Finger Tapping test

In this proof-of-concept study, we demonstrated that a simple keyboard finger tapping test can be used to quantify separate components of distal movement such as speed, akinesia and rhythm. In general, patients with PD performed the DFT test more slowly (fewer key taps per task with higher akinesia time) and more erratically (higher arrhythmicity score) than controls. These differences were noticeable enough to consider each kinetic parameter an accurate measure to distinguish patients from controls.

The combination of DFT and BRAIN test performance including KS, AT and IS parameters from both tests reached the highest level of accuracy. Although both tests assess repetitive movements in the upper limbs, they are focused on movements originating at different anatomic levels. The former assesses distal repetitive movements (hand level) and the BRAIN test assesses proximal repetitive movements (shoulder level). Distal and proximal bradykinesia have been demonstrated to be differentially affected in PD and have distinct responses to therapeutic options.[174,215] Used in conjunction with the BRAIN test, the DFT test would provide a complementary view of proximal and distal upper-limb movement.

The DFT test offers several advantages compared to other technology-based tools designed to assess bradykinesia in PD. The BRAIN test shares all the following advantages of cost, feasibility and compliance. Unlike previous tests, the DFT test is free from specialist equipment or

software and is freely available online, hence it can be accessed by any laptop or computer. Further, the fact that the DFT test requires just less than two minutes in total might facilitate compliance. Results were automatically transferred to a secure and central server for clinicians to view, and data could only be accessed using unique tokens and passwords, adding to the safety of patient data. In practical terms, the DFT and BRAIN tests might serve as a key strength for unsupervised remote monitoring of patients' motor function. This fact could prove particularly useful in the COVID-19 era where face-to-face clinic appointments are substantially reduced.

Despite the potential applicability of the DFT test, it has several limitations. The DFT test is not able to measure amplitude and its decrement. Practically, it is not feasible to capture amplitude with a keyboard alone and using the DFT test as a remote assessment tool would not favour the incorporation of additional equipment, like a camera, to assess amplitude. For that reason, it cannot be said that the DFT test comprehensively measures bradykinesia, as amplitude is an essential component of its definition. Nevertheless, the DFT test was able to accurately capture relevant kinetic aspects of distal movement such as frequency, rhythm and velocity.

It is important to note that the DFT test cannot be considered a holistic measure of motor function, as it cannot account for other components of movement known to be affected in PD, such as walking, speech and facial expression; thus, its aim is not to act as a diagnostic tool but rather to complement clinical assessment. Additionally, IS20 did not correlate with FT-sub-scores. This is possibly due to the crude integer MDS-UPDRS-III, which is not able to capture subtle effects such as the variance of travelling time. Hasan and colleagues described the correlation of MDS-UPDRS is not a good indicator for objective monitoring of tests.[216] Instead, clinimetric properties such as test–retest reliability, sensitivity, specificity, responsiveness, feasibility, and administrative burden should be considered in carrying out a more comprehensive evaluation of these tests, for all of which the DFT test bodes well.

A further limitation faced by the DFT as a monitoring tool is the potential role of confounding factors such as mood and alertness which might influence patients' motor performance. Unfortunately, it was not possible to control these factors. Similarly, the use of different keyboard types with varying key sizes and resistance was not considered in the present study and may have interfered with the performance of the task.

Finally, selecting the most affected side in PD could have magnified the accuracy of the test. However, this was carried out as PD is an asymmetrical condition and thus it would be appropriate to assess the performance of the tapping test in patients' worst affected side.

Future directions for the DFT test include assessing it in combination with the BRAIN test as a form of remote longitudinal monitoring of asymptomatic individuals who are at high risk of developing PD. In that regard, we included the DFT in the PREDICT-PD website. In the next few years, I hope we will have enough data to prove its applicability.

4.4.2 Slow-Motion Analysis of Repetitive Tapping test

The main aim of the study was to prove that beyond bradykinesia there are early repetitive movements in PD which might be difficult to pick up by the 'naked eye' but may be detectable through slow-motion video capture.

The etymological definition of bradykinesia is 'slowness of movement'. In the context of PD, the concept of bradykinesia also involves another classic feature which is the decrement of amplitude and frequency over repetitive movements defined as 'sequence effect'.[116] In this regard, I found that patients with early PD did not fulfil a full definition of bradykinesia in our cohort; although they performed the finger tapping task more slowly and with more pronounced decrement in frequency than controls, they had similar amplitude and decrement to controls. In addition, the SMART test detected a potential early new PD signature beyond bradykinesia, which might explain the lack of decrement in amplitude in our cohort. I found that the amplitude of finger tapping had a common pattern in people with early PD. It was based on a non-linear trend characterised by a 'burst' phenomenon: repetitive cycles of slowing down and becoming smaller followed by a late amplitude increase. In fact, this last reinforcement could have compensated for the decrement and the average of amplitude over the 20-second finger tapping task (see PD case example B in Figure 4.7). This rebound pattern could be explained by a compensatory motor mechanism present in early stages of PD before grinding down to a complete halt in more established PD. In fact, other studies using electronic measures have yielded similar results.[181]

Velocity and frequency were able to distinguish patients from controls with good accuracy, particularly using a combination of both (AUC 0.88). Our findings agreed with another study, with mean velocity and its parameter of variation (CV) found to have high accuracy (see Table 1.3). In contrast, in a study by Růžička and colleagues, who used a contactless 3D motion capture system to compare 22 patients with 20 controls, amplitude was the best marker.[169] The slope of amplitude alone provided an accuracy of 0.87. Since their cases had a longer disease duration (9.3 years) than ours, this might suggest that 'sequence effects' are more predominant later in the disease course.

Amplitude and velocity correlated best with the MDS-UPDRS-III FT-sub-scores. This might suggest a potential role as surrogate markers of disease severity. Although there was a moderate positive correlation with FT-sub-scores, the lack of any strong correlation suggests that the SMART test and the FT-sub-scores of the MDSUPDRS-III are identifying different phenomena. It is important to consider that unlike the SMART test parameters, which are continuous data, the FT-sub-scores are categorical data (from normal to severe). Since the MDS-UPDRS-III FT-sub-score was designed for patients with established PD, in people with early PD we could expect a floor effect defined as a large concentration of kinetic values near the lower limit of the FT-scale. This phenomenon is illustrated in the correlation plots in Figure C4.3 in Appendix C. In contrast, continuous data is not subject to floor effect, offering the

opportunity to define early signatures of PD. Williams and colleagues carried out a project with a similar approach to us.[188] They recorded a 10-second finger tapping task using a smartphone and tracked this movement applying deep learning algorithms (convolutional neural networks) extracted from the DeepLabCut platform.[217] In this study, patients had a longer disease duration (median of 4 years) and were on average 9 years older than our group. Although accuracy was not reported, velocity exhibited a greater correlation with the FT-sub-score of MDS-UPDRS-III than ours (r=−0.74 vs r=−0.60). This may support the notion that the MDS-UPDRSIII is best adapted to patients with established disease rather than earlier stages.[218]

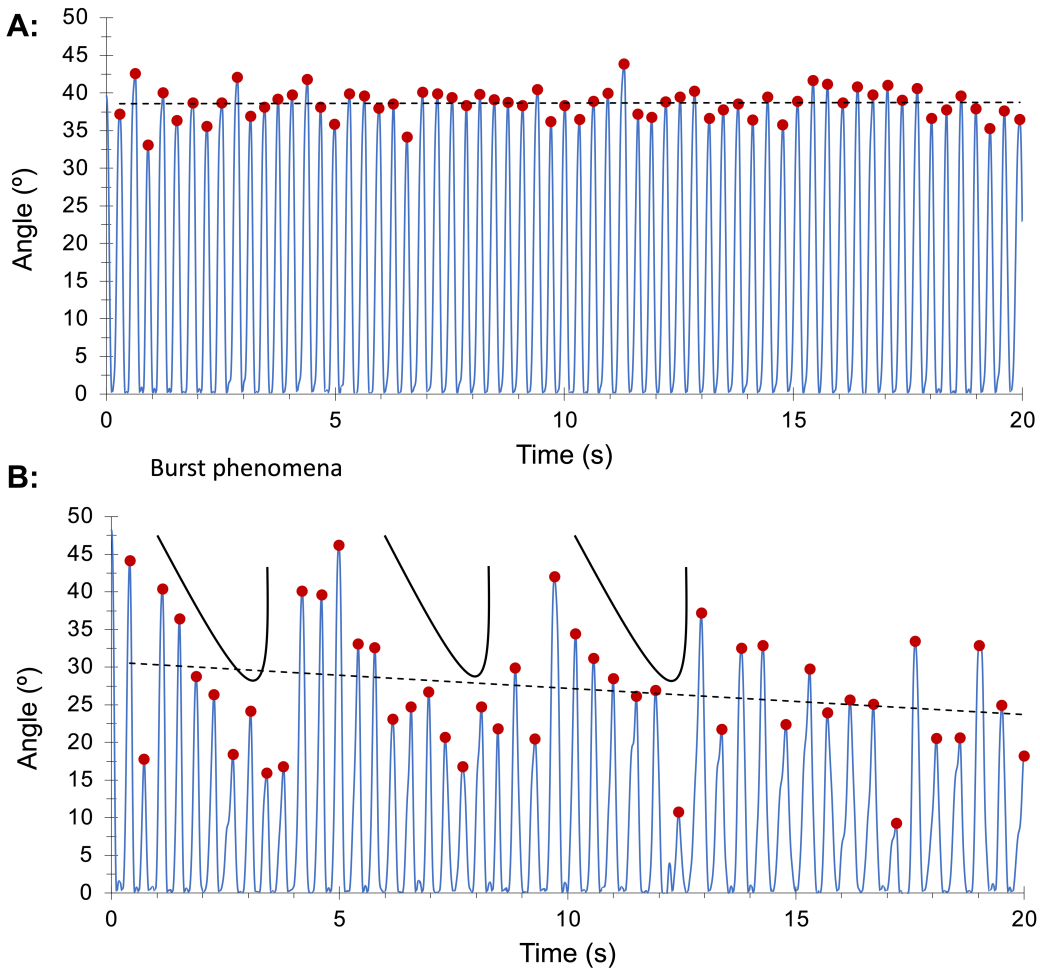

Figure 4.7 Control subject (A) with constant frequency and amplitude compared to patient with PD (B) showing a 'burst phenomena' (repetitive amplitude rebound over 20 seconds task)

Beyond the MDS-UPDRS-III not having been designed for early stages of PD, it has intrinsic clinimetric limitations. It is important to consider that clinical scales are semiquantitative and semi-objective, and they are prone to individual bias, which increases inter- and intra-rater variability.[218] In contrast, technology-based tools are objective quantitative measures less subject to individual bias. To be of practical value, technology should exceed the performance of "Gold Standard" clinical scales or at least be more efficient. Gao and collaborators designed a sensor device able to assess finger tapping and explore whether it could be used to identify

early stages of PD and correlate with disease progression.[183] Readings from the sensors were analysed by using evolutionary algorithms, which are a form of artificial intelligence designed to create classifiers of patterns of movement.[219] Their tool reached a high accuracy (≥89.7%) for detecting different severity degrees of bradykinesia. Moreover, it could discriminate early stages of PD with AUC of 0.90. A recent review gave evidence about the potential role of video-based artificial intelligence in PD diagnosis. It could be particularly useful when classification involves complex and dynamic patterns of movement.[220] These findings should encourage further research to focus on meticulous detection methods of motor dysfunction throughout the disease course, including the early phase of PD.

Most studies of idiopathic anosmia did not find detectable motor dysfunction using the MDS-UPDRS-III.[1,54,221] One longitudinal study showed that whereas subjects with anosmia did not have worse motor scores than individuals with a normal sense of smell, a greater proportion had abnormalities on the DAT-SPECT (11% vs 1%).[54] One systematic review and metanalysis suggested that anosmia was associated with a 3.84-fold risk of developing PD[222] and the MDS Criteria for Prodromal PD show that, based on seven prospective studies, objective smell loss has a positive likelihood ratio of 4.0.[67] Based on these findings, we could expect that individuals with anosmia will have sub-clinical motor features. The fact that UPDRS-III is often normal in patients with anosmia suggests that other assessments adapted for early stages of PD are needed.[223]

The current study is the first to use a technology-based tool to explore subtle motor features in idiopathic anosmia. Although our findings remain exploratory and warrant further investigation in a larger sample, the SMART test appeared able to detect subtle changes in the anosmia group whilst the FT-sub-score of the MDS-UPDRS-III was less able to identify such discrepancies (six out of nine patients had normal FT-sub-scores). Similar to the most affected side of the PD group, individuals with anosmia performed the finger tapping task less quickly than controls. The anosmic group also shared similarities (CV of all three parameters: amplitude, frequency and velocity) with the unaffected side of PD, which may suggest that erratic patterns of movement could be potential sub-clinical motor signatures, like the 'burst' phenomenon described above. Interestingly, mean amplitude and mean frequency showed opposite results. Subjects with anosmia had a smaller finger tapping amplitude with similar frequency compared with controls, whereas PD patients exhibited a reduced frequency with similar amplitude to controls (Figure 4.8). This might suggest distinct compensatory mechanisms (maintaining a bigger amplitude by reducing the frequency and vice versa) at different stages of disease. This could also suggest that amplitude of finger tapping has a longer LD response duration in patients with PD, which might explain why patients with PD had a bigger amplitude than the anosmia group.

One could argue that the PD group was on average younger that would be expected based on population-based studies that cite PD age of onset at around 70 years.[224] QMAP is an MRI-based study, and the contraindications to scanning (e.g. implanted devices such as pace-makers)

will increase with age, and so this may contribute to having a slightly younger group. There may also be a slight bias in the types of people who join research trials that require active travel/engagement. However, there is no clear-cut indicator on the average age of PD onset, and therefore the question is why there is a discrepancy between deep phenotyping studies focused on early PD and the gold-standard post-mortem defined literature, versus the existing epidemiological literature.[225]

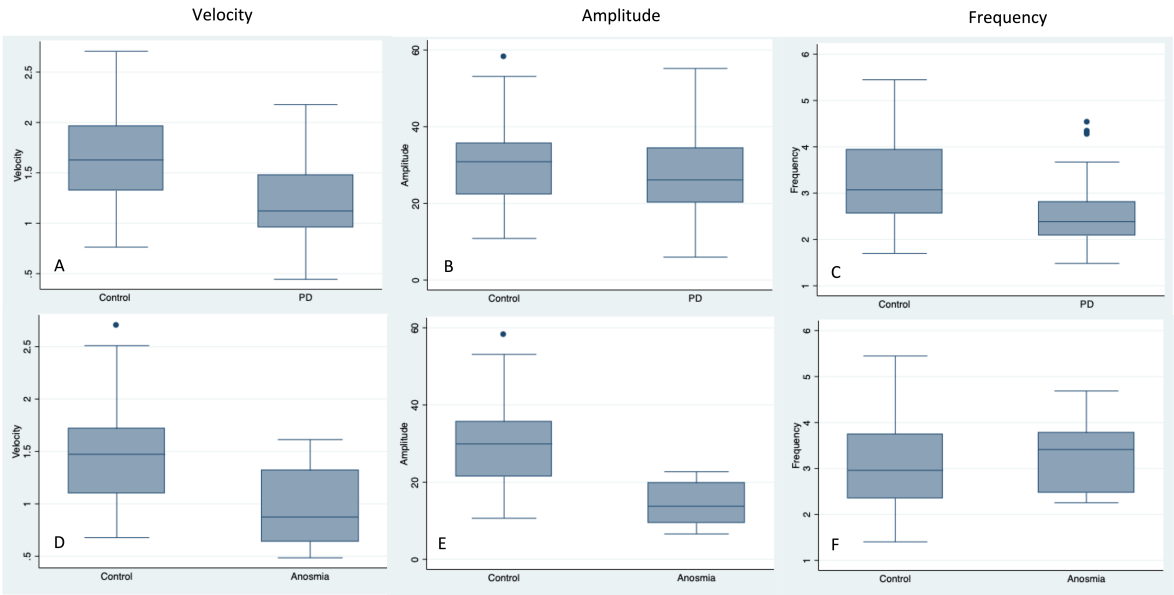

Figure 4.8 Boxplots comparing SMART test performance of PD group with the control group[1] (A-C) and the anosmia group with the control group[2] (D-F)

The SMART test offers several advantages. It is a sensor-free tool; therefore, it does not interfere with the natural range of movement. It is inexpensive with only a smartphone camera being required, which can potentially make it applicable in larger scale studies. However, it also entails several methodological and data processing limitations.

In terms of methodological limitations, one important consideration is that the exclusion of controls scoring more than 6 in the MDS-UPDRS-III (cut-off for SP) may have contributed to artificially increasing the accuracy of the SMART test. In a similar way, the selection of the best scenario comparing the dominant hand in controls and the most affected side in PD could also have magnified the accuracy of the test. Although handedness was reported as a binary variable, degrees of hand dominance amongst controls should be presumed. Pure-right and pure-left handed people are expected to exhibit bigger discrepancies between their dominant and non-dominant hand. However, in this proof-of-concept study, the main purpose was to know whether the SMART test was able to distinguish patients form controls under the best circumstances without potential confounding factors such as handedness. Further studies would need to account for the role of handedness as a continuous variable with scales such as the Edinburgh Handedness Inventory.[226] Sex matching was difficult to accomplish due to our source of recruitment. Most of our controls were the partners of PD cases (who were

predominantly male). One might expect that the lack of sex matching could bias comparisons since men and women's hands have different characteristics. However, there were no differences in terms of their performance between male and female controls. Another methodological limitation to consider would be that by asking not to rotate the hand, which was done to capture the real angle, we might have prevented patients from adopting certain hand postures. This limitation is important, especially considering a possible co-existence of dystonic tremor in patients with action tremor. Finally, the PD, anosmia and control groups were tested by three different clinicians. Although they followed the same instructions, some degree of variability in the finger tapping recording should be expected. To overcome this limitation, Dr Miquel A Galmés and I checked the videos one by one and made sure that they were recorded in a consistent way. Moreover, I scored all the FT-sub-scores to avoid inter-rater variability.

Moving to data processing limitations, I derived relatively simple summary statistics from the derived time series. Using other techniques based on the frequency domain that capture beat to beat variation may be more sensitive, as demonstrated by Biase and colleagues with the tremor stability index.[227] However, the aim of this work was to provide proof of concept, that motion capture using a smart phone could provide metrics sensitive to changes in early PD. There are many non-linear, time-series metrics, and this question will be the focus of future work. Although I used a simple, threshold-based method for discriminating PD from controls, I acknowledge that there are other approaches based on machine learning that may be able to leverage the whole time-series, or indeed the raw video footage, and ultimately prove more accurate. However, in this current work I sought to derive quantitative metrics from video footage, given that these measures have much broader utility beyond mere categorical diagnostics (e.g. treatment biomarkers).

To conclude, I developed two novel methods capable to detect abnormal patterns of repetitive finger tapping movement. The DFT and BRAIN tests seemed to work properly in conjunction, capturing upper limb movements from two separate origins, distal and proximal movement respectively. Moreover, the SMART test showed detectable differences in the motor performance of people with idiopathic anosmia unravelling potential prodromal signatures of bradykinesia. Speed measures, including KS and finger tap velocity, were found to be the most accurate parameters in both tests. In the next chapter, I created a motor battery which included the DFT, BRAIN and SMART tests, amongst other tools. They were tested in an enriched population with increased risk of PD to explore whether they were able to capture a broader picture of movement abnormalities in the prodromal stage of PD.

Chapter 5

Developing and testing a motor battery in people with rapid eye movement sleep behaviour disorder

5.1 Introduction

There is evidence supporting the prominence of parkinsonian signs amongst people with isolated iRBD who go on to develop an α-synucleinopathy such as PD and Dementia with Lewy Bodies (DLB).[37] Motor signatures are appealing clinical biomarkers to use in prediction models for future PD. However, specific protocols, including those using quantitative motor tools are yet to be optimised.

In selecting sensitive clinical biomarkers, quantitative motor tools appear to be more appropriate at early stages of the disease.[108] A study with the largest cohort of people with v-PSG-confirmed iRBD carried out by the International RBD Study Group followed up 1280 patients with iRBD over a median of 3.6 years.[37] They found that, unlike clinical rating scales, the combination of quantitative motor tests (alternate finger tapping, TUG and Purdue PegBoard test) was the most powerful predictive marker of future diagnosis of any sort of α-synucleinopathy (hazard ratio 3.16; 95% CI, 1.86 to 5.37). Arora and colleagues created a set of quantitative motor assessments based on a smartphone which analysed finger tapping, voice, balance, reaction time and tremor.[150] They did an internal validation with machine learning models which showed that the overall set of tools was highly effective in discriminating between people with iRBD, PD patients and controls. However, this study had several limitations that need to be considered. First, motor tasks were restricted to a single device used for data collection; therefore, it was not possible to extract detailed kinematic parameters of gait and finger tapping. Second, these sophisticated algorithms are mathematically complex, and therefore difficult to be interpreted from a clinical perspective. Thus, a high discrimination accuracy of machine learning algorithm does not necessarily denote high explanatory power. It is with that in mind that that there is a need to develop a motor battery that can measure early motor dysfunction, not only in a truly quantitative manner, but this battery also needs to be used in a replicable and interpretable way. To be replicable on a large scale, it is important to use inexpensive and user-friendly tools. Moreover, this battery needs to have a clinically meaningful interpretation to avoid making spurious conclusions.

5.2 Methods

I created a motor battery for the assessment of potential motor prodromes of PD and compared them with the standardised clinical rating scale in PD (the MDS-UPDRS-III). To do that, I tested individuals with iRBD and compared them to age and sex matched healthy controls to identify the most salient motor signatures in people with iRBD. The final goal was to define the most sensitive motor markers of imminent parkinsonism. Given the cross-sectional nature of the study, Subthreshold Parkinsonism (SP) was used as the outcome due to the lack of incident PD cases.

Of note, I was not blind to participants' diagnosis. The assessments took place between April 2021 and August 2021. Patients with iRBD were identified from the Sleep Clinic at Guy's St Thomas Hospital by Dr Laura Pérez-Carbonell, Consultant in Neurology and Sleep Medicine, and Prof Guy Leschziner, Consultant Neurologist and Clinical Lead for Sleep Disorders Centre. All patients had an overnight v-PSG that confirmed the diagnosis of iRBD. Healthy controls were recruited from the lower risk group in the PREDICT-PD study and were matched for age and sex with the iRBD group.

Exclusion criteria for iRBD and controls were having a formal diagnosis of dementia, PD and other neurological condition that could affect their motor performance such as essential tremor, motor neurone disease, multiple sclerosis or polyneuropathy. I also checked for current medication with potential parkinsonian side effects.

I used the same equipment for all the assessments including camera, smartphone and laptop. Due to the COVID-19 pandemic, I adapted the location of face-to-face visits based on participants' preferences. Most of them took place at Wolfson Institute of Population Health or at participants' homes. I put a range of measures (gloves and mask during each visit) in place to guard participants against the risk of coronavirus during visits. Participants wore a mask except for during the facial expression analysis as part of the MDS-UPDRS-III. In that case, I maintained a 2-metre distance between myself and participants.

5.2.1 Motor assessments

I invited each participant to complete a battery of motor tests which encompassed a series of semi-quantitative and quantitative motor tools (Figure 5.1). I included the same quantitative motor tests used in Chapter 3 (the BRAIN test, TUG test and timed handwriting task) and the DFT and SMART tests presented in Chapter 4. It is known that challenging conditions might unmask early motor dysfunction in individuals at risk of developing PD. For that reason, I added three new challenging tasks: a 10-metre walking test while doing a mental task, a 10-second longer (30 instead of 20 seconds) finger tapping test while performing another mental exercise and a rapid blinking task. In addition to the motor battery, I scored each participant's

examination following the MDS-UPDRS-III instructions and asked for motor symptoms using the MDS-UPDRS-II.

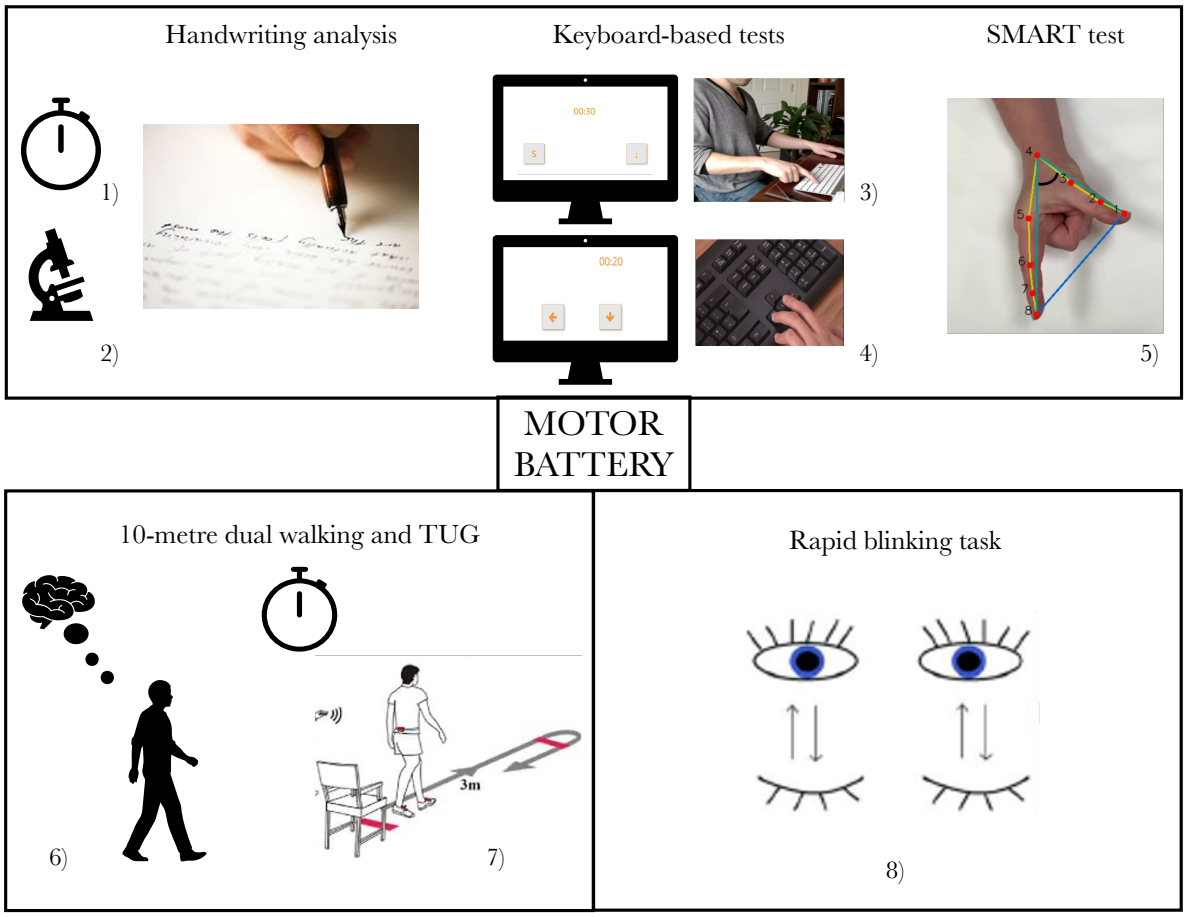

Figure 5.1 Motor battery description. 1) timed handwriting analysis (copying three times a sentence), 2) graphologist impression (8-item scale), 3) BRAIN (BRadykinesia Akinesia INcoordination) test, 4) DFT (Distal Finger Tapping) test, 5) SMART (Slow-Motion Analysis of Repetitive Tapping) test, 6) 10-metre dual walking, 7) TUG (Timed-Up-and-Go test) and 8) rapid blinking task

As described in Chapter 3, the MDS-UPDRS-III is a semi-quantitative scale which covers features known to be part of the clinical picture of PD, such as bradykinesia, rigidity, tremor and axial signs.[163] The scale uses several tasks to evaluate each motor feature. As an example, bradykinesia is assessed via five separate tasks: finger tapping, hand movements, pronation/supination movements, toe tapping and leg movements. The degree of impairment of each task is based on the following scoring system: 0 (normal), 1 (slight), 2 (mild), 3 (moderate) and 4 (severe). I took the same approach as in Chapter 3. I broke down the MDS-UPDRS-III into separate motor domains and gathered all the scores related to that domain together. As the same example given before, I added all the scores from bradykinesia to obtain an overall bradykinesia score. I used the same method for action tremor, which included postural and kinetic tremor, rigidity and axial domains, which covered the following tasks: gait, posture, posture instability, and rising from a chair.

Lower limb function was assessed via walking speed and sequential tasking by the TUG (for more details see Methods in Chapter 3). Moreover, walking speed was also tested under mental task conditions which consisted of listing the months of the year in reverse order.

Fine motor skills were objectively assessed through three separate upper limb motor tests, two of which were based on a keyboard tapping task (the DFT and BRAIN tests) and the other one was based on a finger tapping task tracked with a slow-motion video (the SMART test). For information regarding the BRAIN test instructions, see Chapter 1. Chapter 4 provides detailed information about how the DFT and SMART tests work. It is important to point out that all participants used the same laptop to perform DFT and BRAIN tests. To test our hypothesis about challenging conditions unmasking motor dysfunction at early stages of PD, I added two measures to make the finger tapping task more difficult: I increased the duration of the task from 20 seconds to 30 seconds and asked participants to do a mental exercise (successively subtract '3' from '100' continuously) while performing the motor task.

Additionally, I added another semi-quantitative test to assess fine motor skills. I included the same handwriting task I used in Chapter 3. As a reminder, participants were timed while copying the sentence '*Mary had a little lamb, its fleece was white as snow*' three times using a pen and white paper. Due to the low proportion[IX] of micrographia found in individuals at higher risk (described in Chapter 3), I changed the handwriting analysis approach. I incorporated the expertise of a graphologist, Emma Bache. I invited her to review the handwriting scripts of people with iRBD and controls. Of note, she was blind to participants' diagnosis. Based on her impression, I selected the most prevalent handwriting features considered abnormal and created an 8-item scale (Table 5.1, Figure 5.4 and Figure 5.5). All items were scored 0 or 1 point, reflecting absence or presence of the sign respectively.

Table 5.1 Handwriting scale

Item	Description
1. Micrographia	Abnormal progressive reduction (decrement) in handwriting size
2. Sentence slope	Upwards or downwards sentence slant
3. Hidden tremor	Small signs of hand tremor within the scripts
4. Retracing	Letter formation that has been retraced
5. Resting marks	Presence of dots in the scripts that denote resting pause during writing
6. Irregular shape	Irregular shape of the same letter or word across the three sentences
7. Excessive Pen Pressure	Sign on the paper suggesting that writer pressed very firmly with pen
8. Inconsistent word spacing	Space between words not consistent across the sentences

[IX] Six people had micrographia (based on my clinical impression): one was classified in the HR group and five in the LR group

It is important to point out that I used another control group for the handwriting analysis. I selected previous scripts collected in Chapter 3. The main reason for using old, rated scripts were time and budget constraints. The analysis of one handwriting script done by the graphologist cost an average of £160. Since our time and budget was limited, we decided to re-use the same control group we used for a separate project done in collaboration with the graphologist. The control group was older than iRBD (mean (SD), 74.9 years (5.4) vs 68.9 years (8.1); $p<0.001$) but had a similar proportion of males (23/29 (79.3%) vs 30/33 (90.9%); $p=0.283$).

Finally, I looked for the presence of hypomimia amongst both groups. Reduced blinking is part of facial hypomimia. I objectively assessed blink rate under two separate conditions: informal conversation (involuntary blinking) and challenging conditions (voluntary rapid blinking). The former consisted of asking participants to talk about what they like to do in their spare time for 1 minute, while they were looking at a camera. The latter consisted of asking participants to blink as fast as they could for 15 seconds. Footage was then later reviewed by a medical student (Michaela Francis) who counted the number of blinks in both tasks. Participants were instructed to remove their glasses whenever possible to allow for better visualisation of the eyes.

5.2.2 Systemic symptoms

Participants completed a series of questionnaires asking for non-motor aspects of experiences of daily living (MDS-UPDRS part I), motor symptoms (MDS-UPDRS part II), and symptoms of autonomic dysfunction (SCOPA-AUT). I measured their blood pressure lying and standing up to check for orthostatic hypotension. I invited participants to lie down for 1 minute and measured their blood pressure and then asked them to stand up and repeated the blood pressure measurement after 1 and 3 minutes while they remained standing.

Finally, participants completed the MoCA test, which is a widely used screening instrument to detect cognitive impairment in PD and PD-dementia.[205] It assesses a series of cognitive domains which include visuo-constructional and executive skills, short term memory, attention skills and orientation.

5.2.3 Statistical analysis

Data normality was assessed using the D'Agostino test. Summary statistics were obtained, and data were checked for outliers and skew with histograms and boxplots. The mean and SD were calculated for normally distributed data. IQRs were calculated for non-normal distributed data. Categorical variables were presented by absolute frequency and percentage, and compared with Fisher's exact test. Quantitative data for demographic and motor outcomes were compared using the Welch's test for unequal variances.

I explored whether handedness could influence keyboard kinetic parameters in iRBD and controls separately. To do that, I compared the DFT, BRAIN and SMART tests performance using the dominant hand with the non-dominant hand, applying paired t-test and Mann-Whitney for normally and not normally distributed data respectively. I considered one pairwise discriminatory comparison between participants with iRBD and controls. I used the Wilson/Brown method to generate ROC curves and determine the accuracy (sensitivity and specificity of parameters) of the test in detecting iRBD patients from controls, iRBD with SP from controls and iRBD with and without SP. A cut-off that maximised Youden's J index was selected for each variable. I used logistic regression and ROC curves to define AUC values for each quantitative motor marker and the MDS-UPDRS-III. I repeated the analysis using the combination of the most accurate motor parameters in the motor battery. I did not adjust for age or sex given that both groups were matched for age and sex.

All statistical tests were two-tailed. I accounted for multiple comparisons problem to avoid an increase in type 1 error. I applied the Bonferroni calculation to adjust the cut-off for evidence of association. This was the case of the handwriting scale which included nine variables, therefore I set the p-value at <0.006 (0.05/9). Data analysis was carried out using STATA v.13 (StataCorp, College Station, TX).

Ethics approval was granted by the Queen Square Research Ethics Committee (09/H0716/48). Participants received verbal and written information about the study and appropriately consented.

5.3 Results

I recruited 34 participants with iRBD and 35 age and sex matched controls. One patient with iRBD fulfilled the diagnosis of PD when I saw her in person, therefore I did not include her in the final analysis. I excluded three controls with a diagnosis of essential tremor, two with rest tremor and rigidity on examination and one with cognitive impairment (probably related to HIV infection). In the end, I included 33 patients with iRBD and 29 age and sex matched controls in the analysis. Both groups were comparable in terms of age (mean (SD), iRBD: 68.88 years (8.07) vs controls: 69.65 years (7.74); p=0.701). Male predominance was present in the iRBD group (30/33 -90.9%-) and the control group (25/29 -86.2%-; p=0.696). Table 5.2 summarises relevant demographic and clinical information of both groups.

With respect to medical comorbidities, the iRBD group had a higher proportion of people with T2D (4 (12.1%) vs 1 (3.4%); p=0.360), high cholesterol (18 (54.5%) vs 11 (37.9%); p=0.213) and cardiovascular events in the past (4 (12.1%) vs 3 (10.3%); p=1.000), as well as a lower proportion of people with hypertension (8 (24.2%) vs 12 (41.4%); p=0.181) compared with controls. However, neither of the differences were statistically significant. Three iRBD participants, but none of the controls, were on treatment for depression (selective serotonin reuptake inhibitors). Four participants with iRBD used hearing aids compared with six controls

(p=0.493). Both groups had a similar proportion of osteoarticular problems with none of them having a big impact on their motor performance (6 (18.2%) vs 7 (24.2%); p=0.756). Four patients with iRBD had a first-degree family history of PD compared with six controls (p=0.493). Nine patients with iRBD had a first-degree family history of dementia (two of them were formally diagnosed with AD) compared with four controls (p=0.227).

Patients with iRBD had a mean disease duration from symptoms onset of 10.6 years (SD 6.87). There was a delay in the diagnosis since they started noticing symptoms of 3 years (IQR 1 to 3). Only 13 were taking symptomatic medication to treat iRBD (clonazepam and/or melatonin). Patients with iRBD rated their sleep quality as being poorer than controls (question 1.7 in the MDS-UPDRS-I, iRBD: 13/33 vs control: 3/29; p=0.011) but equally restorative to controls with a similar proportion of people in the iRBD group experiencing daytime sleepiness (question 1.8 in the MDS-UPDRS-I, iRBD: 19/33 vs control: 14/29; p=0.675). Most considered their RBD symptoms to be stable (21 out of 33), four reported their symptoms had improved, one had worsened and two fluctuated. In 29 cases it was possible to corroborate the information with their bed partner.

Table 5.2 Demographic information, risk factors and non-motor manifestations

	iRBD (n= 33)	Controls (n= 29)	p-value
Age, mean (SD)	68.88 (8.07)	69.65 (7.74)	0.701
Male, n (%)	30 (90.9)	25 (86.1)	0.696
T2D, n (%)	4 (12.1)	1 (3.4)	0.360
Hypertension, n (%)	8 (24.2)	12 (41.4)	0.181
High cholesterol, n (%)	18 (54.5)	11 (37.9)	0.213
Cardiovascular events, n (%)	4 (12.1)	3 (10.3)	1.000
Hearing aids, n (%)	4 (12.1)	6 (20.7)	0.493
Osteoarticular problems, n (%)	6 (18.2)	7 (24.1)	0.756
First-degree PD family history, n (%)	4 (12.1)	6 (20.7)	0.493
First-degree dementia family history, n (%)	9 (27.3)	4 (12.1)	0.227

T2D: type 2 diabetes; p-value extracted from two-sample Wilcoxon rank-sum (Mann-Whitney) test, except for 'age' which was extracted from two-sample t test with equal variances

5.3.1 Motor assessments

Table 5.3 summarises the main findings of the motor assessments, except for the DFT, BRAIN and SMART tests, which are presented separately. For each task, I carried out the pairwise comparison between groups and calculated their discriminatory ability to distinguish iRBD from controls. Then, I compared the overall performance of the most discernible motor parameters in the motor battery with the standardised MDS-UPDRS-III scale. Given that the

iRBD group showed a great clinical variability (MDS-UPDRS-III score with a high SD: mean 7.24 points, 4.81 SD), I selected iRBD patients with SP only to make the iRBD group more homogenous, and did a separate ROC analysis for each task.

Table 5.3 Motor performance in the iRBD and control groups

	iRBD (n= 33)	Controls (n=29)	p-value
MDS-UPDRS-II, mean (SD)	2.51 (3.16)	0.83 (1.46)	0.004
MDS-UPDRS-III, mean (SD)	7.24 (4.81)	2.65 (1.80)	<0.001
SP-MDS, n (%)	11 (33.3%)	0	<0.001
TUG (sec), mean (SD)	8.06 sec (1.42)	7.33 (0.97)	0.010
Handwriting speed (sec), mean (SD)	76.70 (30.39)	61 (10.71)	0.004
10m-walking (natural) (sec), mean (SD)	8.22 (2.52)	7.49 (0.84)	0.131
10m-walking (dual task) (sec), mean (SD)	10.30 (4.09)	7.81 (1.42)	0.001
Rapid blinking (blinks/sec), mean (SD)	3.63 (1.07)	4.02 (0.74)	0.047

SD: standard deviation, IQR: interquartile range, MDS-UPDRS-II: motor experiences of daily living, MDS-UPDRS-III: motor examination, SP-MDS: Subthreshold Parkinsonism following MDS Task Force criteria (for more details see Glossary), TUG: Timed Up-and-go test, 10m-walking (natural): timed 10-metre walking under normal conditions, 10m-walking (dual task): 10-metre walking while doing a mental task

a. Clinical scales

Eleven people with iRBD fulfilled criteria for SP following MDS Task Force criteria (for more details see Glossary). In contrast, none of the controls scored more than 6 points in the MDS-UPDRS-III (excluding action and postural tremor). On average, motor scores differed by approximately 5 points (mean MDS-UPDRS-III (SD), iRBD: 7.24 (4.81); control: 2.65 (1.80); $p<0.001$). The MDS Research Criteria for Prodromal PD (≥6 points after excluding action tremor) showed a low sensitivity (42.4%) with high specificity (96.5%). By decreasing the cut-off down to 3 points (excluding action tremor), the sensitivity improved up to 69.7% for 72.4% specificity (AUC 0.81; 95% CI, 0.71 to 0.91). The total MDS-UPDRS-III score (including action tremor) was more accurate in differentiating iRBD from controls than the MDS Task Force, with 81.8% sensitivity and 72.4% specificity for a cut-off ≥4 points (AUC 0.83; 95% CI, 0.72 to 0.93) (Table 5.8).

A breakdown of the MDS-UPDRS-III showed that individuals with iRBD also exhibited higher scores in all motor domains separately. Of note, two of the motor signs required for PD diagnosis (bradykinesia and rigidity) had higher scores in people with iRBD compared with controls. When gathering the items assessing upper and lower limb bradykinesia (MDS-UPDRS-III item 3.4a-3.8b), the iRBD group scored on average 2.12 points (SD 1.99) compared with 0.79 in controls (SD 1.01; $p<0.001$). In the same way, the addition of the total rigidity scores (MDS-UPDRS-III item 3.3a-3.3e) was also higher in the iRBD group (mean score (SD) 1.58 (1.35) vs 0.55 (0.91); $p<0.001$). On examination, I noticed rest tremor in one

participant with iRBD but not in controls. Paying attention to other motor features, the overall action tremor score (MDS-UPDRS-III item 3.15a-3.16b) was on average greater (mean score (SD), iRBD: 1.76 (1.32) vs control 1.03 (1.29); p=0.017) as well as axial features (MDS-UPDRS-III item 3.10-3.14) with the iRBD group scoring 0.79 (SD 1.17) and controls 0.17 (SD 0.47; p=0.004). A higher proportion of individuals with iRBD had an abnormal gait pattern (15 (45.4%)) compared with controls (3 (10.3%); p=0.004), with asymmetric reduced arm swing being the most common feature amongst iRBD patients (13 (39.4%) vs 4 (13.8%); p=0.044).

Assessing each motor domain in the motor symptoms scale (MDS-UPDRS-II) separately, bulbar symptoms (question 2.1 -speech-, 2.2 -saliva drooling-, and 2.3 -chewing difficulties-) were the most frequently reported manifestations by iRBD individuals compared with controls (mean scoring points 2.1-2.3 (SD), iRBD 0.79 (1.24) vs 0.14 (0.58); p=0.005). In contrast, no differences were found in other symptoms such as slowness in daily life activities, symptomatic tremor and walking difficulties.

It was not possible to get blinded scores from all patients but Prof Andrew Lees, who has an extensive experience and expertise in the Movement Disorders field and is one of the authors of the Brain Bank diagnostic criteria of PD, reviewed 22 videos of participants with iRBD and controls. He was blind to their diagnosis. Our scores matched (<5-point difference) in 20 participants.

b. Gait analysis

In line with abnormal gait patterns, patients with iRBD also performed the TUG test more slowly compared with controls (mean (SD), iRBD: 8.06 sec (1.42) vs control: 7.33 sec (0.97); p=0.010). Moreover, dual tasking unmasked differences in walking pace between groups (mean (SD), iRBD: 10.30 sec (4.09) vs controls: 7.81 sec (1.42); p=0.001), which were not detected in the 10-metre walking task in isolation (mean (SD), iRBD: 8.22 sec (2.52) vs control: 7.49 sec (0.84); p=0.131). Dual tasking had an impact on iRBD patients' walking speed. Unlike controls, patients with iRBD slowed down their pace when were asked to do a mental task. A cut-off of 8 seconds in the dual task walking showed a 67.7% sensitivity for 62.1% specificity and AUC of 0.72 (95% CI, 0.59 to 0.85) to distinguish iRBD from controls. No differences, in terms of accuracy, were found to differentiate iRBD patients with SP from controls and from iRBD without SP.

c. Keyboard-based tools: the DFT and BRAIN test

Non-dominant hand vs dominant hand performance

Most of participants were right-handed (30 iRBD and 26 controls). The overall DFT performance of iRBD participants was comparable between the dominant and non-dominant hand (Table D5.1 in Appendix D). In contrast, they performed the BRAIN test more slowly with the non-dominant hand. Additionally, controls performed both tests slower with their non-

dominant hand (Table D5.2 in Appendix D). Interestingly, the parameter measuring incoordination (IS) was similar between the dominant and non-dominant in both group (iRBD and controls) and both tests (the DFT and BRAIN test). The fact that DFT performance was similar between both hands in iRBD could indicate that the dominant hand could have subtle motor impairment which compensates for the performance of the non-dominant hand. For that reason, I did two separate analyses with the dominant and non-dominant hand.

Kinetic parameters comparison

The non-dominant hand of the iRBD group performed the alternate tapping task (BRAIN test) and single finger tapping task (DFT test) more slowly and erratically than the non-dominant hand of controls. People with iRBD tapped on average 10 keys fewer than controls (mean KS-BRAIN (SD), 49.45 taps (15.19) vs 61.03 taps (9.98); $p<0.001$). Although KS-DFT was lower in the iRBD group, the difference between groups was less evident than in the BRAIN test (mean KS-DFT (SD), 83.26 taps (15.76) vs 90.58 taps (11.62); $p=0.020$). Moreover, the iRBD group performed both tests more erratically than controls. That was based on a greater variance of travelling time between keystrokes (IS). In both test IS was significantly higher in the iRBD group (median IS-BRAIN (IQR), 5354.66 msec^2 (2702.75 to 11478.53) vs 2375.19 msec^2 (1640.95 to 3874.55); $p<0.001$; median IS-DFT (IQR), 2210.62 msec^2 (1049.74 to 3265.58) vs 800.48 msec^2 (329.57 to 1364.56); $p=0.006$). Although patients with iRBD spent slightly longer dwell time on each key (AT), the discrepancy between groups was not as noticeable as with other parameters (mean AT-BRAIN (SD), 131.43 msec (50.56) vs 109.86 msec (25.89); $p=0.018$; mean AT-DFT (SD), 110.70 msec (27.56) vs 102.64 msec (23.03); $p=0.107$) (Table 5.4 and Table 5.5).

The comparison of the iRBD group with controls using their dominant hand showed similar results to those with the non-dominant hand. Again, the iRBD group performed both tasks less quickly and more erratically with their dominant hand. The differences between groups were equally significant across all parameters and tests except for IS-DFT, which were slightly smaller (median IS-DFT (IQR), 1095.33 (370.98 to 2951.68) vs 361.66 (256.84 to 853.01); $p=0.021$) (see Table D5.3 in Appendix D).

Keyboard test accuracy for iRBD detection

All parameters performed with the non-dominant hand discriminated between iRBD and controls. The number of alternated key taps (KS-BRAIN) and incoordination of single taps (IS-DFT) showed the best discriminatory power. The former had 72.7% sensitivity for 62.1% specificity (cut-off=57 taps) and AUC of 0.77 (95% CI, 0.65 to 0.89). The latter had 81.8% specificity detection rate of iRBD for 69.0% specificity (cut-off=950.9 msec^2) and AUC of 0.76 (95% CI, 0.64 to 0.89). (Table 5.4 and Table 5.5). The combination of the three parameters (KS, AT and IS) of both tests noticeably improved the accuracy of the keyboard tests with 78.8% sensitivity and 72.4% specificity, with an AUC of 0.81 (95% CI, 0.70 to 0.92) (Figure 5.2).

The dominant hand obtained a similar overall accuracy (for more details see Table D5.3 and D5.4 in Appendix D). The combination of KS, AS and IS parameters from both tests (DFT and BRAIN test) reached a similar discriminatory power with 78.8% sensitivity for 75.9% specificity and AUC of 0.84 (95% CI, 0.74 to 0.93). There were minor differences when analysing each parameter separately. In brief, the dominant hand was more accurate in detecting iRBD based on the alternating tapping incoordination (IS-BRAIN) than the non-dominant hand. Applying the same cut-off (2964 msec^2), the accuracy of the non-dominant hand was approximately 60% sensitivity and specificity, whereas with the dominant hand the detection rate was higher (81.8%) for 62% specificity and AUC of 0.78 (95% CI, 0.66 to 0.90). On the other hand, the IS-DFT extracted from the dominant hand had a worse overall accuracy (AUC 0.65 vs 0.76) with lower true positive (72.7% vs 81.8%) and negative rate (51.7% vs 70.0%) compared with the non-dominant one.

Table 5.4 Comparison of BRAIN test kinetic parameters in the non-dominant hand of iRBD and controls, and corresponding ROC analysis

	Mean KS-BRAIN (SD)	**Mean AT-BRAIN (SD)**	**Median IS-BRAIN (IQR)**
iRBD (n=33)	49.45 (15.19)	131.43 (50.56)	5354.66 (2702.75 to 11478.53)
Controls	61.03 (9.98)	109.86 (25.89)	2375.19 (1640.95 to 3874.54)
p-value	<0.001[a]	0.018[a]	<0.001[b]
	KS-BRAIN Sensitivity	**AT-BRAIN Sensitivity**	**IS-BRAIN Sensitivity**
Specificity 90% (cut-off)	57.6% (50)	27.3% (155.0)	45.5% (6830.0)
Specificity 85% (cut-off)	60.6% (51)	36.4% (137.5)	54.6% (4691.0)
Specificity 80% (cut-off)	57.5% (52)	45.5% (128.65)	54.6% (4368.86)
Best combination (cut-off)	72.7% sensitivity for 62.1% specificity (57)	60.6% sensitivity for 55.7% specificity (108.94)	63.6% sensitivity for 62.1% specificity (2903)
AUC (95% CI)	0.77 (0.65 to 0.89)	0.62 (0.48 to 0.76)	0.73 (0.60 to 0.85)

KS, kinesia score; AT, akinesia time; IS, incoordination score; CI, confidence interval; IQR, interquartile range; SD, standard deviation; a) Welch's test for unequal variances; b) Mann-Whitney test. ROC, Receiver Operating Characteristic, AUC: Area Under the Curve

Table 5.5 Comparison of DFT test kinetic parameters in the non-dominant hand of iRBD and controls, and corresponding ROC analysis

	Mean KS-DFT (SD)	Mean AT-DFT (SD)	Median IS-DFT (IQR)
iRBD (n=33)	83.26 (15.76)	110.70 (27.56)	2210.62 (1049.74 to 3265.58)
Controls	90.58 (11.62)	102.64 (23.03)	800.48 (329.57 to 1364.56)
p-value	0.020[a]	0.017[a]	0.006[b]
	KS-DFT Sensitivity	**AT-DFT Sensitivity**	**IS-DFT Sensitivity**
Specificity 90% (cut-off)	45.5% (79)	24.3% (125.7)	21.2% (4727.95)
Specificity 85% (cut-off)	48.5% (82)	30.3% (118.4)	27.3% (3153.0)
Specificity 80% (cut-off)	48.5% (81)	36.4% (113.3)	60.6% (1884.0)
Best combination (cut-off)	72.7% sensitivity for 51.7% specificity (92.0)	51.5% sensitivity for 62.1% specificity (105.4)	81.8% sensitivity for 69.0% specificity (950.9)
AUC (95% CI)	0.66 (0.52 to 0.80)	0.58 (0.43 to 0.72)	0.76 (0.64 to 0.89)

KS, kinesia score; AT, akinesia time; IS, incoordination score; CI, confidence interval; IQR, interquartile range; SD, standard deviation; a) Welch's test for unequal variances; b) Mann-Whitney test. ROC, Receiver Operating Characteristic, AUC: Area Under the Curve

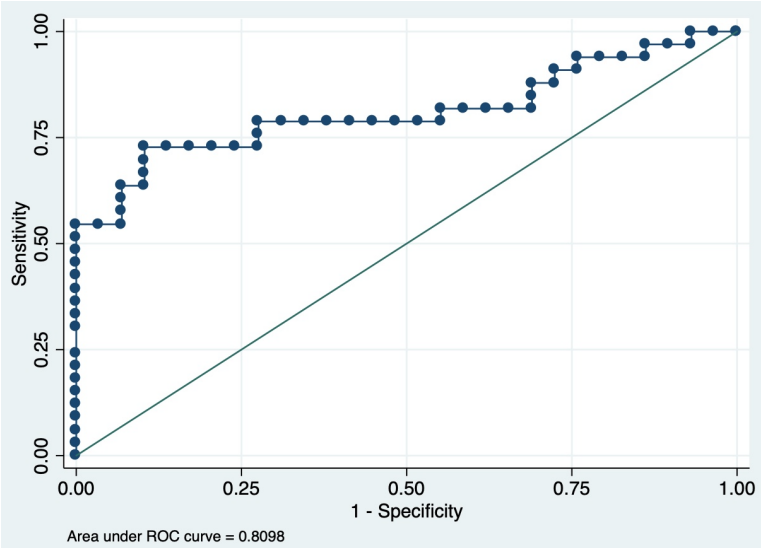

Figure 5.2 ROC curves for the combination of DFT and BRAIN test to distinguish patients with iRBD and controls

Keyboard tests accuracy for detection of subthreshold parkinsonism

As detailed in Table 5.3, 11 people with iRBD and no controls fulfilled the criteria for SP. Both tests showed a high discriminatory power to detect people with SP. The number of alternate taps (KS-BRAIN) and the single tap incoordination parameter (IS-DFT) again showed the best discriminatory power, with both kinetic markers reaching 80% detection rate with high specificity: the KS-BRAIN had 93.1% specificity (cut-off=49 taps) and an AUC of 0.85 (95% CI, 0.66 to 1.00) and IS-DFT showed 82.8% specificity (cut-off=2210 msec^2) and an AUC of 0.84 (95% CI, 0.72 to 0.97). When combining the three parameters together (KS, AT, IS) the overall accuracy of the BRAIN test improved (AUC 0.90) up to 90% sensitivity for 96.5% specificity whereas the DFT test did not change. The absence of controls classified as having SP could have overestimated the accuracy of both tests. For that reason, I carried out a second analysis using data form the iRBD group only. Overall, both tools were still able to distinguish iRBD patients with SP amongst those without SP. Accuracy did not decrease when using a smaller sample size (only including 33 iRBD participants). The combination of both tests (including all three parameters) showed a similar degree of accuracy (80% sensitivity for 91.3% specificity) and AUC of 0.86 (95% CI, 0.68 to 1.0) (Figure 5.3). A separate analysis of each parameter did not show meaningful differences. The KS-BRAIN had 70% sensitivity for 73.9% specificity (cut-off=48 taps) and AUC of 0.72 (95% CI, 0.49 to 0.96). The IS-DFT showed 80% sensitivity for 60.9% specificity (cut-off=2210 msec^2) and AUC of 0.64 (95% CI, 0.44 to 0.83).

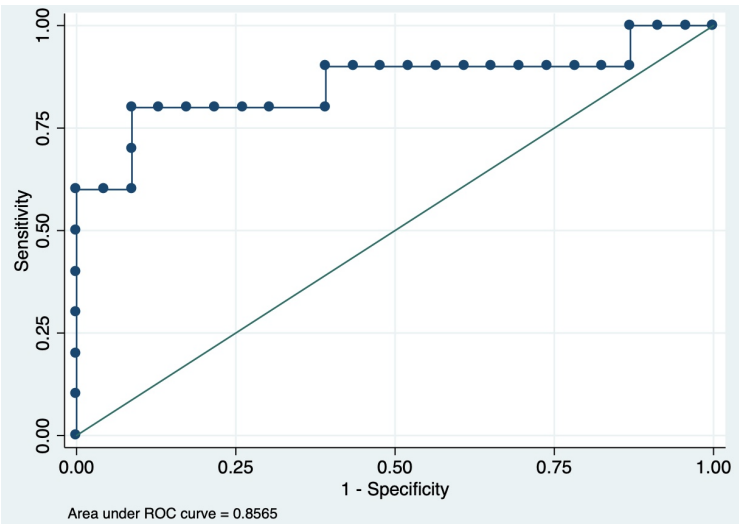

Figure 5.3 ROC curves for the combination of DFT and BRAIN test to distinguish iRBD patients with and without SP

d. SMART test

I compared the finger tapping performance of the dominant hand with the non-dominant in both groups. I did not find any differences. I focused on non-dominant hand results to be consistent with the results presented in the other motor tasks.

Slow-motion analysis of repetitive finger tapping was similar in iRBD and in controls under natural conditions. In contrast, under challenging conditions (mental task), patients with iRBD decreased their finger tapping amplitude and velocity to a greater extent than controls. Similarly, their amplitude variation (CV amplitude) was greater than controls (Table 5.6). On the other hand, the number of taps per second was similar in both groups.

Table 5.6 SMART test parameters under challenging conditions

	iRBD (n=32) *	Controls (n=27) *	p-value
Amplitude (a.u.)			
• Mean	0.54 (0.49 to 0.59)	0.65 (0.60 to 0.71)	0.001
• CV	0.32 (0.28 to 0.35)	0.22 (0.18 to 0.26)	<0.001
• Slope	$-5.80x10^{-3}$ ($-8.40x10^{-3}$ to $-3.20x10^{-3}$)	-3.04x10-3 ($-6.16x10^{-3}$ to $0.07x10^{-3}$)	0.170
Frequency (taps/sec)			
• Mean	2.63 (2.50 to 2.76)	2.60 (2.41 to 2.80)	0.814
• CV	$11.11x10^{-2}$ ($9.52x10^{-2}$ to $12.69x10^{-2}$)	9.30x10-2 ($7.43x10^{-2}$ to $11.17x10^{-2}$)	0.137
• Slope	$-8.67x10^{-3}$ ($-16.86x10^{-3}$ to $-0.48x10^{-3}$)	-3.49x10-3 ($-8.82x10^{-3}$ to $1.84x10^{-3}$)	0.284
Velocity (a.u/sec)			
• Mean	$1.89x10^{-2}$ ($1.70x10^{-2}$ to $2.10x10^{-2}$)	$2.23x10^{-2}$ ($2.03x10^{-2}$ to $2.44x10^{-2}$)	0.007
• CV	0.38 (0.31 to 0.45)	0.28 (0.22 to 0.35)	0.023
• Slope	$-2.47x10^{-4}$ ($-3.69x10^{-4}$ to $-1.25x10^{-4}$)	$-1.78x10^{-4}$ ($-3.04x10^{-4}$ to $-0.56x10^{-4}$)	0.417

Between brackets: 95% CI. Amplitude was normalised. a.u.: arbitrary unit, p-value extracted from Welch's test for unequal variances. * Three finger tapping video recordings (one iRBD and two controls) were excluded due to technical issues related to hand image processing

The SMART test alone did not exceed the accuracy of the keyboard-based tapping tasks (DFT and BRAIN tests). Amongst all parameters, amplitude (mean) combined with velocity (mean) showed the highest discriminatory power in distinguishing iRBD patients from controls, with 78.8% sensitivity and 60.71% specificity (AUC 0.757; 95% CI, 0.63 to 0.88). Similarly, the combination of both parameters was able to differentiate between iRBD patients with SP and without SP, with 72.73% sensitivity and 71.43% specificity (AUC 0.802, 95% CI, 0.64 to 0.96).

e. Handwriting analysis

Handwriting speed clearly distinguished people with iRBD from controls. The iRBD group took 10 seconds longer to write three sentences than controls (mean time (SD), iRBD: 76.70 seconds (30.39) vs control 61 seconds (10.71); p=0.004). In contrast, handwriting size was similar between groups. The proportion of people with micrographia in the iRBD group did not differ from controls (15 people with iRBD (45.4%) and 12 controls (41.4%); p=0.801). In addition to size, other handwriting markers were found to be abnormal in the iRBD group. This was the case with excessive pen pressure, sentence slope and hidden markers of tremor in handwriting scripts (Table 5.7). Markers of tremor were found to be the most common feature amongst the iRBD group (72.0% vs 34.5%; p=0.005), followed by sentence slope (60% vs 24%;

p=0.005) and increased pen pressure (48% vs 14%; p=0.006). Interestingly, tremor was only visible in handwriting scripts, but not with the naked eye, in 10 of the participants with iRBD and six of the controls. Figure 5.4 and Figure 5.5 illustrate several examples of handwriting markers that were considered abnormal following graphologist impression criteria.

Table 5.7 Comparison of the handwriting scale in iRBD patients and controls

	iRBD (n=33)	Controls (n=29)	p-value
Handwriting scale (1-8); mean score (SD)	3.18 (1.44)	1.69 (1.58)	<0.001[1]
Micrographia; n (%)	15 (45.40)	12 (41.38)	0.801[2]
Sentence slope; n (%)	20 (60.60)	7 (24.14)	0.005[2]
Tremor; n (%)	24 (72.73)	10 (34.48)	0.005[2]
Retracing; n (%)	9 (27.27)	4 (13.79)	0.227[2]
Resting marks; n (%)	4 (12.12)	2 (6.90)	0.676[2]
Excessive pen pressure; n (%)	16 (48.48)	4 (13.79)	0.006[2]
Irregular shape; n (%)	6 (18.18)	0	0.026[2]
Word spacing; n (%)	9 (27.27)	3 (10.34)	0.116[2]
Handwriting speed; mean seconds (SD)	76.70 (30.39)	61 (10.71)	0.004[1]

Handwriting scale based on the presence (score 1) or absence (score 0): overall score includes items 1-8; Handwriting speed: handwriting task duration (including the three sentences); 1) Welch's test for unequal variances. 2) Fisher's exact test

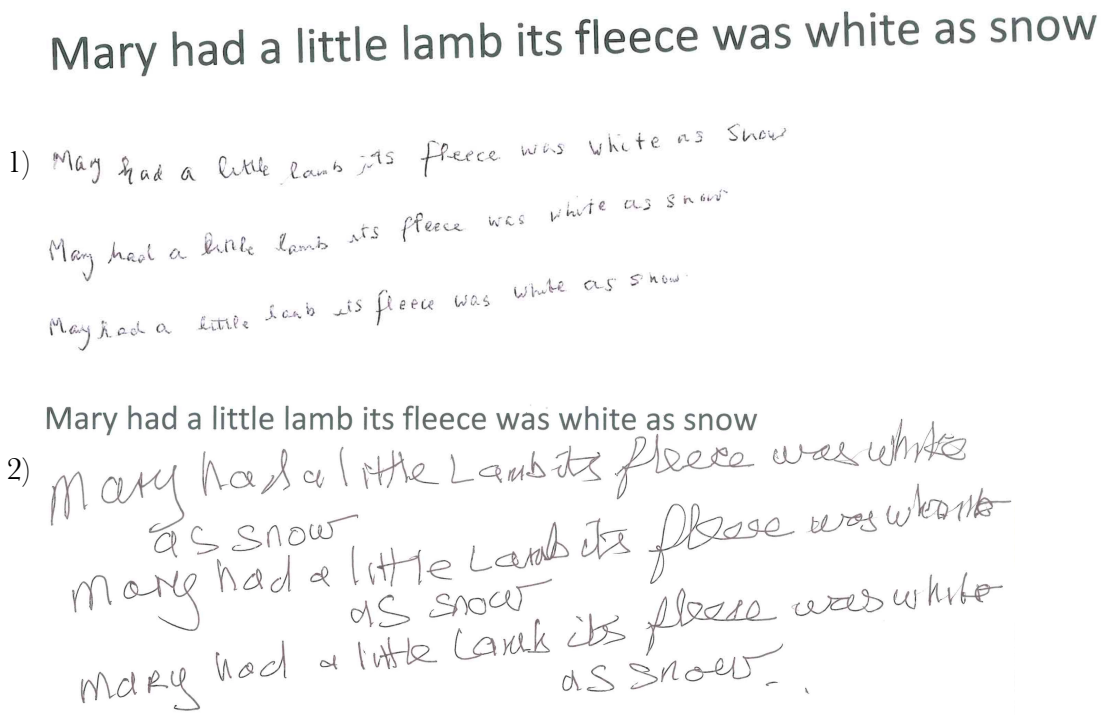

Figure 5.4 Two examples of abnormal handwriting. 1) Patient with established PD and micrographia: progressive reduction in handwriting size across sentences (e.g. 'white' and 'snow'). 2) Patient with iRBD and dysgraphia: untidy (irregular letter and word shape). Note sentence slope in both examples.

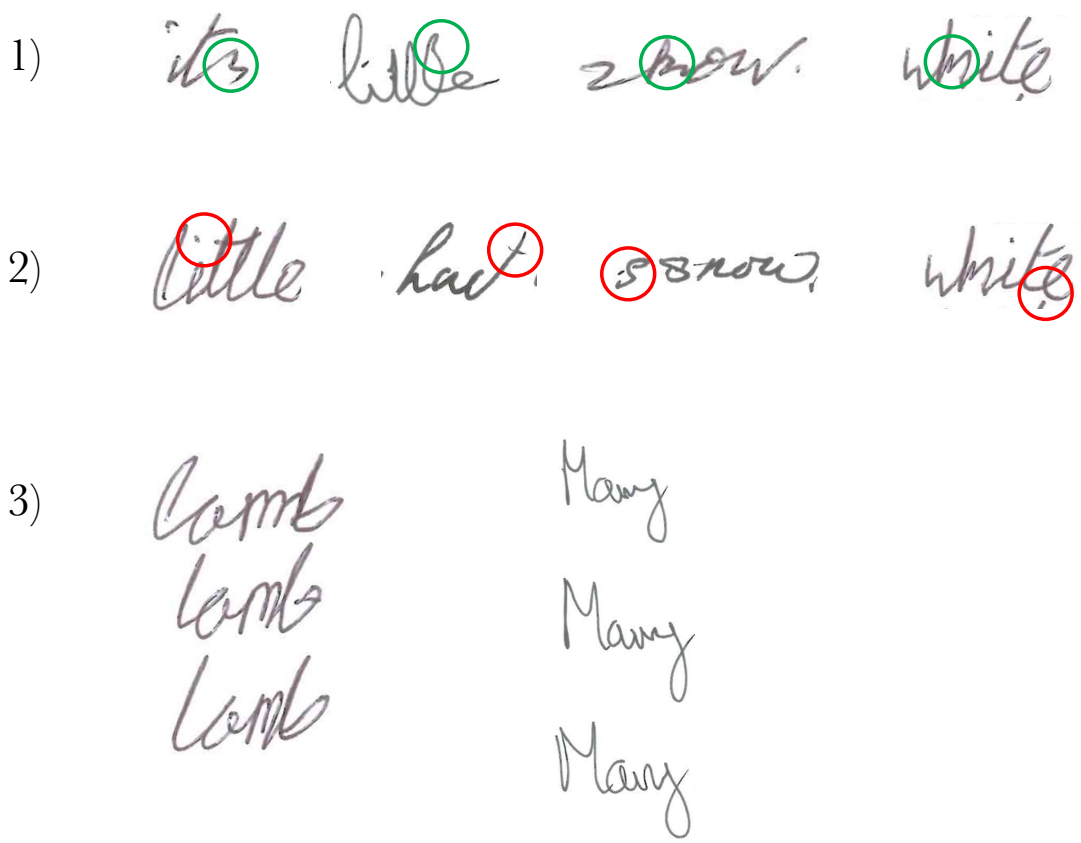

Figure 5.5 Handwriting markers (examples): 1) hidden tremor in green, 2) resting marks in red and 3) irregular word shape across sentences

In contrast with micrographia, handwriting speed was able to correctly detect iRBD patients from controls with 63.6% sensitivity for 75.9% specificity and using a cut-off of 21 seconds (AUC 0.71; 95% CI, 0.58 to 0.84). When increasing the cut-off up to 24 seconds, it was found that handwriting speed was also able to accurately distinguish iRBD with SP from those without SP, with a detection rate of 81.8% for 82.8% specificity (AUC 0.86; 95% CI, 0.68 to 1.00).

When combining the handwriting markers, the test's ability to correctly detect iRBD had 75.8% sensitivity for 69% specificity (cut-off: three items altered; AUC 0.77; 95% CI, 0.64 to 0.89). If four items were found to be abnormal, the scale was also capable of detecting iRBD with SP with a detection rate of 72.7% for 70.6% specificity (AUC 0.74; 95% CI, 0.60 to 0.88).

f. Rapid blinking

People with iRBD blinked on average slightly less than controls but without meaningful differences (mean blink rate/sec (SD), iRBD 3.63 blinks (1.07) vs control 4.02 blink rate/sec (0.74); p=0.047). During informal conversation both groups had a similar blink rate (iRBD 0.33 blinks (0.50) vs control 0.46 (0.35); p=0.240).

g. Motor battery vs clinical rating scale

The BRAIN test and DFT test offered a slightly higher accuracy than MDS-UPDRS-III with the advantage that they can be applied remotely (Table 5.8). In contrast, rapid blinking was similar between groups. When combining the most discernible quantitative motor parameters (BRAIN and DFT test: KS and IS parameter, 10-metre walking task and handwriting speed) the overall accuracy was comparable to the BRAIN and DFT test alone: 74.2% sensitivity for 82.8% specificity (AUC 0.81; 95% CI, 0.67 to 0.93).

Table 5.8 ROC analysis of remote keyboard tapping tests and MDS-UPDRS-III

	MDS-UPDRS-III (minus action tremor) sensitivity	DFT & BRAIN test sensitivity	MDS-UPDRS-III sensitivity
Specificity 90%	48.5%	69.7%	54.5%
(cut-off)	(5)	(0.58)	(7)
Specificity 85%	57.6%	72.73%	63.6%
(cut-off)	(4)	(0.50)	(5)
Best combination	69.7% sensitivity for	78.8% sensitivity and 72.4%	81.8% sensitivity for
(cut-off)	72.4% specificity	specificity	72.4% specificity
	(3)	(0.40)	(4)
AUC	0.81	0.81	0.83
(95% CI)	(0.71 to 0.91)	0.70 to 0.92	(0.72 to 0.93)

Accuracy to distinguish iRBD (n=33) and controls (n=29), *MDS-UPDRS-III excluding action tremor (AT) from MDS-UPDRS-III following MDS Task Force criteria for subthreshold parkinsonism. DFT and BRAIN test including KS20, AT20, IS20, KS30, AT30 and IS30. ROC, Receiver Operating Characteristic, AUC: Area Under the Curve

5.3.2 Systemic symptoms

Overall, people with iRBD scored higher in all questionnaires assessing symptoms not related to movement but in other systemic domains (Table 5.9). This is the case of SCOPA-AUT (iRBD 9.51 (6.84) vs 6.71 (4.87); p=0.034) and MDS-UPDRS-I (iRBD 8.03 (5.47) vs 3.95 (3.63); p<0.001). Among all symptoms assessed, constipation and neuropsychiatric symptoms (depression, anxiety, apathy) were more common in iRBD than in controls. Gathering all questions asking about constipation in the MDS-UPDRS-I (question 1.11) and SCOPA-AUT (question 5 and 6), the average score in the iRBD group was 3 (SD 3.19) and in the control group it was 0.41 (SD 0.94; p<0.001). Bringing together all questions on mood disturbances in the MDS-UPDRS-I (question 1.3 -depression-, 1.4 -anxiety-, 1.5 -apathy-), the iRBD group scored higher compared with controls (1.27 (1.72) vs 0.41 (0.63); p=0.005).

People with iRBD were more likely to have an abnormal cognitive test: eight iRBD participants scored less than 26 in the MoCA test (seven patients were classified as having mild cognitive

impairment [25-22 points] and one as having moderate cognitive impairment [21-19 points]). In contrast, no controls got a score less than 26 (p=0.005). A higher proportion of patients with iRBD reported having mild memory difficulties than controls (20/33 (60.6%) vs 10/29 (34.5%); p=0.047). A breakdown of cognitive domains assessed in the MoCA test showed that iRBD participants had a worse performance (lower score), especially in the visuospatial (1.42 vs 1.79; p=0.005), executive (2.48 vs 2.93; p=0.002) and delayed memory (3.70 vs 4.41; p=0.003) domains than controls. On the other hand, both groups had similar scores in attention (5.30 vs 5.59; p=0.278), language (2.67 vs 2.86; p=0.122), abstraction (2.00 vs 1.96; p=0.662) and orientation (5.85 vs 6.00; p=0.134) domains. Of note, both groups had similar formal education duration (iRBD 19.42 years (6.35) vs 20.14 (3.31); p=0.575).

A higher proportion (39.40% vs 10.34%; p=0.011) of people with iRBD had orthostatic hypotension based on lying and standing blood pressure, with a drop of 20 millimetres of mercury (mm Hg) or more in their systolic blood pressure in 13 of them. In contrast, only two patients mentioned symptoms such as light headedness.

Urological problems such as urinary symptoms and sexual dysfunction were similarly prevalent in both groups. When gathering questions related to the intensity (question 1.10 in the MDS-UPDRS-I) and frequency (from question eight to 13 in the SCOPA-AUT scale) of urinary symptoms, both groups had a similar score (iRBD 7.30 (7.74) vs 7.48 (7.32); p=0.925). Similar results applied for sexual dysfunction (from question 22 to 25 in the SCOPA-AUT scale) with iRBD scoring on average 1.39 (SD 1.98) and controls 0.86 (SD 1.46; p=0.230).

Table 5.9 Systemic symptoms

	iRBD (n= 33)	Controls (n=29)	p-value
MDS-UPDRS-I, median (IQR)	6 (3 to 11)	3 (1 to 6)	<0.001
SCOPA-AUT, median (IQR)	8 (5 to 12)	6 (3 to 9)	0.034
Orthostatic hypotension (%)	12 (36.4)	3 (10.3)	0.011
Abnormal MoCA score, n (%)	8 (24.2)	0	<0.001

MDS-UPDRS-I: non-motor experiences of daily living; SCOPA-AUT: SCales for Outcomes in PArkinson's disease - Autonomic Dysfunction; MoCA, Montreal Cognitive Assessment; Abnormal MoCA cut-off: <26; IQR: interquartile range; 1) Two-sample t test with equal variances, 2) Two-sample Wilcoxon rank-sum (Mann-Whitney) test

5.3.3 Incident PD case

Once I finished data collection and analysis, one participant with iRBD was diagnosed with PD. He was a 66-year-old right-handed male with a 16-year history of iRBD. He started with RBD symptoms in 2006 but did not receive the formal diagnosis until 5 years later. He did not have any motor symptoms when I saw him in person (MDS-UPDRS-II: 0). However, he fulfilled criteria for SP (total score in the MDS-UPDRS-III -after excluding action tremor-: 13).

On examination he exhibited facial hypomimia and low volume speech. He also had mild bradykinesia in both hands although it was more noticeable in his left hand. When walking, he exhibited reduced right arm swing. He did not have rest tremor but bilateral mild action tremor which was also noticeable in his handwriting script (Figure 5.6, circles in red). Eight months after my assessment, he started noticing motor symptoms which included right hand tremor, walking difficulties and short-term memory symptoms. It took four months to receive the diagnosis of PD. Looking back to his motor performance, his scores surpassed the cut-off established to distinguish iRBD with SP in four tasks: KS-BRAIN 48 taps (cut-off ≤ 49 taps), IS-DFT 5346.8 sec^2 (cut-off ≥ 2210 sec^2), 10-metre dual walking task 15.7 sec (cut-off ≥ 8 sec), handwriting score 4 (cut-off ≥ 3 points) (Figure 5.6). In contrast, he spent less time than the established cut-off for SP (21 sec vs 24 sec). He scored 24/30 in the MoCA test at expenses of visuo-spatial and executive domain (2/5), delayed recall (3/5).

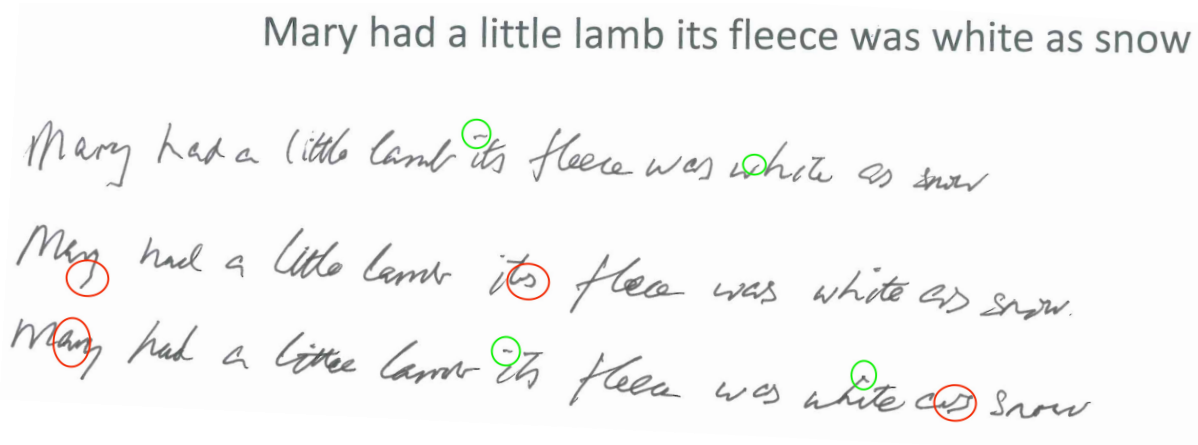

Figure 5.6 Incident PD handwriting (8 months before receiving diagnosis of PD). Circle in red: hidden tremor, circle in green: resting marks. Note the irregular word 'Mary' shape across the three sentences and downwards slope.

5.4 Discussion

There remains a need for quantitative tools that are accurate enough to detect fine motor impairment at early stages of PD. Although clinical scales are widely used in PD, they are not designed to measure early motor impairment. Their floor effect and insensitivity at prodromal stages of PD are the most important limiting factors.[118] Here, I created a motor battery which was tested, together with the standardised MDS-UPDRS-III scale, in people with v-PSG-confirmed iRBD and compared it with age and sex matched controls. The iRBD group showed a greater motor dysfunction based on higher motor scores in the MDS-UPDRS-III. In fact, two participants with iRBD were newly diagnosed with PD[x]and a third of the iRBD group fulfilled criteria for SP. Several objective tests in the motor battery detected motor signatures,

[x] One participant fulfilled criteria for PD and was not included in the analysis. The other one was diagnosed with PD 8 months later my assessment

not measured in the MDS-UPDRS-III, which were more prevalent in patients with iRBD than controls. The iRBD group performed the alternate finger tapping (the BRAIN) and single finger tapping (the DFT) tests more slowly and erratically than controls. Moreover, the iRBD group tended to write more slowly and showed abnormal handwriting makers (hidden tremor, sentence slope and excessive pen pressure). Finally, challenging conditions were able to unmask motor dysfunction not seen when using a single motor task in iRBD. This is the case of the 10-meter dual task and a 30-second dual finger tapping task where people with iRBD decreased their walking pace and finger tapping performance (amplitude and velocity) to a greater extent than controls.

The existence of compensatory mechanisms has been proved using functional neuroimaging.[228] Non-manifesting carriers of Parkin mutation were studied to map preclinical compensation in PD. Participants were instructed to perform two separate motor tasks; simple motor task selected by participants (internally cued) and a motor task specified by a visual cue (externally cued). They found that different sets of pre-motor areas were recruited depending on the specific task. The anterior cingulate motor area and left rostral pre-motor cortex were activated during internally cued movement, whereas the rostral supplementary motor area and right dorsal pre-motor cortex were strongly activated during externally cued tasks. Considering none of the mutation carriers were clinically impaired, the activation of these motor cortical areas could be explained by mechanism that effectively compensate for nigrostriatal dysfunction. In this current study, people with iRBD were more susceptible to challenging conditions which might be indicative of having compensatory mechanisms that when challenged, they unmasked a true motor dysfunction.

There is increasing evidence showing that people with iRBD have motor dysfunction years before PD or DLB diagnosis. Higher motor scores in clinical rating scales,[37,164,229–231] slow finger tapping,[37,150,164,229,232] decreased gait velocity with greater step variability[164,233,234] and speech abnormalities[235] are some of the examples described in the literature. Apart from supporting the existing evidence of parkinsonian signs being prevalent in iRBD, the current study provides novel findings of motor impairment in iRBD detected by tools that have never been tested in people with iRBD. This is the case with the DFT and BRAIN test, as well as timed handwriting and dual task finger tapping. These tools appeared to be highly accurate in distinguishing iRBD with SP from controls. Previous literature found that the accuracy and predictive value of motor signs increased exponentially 2 years prior to PD diagnosis.[3,86] This suggests that motor dysfunction does not progress in a linear fashion, and it is a rapid progression that might be a proximity marker of phenoconversion instead of the motor manifestation per se.[108] Therefore, having an objective tool able to detect early motor decline will have important implications when neuroprotective drugs become available.

Accounting for potential confounding factors such as age and sex is important to define the boundaries between neurodegeneration and natural variability (ageing process, sex-related differences). With that in mind, I compared the iRBD with age and sex matched controls.

Therefore, it can be argued that the motor signatures seen in iRBD are likely to be explained by an underlying neurodegenerative process which goes beyond ageing. Other cohorts (e.g. Christine Lo and colleagues[164] and Fereshtehnejad and collaborators[3]) accounted for the same confounding factors. Both studies compared the motor performance of people with v-PSG-confirmed iRBD with age and sex matched controls. The former found that quantitative motor tools were more accurate and consistent in capturing motor change over time than standardised clinical scales. The latter concluded that motor symptoms (handwriting changes, axial, and bulbar symptoms) were reported long before (7-11 years) the clinical signs of PD were detected. Amongst the clinical signs, slow alternate tap test had the longest period prior to diagnosis (8 years), followed by rigidity (3 years) and tremor (2 years).

Upper limb repetitive movements

Patients with iRBD had a higher proportion of bradykinesia in the repetitive finger tapping task included in the MDS-UPDRS-III. These findings were supported by objective parameters which showed slower and more erratic finger tapping in patients with iRBD than controls. Supporting this notion, I found detectable differences in motor performance between participants with iRBD and controls. Keyboard tapping was found to be slow and erratic in people with iRBD, which suggests that speed and incoordination might be potential motor prodromes of neurodegeneration. In fact, rhythm disturbances have already been suggested as a potential early motor marker in iRBD.[236]

Finger tapping is a promising clinical biomarker of future diagnosis of PD in people with iRBD. Several longitudinal studies have found that people with iRBD had slow finger tapping, suggesting prodromal bradykinesia could be used as a prediction marker[3,37,164] reaching a sensitivity and specificity of 80% to identify the presence of iRBD who converted to PD or dementia.[237] The Purdue pegboard test, a keyboard alternate tap,[117,238] and a 3D contactless motion capture of finger tapping are some examples of available quantitative tools able to capture early signs of upper limb bradykinesia. The alternate tap test was found to be one of the earliest motor signs in iRBD prior to PD diagnosis,[3] showing 66.7% sensitivity for 77.3% specificity 2 years before phenoconversion which decreased at a longer (6 years) prodromal interval down to 55% for the same degree of specificity. In line with the study carried out by Fereshtehnejad and colleagues, I found that people with iRBD performed the BRAIN test less quickly than controls. The number of alternate taps per task in the BRAIN test alone had a slightly lower sensitivity than the alternate tap test used by Fereshtehnejad 2 years prior to phenoconversion (61% vs 66.7%). However, the BRAIN test had a higher specificity (85% vs 77.3%) than the alternate tap test. Altogether, this appears to support the notion that slow alternate finger tapping could be an early motor marker with high prediction power of conversion to parkinsonism or dementia.

Finger tapping movements have also been assessed as per the MDS-UPDRS-III instructions[XI]. Růžička and collaborators used a contactless 3D motion capture system to track the finger tapping task in the MDS-UPDRS-III. They tested 40 v-PSG-confirmed iRBD patients, 25 de novo PD patients and 25 healthy controls. They found that people with iRBD had a more pronounced decrement in the amplitude of finger tapping than controls. The instrumental analysis of finger tapping was able to distinguish iRBD from controls with 76% sensitivity and 63% specificity, which is comparable to the accuracy of the SMART test under challenging conditions presented in this chapter. In the previous chapter, I assessed the SMART test in people with idiopathic anosmia, which is also known to be a risk factor for PD. Similar to people with iRBD, they performed the finger tapping task significantly more slowly and with smaller amplitude than controls. Unlike people with iRBD, these differences were seen under natural conditions. Unfortunately, in the anosmia group finger tapping was not assessed under a mental task. Of note, most people with idiopathic anosmia had a normal finger tapping sub-score in the MDS-UPDRS-III, indicating that the SMART test could detect subtle motor signatures difficult to pick up with the naked eye. The DFT test mirrored the finger tapping task assessed in the MDS-UPSRS-III. Unlike the SMART test and 3D motion capture test, the DFT is a simple keyboard-based tap test. It can be used remotely, which facilitates its applicability on a large scale. In line with previous studies, people with iRBD had slower repetitive finger tapping rate than controls. In addition, higher incoordination appeared to be common in our iRBD group, not only when performing the DFT but also with the BRAIN test denoting a potential novel motor signature in iRBD.

Gait abnormalities

Whereas patients with iRBD did not show a slower walking pace under normal conditions than controls, they performed a sequential gait task (TUG) and walking under challenging conditions less quickly than controls.

Subtle gait abnormalities and balance impairment have been described as potential motor prodromes in iRBD.[40,233,239] Decreased gait speed, cadence and step variability are some examples of reported gait features in iRBD.[237] Moreover, gait has been proposed as an important determinant factor of motor phenotypes. For example, postural-instability-gait-predominant phenotype has been proved to have a faster cognitive decline and more aggressive progression. Therefore, having an accurate gait assessment tool will have important implications in terms of clinical prognosis.

Kaylena and collaborators assessed 24 v-PSG-confirmed iRBD and 14 age-matched controls. Participants were invited to walk across a pressure sensor carpet under five different conditions: normal pace, fast pace and under three mental tasks ranging in difficulty.[40] Whereas both groups had a similar walking pattern in terms of pace and step height and width under natural

[XI] Finger tapping task: *"tap the index finger on the thumb as fast and wide as you can for 10 seconds"*.

conditions, under challenging conditions (dual tasking) people with iRBD increased their footstep asymmetry. In contrast with my findings, the control group assessed by Kaylena and colleagues also decreased their pace under challenging conditions in the same way iRBD did.

Gait performance can be affected by a wide range of confounding factors. This is the case with neuropsychiatric symptoms (depression and cognitive impairment), osteoarticular conditions (joint problems in the lower limbs and lumbar spine disease), peripheral neuropathy and also drugs. Although the iRBD group and controls were matched for age and sex, motor impairment was more prevalent in the iRBD group, therefore a confounding effect on gait performance could not be excluded. However, a study carried out by McDade and colleagues which adjusted for cognitive impairment found that slow pace and cadence remained lower in the iRBD group.

Sensor free tools might serve as an alternative method to distinguish iRBD from controls. TUG test has been extensively used in PD as described in Chapter 1 (1.7. Motor prodromes/Gait). TUG test was part of a motor quantitative battery which was used in two relevant longitudinal studies in iRBD, one carried out by Postuma and collaborators[37] (the largest sample size so far) and the other by Fereshtehnejad and colleagues[3] (comprehensive longitudinal phenotyping approach). Whereas the former did not analyse the test separately, the latter found that the 3-metre Up and Go test had lower accuracy compared with finger tapping. Future directions are towards finding more accurate tools able to capture walking patterns in a more natural environment.

The main downside of analysing gait patterns in a lab environment is that they do not reflect motor impairment in a real-world setting. Data extracted from wearables that monitor movement continuously are closer to capture true walking abnormalities. For example, a study led by Silvia Del Din and collaborators recruited 63 RBD patients from the Oxford Parkinson's disease Centre Discovery cohort and monitored their gait patterns using wearable sensors.[234] They found that step velocity had the maximum accuracy of 67 % sensitivity for 60% specificity to distinguish iRBD from controls. Unfortunately, their applicability was constrained by their cost and accessibility. Here, I present a timed walking task which is a simple and inexpensive test which appears to be as accurate (67.7% sensitivity for 62.1% specificity) as the wearable monitoring devices used by Silvia Del Din and colleagues.

Handwriting

Micrographia has been well described as a common clinical sign in people with PD.[145] Moreover, mild motor symptoms including reported alterations in handwriting were reported 7 to 11 years prior to PD diagnosis.[3] This observation encouraged me to explore objective ways to assess handwriting. The first approach was easy to perform. I found that iRBD participants spent on average 10 more seconds to write three sentences than controls. I took this a step further and with the help of a graphologist we found abnormal handwriting patterns beyond micrographia. This is the first study exploring handwriting signatures in people with iRBD.

Dysgraphia, defined by sentence slope, irregular shape and increased pen pressure, together with indirect signs of hidden tremor and slow writing, could be early motor signatures of PD. Although these findings remain exploratory, they warrant further investigation towards developing objective tools able to measure script irregularities.

Quantitative motor signatures in iRBD

Slow and erratic movement were the common denominator across all quantitative motor tasks carried out by patients with iRBD. Considering the high prevalence of cognitive dysfunction in iRBD, it is difficult to determine to what extent 'cognitive slowing' or bradyphrenia could have contributed to slowing down the movement in patients with iRBD. Cognitive and motor domains are closely related. Little is known about whether patients with higher cognitive burden are more susceptible to being affected by motor challenging conditions. Having cognitive impairment might affect compensatory mechanisms that are usually activated to overcome challenging conditions in motor tasks. This fact could explain why, apart from being slower, movements were more erratic in patients with iRBD, reflecting patient effort during a difficult motor task performance.

There is an unmet need to develop a simple prediction model composed of a set of quantitative tools designed to assess separate motor domains. This approach offers two advantages: replicability and accuracy. By using a simple set of motor tools, further cohorts will be able to replicate the same approach on a large scale. Not only that, but new hypotheses will also be tested using existing data. By gathering information from separate motor domains, the overall accuracy of the motor battery will increase. Based on the main findings summarised above, three motor markers could be potential candidates:

1. Remote keyboard-based tapping tests: the BRAIN test in conjunction with the DFT test. They offer the advantage of large-scale applicability since they are available online and do not require sophisticated equipment. Moreover, when used in combination, they have been found to have a high degree of accuracy.
2. Handwriting speed. This can be easily applied. Remote implementation could be achieved by using a timed keyboard-based typing task instead, but first we need to prove that this new approach shows similar results.
3. Walking speed and finger tapping under challenging conditions. Multi-tasking, such as checking our phone while walking on the street, is part of our routine these days. Continuous monitoring of our walking pace while doing other mental tasks might offer a good opportunity to measure walking pace in people at risk under real-world conditions. Similarly, the finger tapping task, which is part of our daily clinical practice, should be assessed in isolation and combined with a mental task.

Limitations

The present study is not without limitations. Firstly, maintaining a strict consistency between assessments was difficult to achieve due to intrinsic constrains of the Coronavirus pandemic;

although Queen Mary University of London approved that our project could return to an adapted face-to-face routine and I put a range of safety measures to guard participants against the risk of coronavirus during the visits, each assessment was adapted to individual preferences. Based on that, I could not perform some of the assessments. This was the case with facial expression in the MDS-UPDRS-III. Two participants did not feel comfortable having to take their mask off, therefore, hypomimia score in the MDS-UPDRS-III could not be properly assessed. In the same way, examining participants in the same conditions was not possible in several cases; although most of the assessments took place at the Wolfson Institute of Population Health, 20 participants preferred to have a face-to-face visit at their home. This fact did not affect the performance of most of the assessments, albeit I had to adapt others. For example, finding a 10-metre-long space free of furniture at home was not always possible. In 10 cases the timed 10-metre walking test was performed on the street or in the garden instead.

Secondly, an observer bias in the MDS-UPDRS-III scoring could not be ruled out due to the lack of a blinded assessment. To mitigate this limitation, I took a representative sample of 11 iRBD patients and 11 controls, and contrasted my unblinded evaluation with a Movement Disorder clinical expert who was blind to people diagnosis (Prof Andrew Lees). Our evaluation matched in 20 out of 22 participants. The other two had iRBD and exhibited abnormal motor features (rest tremor and shuffling gait) that were not captured by video camera. Moreover, it is important to consider that my clinical impression was also supported by other objective motor tools (e.g. keyboard-based tapping tests and handwriting speed).

Thirdly, seven controls had a first-degree family history of PD compared with four people with iRBD. This is particularly important considering that first-degree family history of PD is a well-known risk factor of PD (LR+2.5).[47,67] In that sense, it could be argued that seven controls had a higher risk of developing PD.

Finally, clinical trajectories in iRBD are diverse. Although PD has been described to be the most common final diagnosis in patients with iRBD, in the largest multicentric longitudinal study, it was found that 43.5% patients with iRBD eventually developed DLB and 4.5% to MSA.[37] Due to the cross-sectional nature of my study, I could not explore how and when motor markers progress. Following up this cohort will be crucial to knowing which markers will predict a future diagnosis of PD or other alpha-synuclein disorders.

To conclude, speed and elements of chaotic movement might be early motor signatures in iRBD, which could have a potential role as motor prodromes of neurodegeneration. Two remote keyboard tapping tests (the DFT and BRAIN test) together with handwriting speed and walking speed under challenging conditions had the highest ability to distinguish people with iRBD and SP from healthy controls with slightly higher accuracy than the MDS-UPDRS-III with the advantage that they could be applied remotely.

This motor battery covering a range of separate motor domains provides a comprehensive overview of motor dysfunction in people with iRBD who may be in the prodromal stage of PD. The goal will be to work towards applying the current motor battery on a longitudinal scale to

explore whether it could be a reliable and sensitive method to quantify early motor changes with the aim to implement it in clinical trials of neuroprotective drugs.

Chapter 6

Overall discussion

6.1 Main results overview

This thesis aimed to characterise the motor prodromes of PD. I tried to answer three main questions: 1) **when** do motor prodromes start? To answer that question, we looked for evidence of motor manifestations reported prior to PD diagnosis (Chapter 2); 2) **how** do motor prodromes evolve and therefore how can they be predicted? To answer that question, I explored whether the PREDICT-PD algorithm was able to estimate a more pronounced motor decline in individuals at risk of PD (Chapter 3); and 3) **which** motor markers do we need to investigate? To do that I developed quantitative methods able to capture motor signatures in the prodromal phase of PD (Chapter 4) and tested them in an enriched group with iRBD (Chapter 5).

In brief, this thesis provides evidence that motor prodromes may be present long before the diagnosis of PD. Moreover, it identifies novel methods to capture prodromal motor signatures of PD in a quantitative manner.

In the first project, we performed a nested case-control study in a large primary care dataset from East London to determine associations between risk factors and pre-diagnostic presentations with subsequent PD. Tremor and memory symptoms were reported up to 10 and 5 years before diagnosis respectively, and both were strongly associated with PD. In contrast, symptoms of bradykinesia were not recorded. Similarly, rigidity was rarely reported by patients probably because it is a sign rather than a symptom. The fact that shoulder pain was more common in people who went on to develop PD than controls could suggest that it might be an indirect sign of rigidity. These findings support that there is a broad range of symptoms that prompt people to visit their GP a decade or more before PD diagnosis. Practitioners in primary care should be aware of a range of early presentations and consider PD as a possible cause. It is easy to argue that patients presenting in primary care with tremor should be referred to a movement disorders specialist but the fact that people could wait up to 10 years to receive PD diagnosis suggest that it might not always be the case. Some manifestations of PD (e.g. constipation, erectile dysfunction, and depression) are commonly encountered. Hence, they are typically underreported by patients, unless clinicians ask directly about them. In summary, this study provides further evidence that a range of comorbidities and symptoms are encountered in primary care prior to PD diagnosis, but for the first time in such a diverse and deprived population.

In the second project, I took a further step and prospectively investigated the course of motor prodromes in individuals stratified for future risk of PD. I examined them according to a widely

used standardised clinical rating scale (the MDS-UPDRS-III) which is also used to apply the clinical criteria of subthreshold parkinsonism (SP) established by the MDS Task Force for Research Criteria for Prodromal PD.[67] Next, I compared the motor scores of the follow-up with the baseline assessment, which was carried out 6 years before. My aim was to see whether participants in the higher risk (HR) group had a more pronounced motor decline than those in the lower risk (LR) group. In the baseline study, individuals stratified online for future PD risk exhibited an increased severity of motor disturbances and a greater proportion fulfilled clinical criteria for SP. Six years later, participants in the HR group had greater progression in their motor scores compared to the LR group. Due to the limited number of incident PD cases, I could not demonstrate the temporality of the effect that motor markers had on an outcome such as PD diagnosis, but this is an aim in the future. Given the low incidence of PD in the population, I changed the outcome and looked for the incidence of SP. I found that individuals classified in the HR group at baseline were twice as likely to develop SP as participants in the LR group and had 4-fold greater odds of having bradykinesia in the future, which is considered a cardinal sign for PD diagnosis. The PREDICT-PD approach offers several advantages that make it an appealing method to be applied in a large scale. First, it is a low intensity assessment with a cost-efficient approach (online, lack of sophisticated tools, easy to undertake). Second, I found that PREDICT-PD prediction algorithm can estimate the occurrence of motor disturbances in the future, in particular bradykinesia. Finally, it can also predict more pronounced motor decline over time. What remains unresolved is defining the boundaries between motor decline due to ageing and related to a neurodegenerative process. Future directions need to be towards finding markers of progression instead of phenoconversion.

In Chapter 4, I developed two quantitative tools focused on the analysis of repetitive finger tapping, the DFT and SMART test. I undertook a proof-of-concept approach in both tests. The former was tested on patients with established PD and compared with controls. The latter was tested on recently diagnosed patients with PD, individuals with idiopathic anosmia, and healthy controls. There are several reasons for focusing on creating an accurate method to measure bradykinesia. Firstly, bradykinesia is the cardinal sign of PD diagnosis.[116,210] Secondly, I demonstrated that people at higher risk of PD seemed to be more prone to developing bradykinesia in the future (Chapter 3). Thirdly, unlike other motor markers such as rigidity and shuffling gait, bradykinesia can be measured remotely without the need for sophisticated equipment. Repetitive finger tapping is one of the tasks used in routine clinical practice to assess bradykinesia. Moreover, slow alternate finger tapping has been found to be a consistent motor sign in people with iRBD with a high prediction of rate of future PD.[3] For these reasons, repetitive finger tapping is an appealing motor task to study in an objective manner. I found that both tests (the DFT and SMART test) were accurate methods to distinguish PD cases from controls. Slow finger tapping (reduced KS-DFT and finger tapping velocity captured by the SMART test) was the common denominator in both tests. Moreover, the SMART test introduced a new motor signature in early PD. Many people with PD showed a non-linear trend with a "burst" phenomenon defined as repetitive cycles of amplitude rebound over the

20-second task. Moreover, individuals with idiopathic anosmia performed the finger tapping more slowly and with smaller amplitude than controls. The fact that the finger tapping sub-score in the MDS-UPDRS-III was normal in some of them suggested that the SMART test was able to capture subtle motor dysfunction not seen at first sight. These results support the need for technology-based tools capable of quantifying early motor patterns in a more granular way than standard clinical scales. The fact that both tools are sensor free and can be studied remotely facilitates their applicability on a large scale.

I concluded my thesis by creating a motor battery to assess different motor domains using a set of quantitative and semi-quantitative motor tools. I tested this motor battery on individuals with iRBD, which is an enriched group known to have a high risk of developing PD. I compared their performance with age and sex matched controls. I was inspired by the notion that motor prodromes are strongly associated with imminent risk of PD diagnosis, and encouraged by the lack of standardised protocols for measuring motor prodromal signs in PD. I accounted for age and sex to minimise the chance of confounding the motor performance in the control group. I tested people with iRBD because, despite the fact it is a rare condition, it has a strong association with PD with high conversion rates, therefore a smaller sample size might suffice with adequate statistical power. I found that people with iRBD were more likely to have motor dysfunction than controls. Slow and erratic keyboard-based finger tapping, dygraphia and reduced handwriting pace were the most common motor features amongst patients with iRBD. Unlike controls, the iRBD group was susceptible to challenging conditions which were able to unmask motor dysfunction in two separate tasks, finger tapping and 10-metre walking task. The combination of remote keyboard-based tapping tests (the DFT and BRAIN test) discriminated people with iRBD from controls with 70% accuracy. Together, both tests were able to classify correctly with more than 80% accuracy those participants with iRBD and SP. This study gives further evidence that motor prodromes exist in people with iRBD and are visible enough to be captured by objective motor tools. However, there is a lack of a methodological consensus across longitudinal studies focused on the prodromal phase of PD. The heterogeneity of the methods (markers assessed, tools used, and study design) used in each study encumbers any comparison and reduces generalisability. For that reason, there is a need to establish a universal protocol to assess early motor signs in PD. Heinzel and collaborators systematically evaluated the limitations revealed by 35 longitudinal studies of the prodromal phase of PD.[74] The assessment of prodromal makers was one of the most encountered limitations. Further validation in population-based studies is needed.

6.2 Limitations

I acknowledge some limitations in the present thesis. They can be classified into three main categories: recruitment, data collection and statistical analysis. Most of the limitations entail different types of bias that will be listed throughout this section. Of note, each chapter contains

a more thorough explanation of the limitations encountered in the study. This section is mainly focused on summarising the overall limitations of this thesis.

Recruitment

This thesis includes three types of study design: a nested case-control study in health records data (Chapter 2: East London GP dataset), a prospective cohort study (Chapter 3: PREDICT-PD study), and three cross-sectional studies (Chapter 4: DFT and SMART test proof-of-concept, Chapter 5: motor battery test in people with iRBD). Selection bias is a common denominator in all studies. As with any study, the method of sampling intrinsically involves a selection bias. The net effect of a selection bias might be a lack of external validity since the sample obtained many systematically differs from the population it was intended to represent.

Due to elective selection process of candidates, case-control studies tend to be more susceptible to selection bias than longitudinal cohort studies where candidates are typically selected randomly.[240] Although the first project presented in this thesis (Chapter 2) was a nested case-control study, all PD cases and controls were selected randomly from routinely collected electronic healthcare records which were independent of any research cohort. Moreover, it is important to consider that most of research into the causes of PD has been carried out in White affluent population which do not represent the worldwide PD community. This project aimed to explore early presentations of PD in a diverse and deprived population. Although ethnicity and IMD were not found to be associated with PD, we have improved the representativeness of our knowledge of PD prodromes.

Moving to the second project, the recruitment source and sampling method used in PREDICT-PD might have resulted in selection bias. Although the source of recruitment was heterogeneous (local radio, print media), a considerable proportion of volunteers were recruited from Parkinson's UK. This resulted in recruiting many relatives and spouses of patients with PD, which explained the higher prevalence of positive family history in PREDICT-PD cohort compared with what would be expected in the general population. Although this could have increased the overall PD risk in our cohort, to date the incidence of PD in our cohort has been consistent with the incidence expected in the general population. Volunteer bias is intrinsic to most research studies. It has been reported that volunteers tend to be more educated and come from a higher social class than those who do not participate, leading to further selection bias in the end.[241,242]

Similar to case-control studies, obtaining a representative sample in cross-sectional studies, such as those presented in Chapters 4 and 5, is not always easy and can also result in selection bias. This was particularly relevant in the last project of this thesis where I compared a group of people with iRBD with age and sex matched controls. Firstly, the fact that I selected individuals with a special enriched risk of PD, such as iRBD, may have affected the generalisability of my findings. In fact, not all patients with PD have iRBD and not all patients with iRBD will develop PD; others will present Dementia with Lewy Bodies (DLB) and, in rare cases, Multiple System

Atrophy (MSA).[243] Moreover, it is known that iRBD represents a specific pre-diagnostic phenotype of PD.[29] Thus, patients with iRBD might not necessarily follow the same clinical course as most patients with idiopathic PD do. Further validation of my results in population-based groups is needed. Secondly, the matching process of controls could have included other unknown confounding factors. Of note, the control group had on average a higher proportion of relatives with PD than the iRBD group. This in turn may have elevated their risk to develop PD, which is not preferable, considering they were selected to be part of the control group.

Data collection

Data collection is prone to ascertainment bias. Such bias occurs when cases or controls are not identified correctly. When ascertainment bias comes from participants, it is referred to as response bias, whereas when it originates from the observer, it is referred to as observer bias.

Ascertainment bias was particularly important in the first study (Chapter 2). Data were derived from routinely reported primary care data which were already collected without an active enquiry about pre-diagnostic manifestations in PD. Thus, it was difficult to be certain about the diagnostic accuracy of PD and the correct labelling of manifestations. The lack of information regarding drug prescription was also a limiting factor in our study, meaning that we could not achieve a more robust PD definition supported by antiparkinsonian medication or exclude those with possible pharmacological parkinsonism. Another caveat to this study is that data were extracted in a cross-sectional manner. For that reason, the occurrence time of an effect (risk factor) relative to the onset of the disease was difficult to know. As such, we could only infer an association between prodromal markers and future PD but not a true relationship.

Another concern about ascertainment was the high proportion of dropouts (17.7%) in the second study (Chapter 3). The possibility of some of them having received the diagnosis of PD after baseline assessment could not be ruled out. In fact, apart from motivational aspects, which tend to decrease over time, those people who are more concerned about having developed PD could be the ones who more often drop out of a study.[244] In some cases, symptom perception is the justification for them to be more concerned. Thus, it could be expected that amongst those people who drop out of the study, there were few unreported incident PD cases. This in turn might have underestimated or overestimated the prediction power of our algorithm. Unfortunately, this possibility could not be confirmed since we lost contact with everyone who dropped out the study.

The PREDICT-PD algorithm stratified participants into higher and lower risk based on the above 15th centile and below 85th centile of risk scores respectively. Dichotomising a continuous variable such as risk estimates based on an arbitrary cut-off has the caveat of not accounting for the "dose effect" of risk estimates. Those participants in the middle risk were included in the LR group, making the LR a more heterogeneous group in terms of risk score ranges. Thus, it was plausible that some participants close to the 15th centile were classified in the LR group (false negative), leading to an ascertainment bias. I used the same sampling approach to baseline

study to maintain methodological consistency across the studies. As we gather more longitudinal data, 15th centile cut-off should be validated and might be adjusted based on incident PD cases.

Observer bias was mitigated in the second project (Chapter 3) given that I was blind to risk scores when I examined participants at both baseline and follow-up assessments. It was not the case in the last project (Chapter 5) when I was not blind when I assessed people with iRBD and controls. I contrasted my ratings by using objective quantitative tools which agreed with my clinical impression and asked an external Movement Disorder expert, who was blind to participants' diagnosis, to rate a representative sample of iRBD and controls.

The MDS-UPDRS-III has been designed as a tool for disease progression and treatment response in people with established PD.[245] It is not well adapted for people at risk of PD or early stages of the disease. This in turn may have caused instrumental errors when comparing people with higher and lower risk of PD (Chapter 3) and iRBD people with controls (Chapter 5) since none of the participants had established PD. Research criteria proposed by the MDS Task Force for Prodromal PD include the concept of SP in the definition of prodromal PD. They aim to represent the motor domain in the prodromal phase. The main issue here is that the concept of SP relies on a clinical scale that was not designed for early stages of the disease, meaning that the concept of SP per se was created by the wrong tool and so it might need to be re-defined. Given that there were limited cases of incident PD, I used SP as the outcome to test our algorithm. I used the definition of SP suggested by the MDS Task Force because, despite the intrinsic caveats mentioned above, it has a widely used definition which enabled us to make comparisons with other studies. However, there exists a widespread measurement bias across most of the studies focused on the motor prodromal phase of PD by using the wrong gold standard method to define the motor prodromes of PD. In Chapter 3 I mitigated this limitation by using a ≥ 5-point change in the MDS-UPDRS-III as a marker of motor progression. I also included objective tests such as a timed handwriting and walking task. Unlike the timed walking test, timed handwriting distinguishes people in the HR group from the LR group. Finally, with the last project of this thesis (Chapter 5), I aimed to overcome the MDS-UPDRS-III limitations at an early stage of the disease by creating a motor battery of quantitative tools. I found results in line with those seen in the HR group (Chapter 3), with slow handwriting being again a common feature in people with iRBD. Moreover, I found that the iRBD group had slower and more chaotic finger tapping as well as their walking pace and finger tapping being more susceptible to challenging conditions.

Measurement bias could have also arisen from the unsupervised collection of BRAIN test data in the second project of this thesis (Chapter 3). As a reminder, the BRAIN test is part of the online assessment of PREDICT-PD study. Motor performance may have been altered if participants sought help from others or used two hands to complete the alternate test. Although we anticipated both possibilities and took some steps to minimise them (giving clear instructions and excluding improbable speedy scores), this could have contributed to diluting differences

between the HR and LR groups, where the test was administered remotely. In contrast, when the BRAIN test was administered in person (as part of the motor battery presented in Chapter 5), it showed clear differences between iRBD and control groups. This fact could be explained by the BRAIN test having some limitations when it is applied in an unsupervised manner or by iRBD participants having a greater motor dysfunction than the general population at risk included in the second project. Next steps will be including the DFT test in PREDICT-PD online platform to see whether it is more accurate than the BRAIN test and comparing the performance of people with different degrees of risk (iRBD and general population with higher risk).

Statistical analysis

The lack of incident PD cases in the second study (Chapter 3) limited the scope of the statistical analysis, meaning that survival analysis was not possible to be calculated to extract the prediction power of motor prodromes. Although the results are promising, as we gather more longitudinal data, I expect the incident PD cases will increase and unravel the trajectories of motor prodromes prior to PD diagnosis.

Confounding was a common limitation across all the studies. It can result in Type 1 error (incorrect rejection of the null hypothesis, a false positive). In PD, age is an important confounding factor difficult to adjust as age is also highly associated with the prevalence of PD as well as the presence of mild parkinsonian signs.[108] For that reason, there is a need to adjust research criteria of SP to separate age ranges. For example, a score of 7 in the MDS-UPDRS-III in a 50-year-old male is more meaningful in terms of PD risk than the same score in an 80-year-old male. Sex is also an important confounding factor with different prevalence occurring in males and females. In Chapter 2 we carried out two separate analysis, one matched for age and sex and another unmatched with subsequent adjustment for both confounding factors. In Chapter 3 we excluded age and sex from the algorithm and adjusted the linear regression analysis. In Chapter 4 both groups were comparable in terms of age although no strict matching process was carried out. In chapter 5 both groups were matched for age and sex. It is important to account for other factors that can influence motor performance such as cognition, osteoarticular problems, and depression. Cognitive impairment and depression are also known to be part of non-motor prodromes of PD. They can cause walking abnormalities and slowness.[246] In fact, in both studies (Chapter 3 and 5), individuals at risk were found to have a higher proportion of cognitive impairment. Therefore, it is difficult to know whether some of the motor differences might be also explained by the presence of concomitant cognitive dysfunction. That said, in Chapter 3, I adjusted for cognitive scores, and they did not seem to have a confounding effect between motor and risk scores.

6.3 Further work

Ongoing work and further steps to take in the future are listed below.

Current work:

1. I have written the manuscript for Chapter 3. We are waiting to include the replication analysis using Parkinson's disease Progressive Markers Initiative (PPMI) cohort.
2. I am working on applying more sophisticated methods for non-linear times-series metrics originated from the SMART test. For example, using a statistic machine learning method (random forest analysis) to separate generic data into several classes to see whether we can sub-classify different "abnormal" patterns of movement. Finally, I am planning to correlate finger tapping analysis between kinetic parameters and the expert clinical impression of three Movement Disorder specialists.
3. I am planning to reduce potential observer bias in Chapter 5 by involving three separate Movement disorders specialists. They will be blind when they rate iRBD and controls, and will follow the same clinical scale (MDS-UPDRS-III instructions). One of them has already scored the participants. I used their score to do a preliminary comparison with my unblinded scores (already mentioned in the limitations above). Twenty out of 22 blind scores matched with my unblinded scores.
4. I selected participants from PREDICT-PD who fulfil the criteria for subjective RBD based on their answers in the RBDSQ (>5 points), which is part of the online battery of questionnaires in the PREDICT-PD platform. Then, I will compare their remote BRAIN and DFT test performance. So far, there are 842 participants with subjective RBD. The main objectives are to: 1) validate the DFT and BRAIN tests on a large scale by substantially increasing the sample size; 2) explore whether people with subjective RBD had a similar motor dysfunction to people with v-PSG-confirmed iRBD. If that is confirmed, it will have potential implications in terms of RBD recruitment in large-scale studies. V-PSG is required to confirm iRBD diagnosis. However, it is not widely available due to economic constraints and limited access to Sleep Disorders Centres. These factors limit the number of people with RBD who can have a diagnostic confirmation, which in the end affects the recruitment numbers of people with v-PSG-confirmed RBD who get involved in research studies. Thus, there is a need to prove that people with unconfirmed, but subjective RBD, are comparable in terms of motor impairment with people with a confirmed diagnosis.
5. I am planning to explore the reasons that prompt participants to drop out of the study. To do that, I will use the NHS Spine platform to find out if they are still alive, and NHS numbers, if they are available, to check diagnoses via linkage.

Future work:

The main findings of this thesis aim to encourage further projects in the future. Research targets should be: 1) large scale applicability, 2) developing quantitative motor tools, 3) redefinition of phenoconversion. The following ideas plan to reach the three future goals mentioned above:

1. Expanding the RBD cohort from two different sources (sleep disorders clinic and PREDICT-PD platform) and continuing longitudinal assessment.
2. Close monitoring of people in the HR group with motor impairment at baseline ("motor-enriched" group). The main goal is to define motor trajectories. Instead of taking an outcome-focused approach, we should aim to take a more dynamic approach focused on progression. By gathering consecutive assessments, we will be able to know which people progress, remain stable, and regress. First, we need to define the motor trends: aggressive (concomitant cognitive decline, atypical PD), progressive steady worsening (PD), stable (age-related), fluctuant (confounding factors) and regression (protective factors). Then we need to create a composite estimation model with a set of different prediction markers of progression.
3. Before implementing the motor battery on a larger scale, further research needs to be done in terms of creating a minimal set of motor assessments where motor markers could be accurately captured remotely. So far, the candidates are the keyboard-based tapping tests, handwriting speed and dual tasking (waking and finger tapping). Further research is needed to develop a software tool able to time a handwriting task remotely. An alternative test could be a timed keyboard typing task, although first we would need to prove that a typing speed on a keyboard is affected at early stages of the disease. Handwriting analysis could be complemented by examining digital handwriting scripts, recorded by an electronic pen and tablet and using artificial intelligence models to detect discriminatory handwriting features. The final goal will be to simplify the PREDICT-PD algorithm using a shorter remote assessment which will include an online questionnaire collecting risk factor information and prodromal manifestations, a smell test and a simple motor battery. The main reasons for reducing the algorithm would be to ensure high retention rates followed by selecting the appropriate candidates to be seen in person. The ideal candidate will be one with higher risk and evidence of motor dysfunction (Figure 6.1).

6.4 Ethical issues

Participants from PREDICT-PD study did not receive any information about their risk scores and test results. Similarly, people with iRBD were not informed about their performance in the motor battery and memory test. However, they gave written consent to be informed and contact their GP if PD diagnosis was suspected. This was the case with one participant with iRBD. By the time I saw her in person, she had already fulfilled the criteria for PD and had

motor symptoms. I informed her about my clinical suspicion. I also contacted her GP and suggested that she be referred to a Movement Disorder Specialist.

Risk disclosure in people with iRBD is particularly difficult. When we communicate with patients it is important bear in mind that iRBD is highly associated with an increased risk of later developing PD or a related condition for which there is currently no preventive treatment. There is controversy about what information is disclosed to patients with regards to potential future implications, how this is done and when.

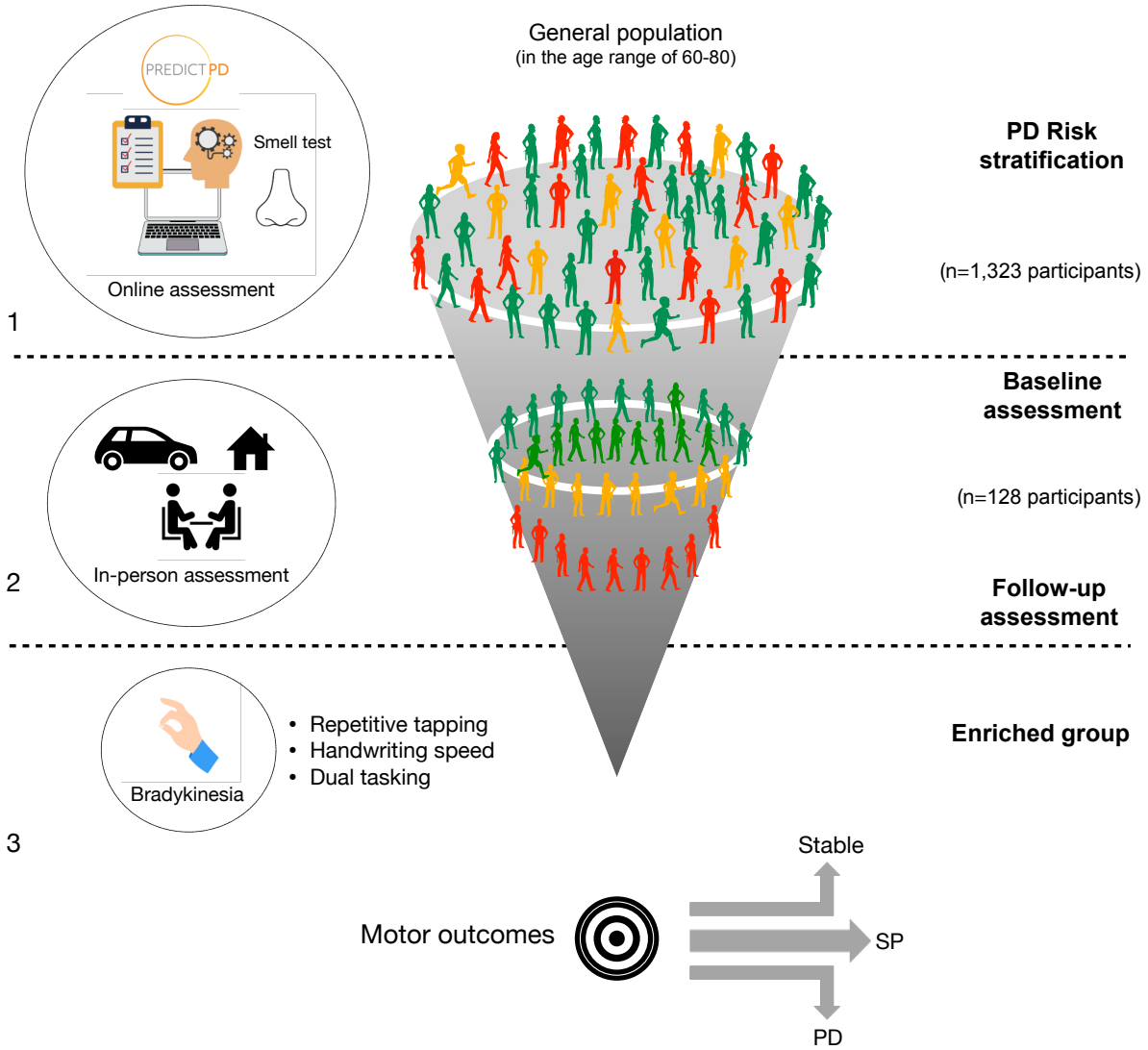

Figure 6.1 Schematic of PREDICT-PD study from population-based online risk stratification to motor outcomes: stable (MDS-UPDRS-III <5), SP (Subthreshold Parkinsonism) and PD (Parkinson's Disease). **Stage 1**) online assessment. Red, orange and green colours represent participant stratification based on risk estimates: higher risk in red, middle risk in orange and lower risk in green (for more details, see Chapter 1). **Stage 2**) a representative group of participants (n= 128) was seen in person at baseline and followed up 6 years later. **Stage 3**) future directions towards remote tools to quantify bradykinesia (potential motor prodrome of PD) to enrich the PREDICT-PD algorithm

I wanted to understand the preferences of patients with iRBD around receiving information about the link between iRBD and other neurodegenerative conditions. In collaboration with Dr Laura Pérez-Carbonell from the Sleep Clinic at Guy's St Thomas Hospital, we developed a 9-question survey asking patients with iRBD about what information they would like to receive about the risk of developing a neurodegenerative condition in the future, how they would like to be informed and when this should take place (Appendix E).

We interviewed 31 patients (28 males, 70yo, SD 8.7y) with iRBD (mean 8.7-year disease duration, SD 6.4). A third had not received any information about the link between iRBD and other conditions by healthcare professionals. More than half (61%) had searched for that information online. Most patients (87%) wanted to receive prognostic information to help them make informed decisions about their future. The preferred time for this was when a diagnosis of iRBD was made rather than when parkinsonism starts (61% vs 7%). Most wanted this information to come from their iRBD specialist (93%) combined with other sources of information (50%) such as patient information leaflet and scientific associations websites. In contrast, only 20% of patients wanted to receive risk information from their GP. Based on these results, it seems that there is a need to raise awareness in other healthcare professionals including those in primary care and create reliable sources of information.

In summary, patients with iRBD mostly wished to receive information regarding the potential future implications of having iRBD when the diagnosis of iRBD is made. Taking account of patients' preferences is relevant in deciding what, when and how to disclose prognostic information to patients with iRBD.

6.5 Concluding comments

The work presented in this thesis demonstrates the existence of prodromal motor features in the pre-diagnostic phase of PD in three separate groups of risks from three different clinical settings: patients without PD in primary care, HR volunteers participating in a population-based study (PREDICT-PD) and an enriched group with iRBD attending the Sleep Clinic. Apart from giving evidence about the presence of motor prodromes, I developed quantitative tools able to define early motor signatures in PD. Reduced velocity and erratic movements seemed to be the common denominators amongst the motor tests evaluated. Finally, the creation of a minimal set of motor assessments to be applied remotely and in a large scale will be crucial to select those individuals with the highest risk who require close monitoring.

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Appendix A

Details of the study in Chapter 2

Definition of other exposures

Vascular risk factors. Coded diagnoses of hypertension, cholesterol, and type 2 diabetes (T2D) were defined on four levels depending on whether the risk factor was never recorded, first recorded prior to PD diagnosis (or dummy date of diagnosis for controls), first recorded after PD diagnosis, or unknown where the data were missing. For each risk factor, the status was determined by the earliest record, unless this was missing, in which case the status at the latest date was used. Total cholesterol levels were taken from the clinical data and were considered valid if they ranged from (0.5-50.0) mmol/L. Values >5.0 mmol/L were indicative of hypercholesterolaemia. Hypertension and T2D were recorded according to the presence of a coded morbidity record. Patients with a diagnosis of type 1 diabetes were recorded as "normal". Although being overweight is considered a vascular risk factor, it is described separately below.

Smoking. Smoking status was defined as being coded as a current, ex-smoker or never smoker, but time before PD diagnosis was not considered, given that smoking initiation in later life is rare. For that reason, data related to smoking will cover all pre-diagnostic periods.

Body Mass Index (BMI). BMI was calculated from clinical data using height and weight measurements and categorised as follows: normal (BMI 20.0-24.9 kg/m2), underweight (10.0-19.9 kg/m2), and overweight (25.0-50.0 kg/m2). For height and weight, the ranges were 100-250 cm and 30-250 kg respectively. Where height or the calculated BMI was outside ranges, these data were deemed unlikely and were reclassified as unknown. In the same way, participants with missing BMI data were classified as unknown, unless they had a diagnosis of obesity recorded. In which case they were classified as overweight. Unlike overweight, underweight is not linked to coexistent vascular disease. We therefore listed it under a 'metabolic prodrome' category.

Proportion of practice/population coverage

For the Hackney & City of London, Newham and Tower Hamlets, 100% of patients were included. For Waltham Forest there were 4 practices that used a different IT system (SytstmOne) that we could not have at the time of access. This has 48,299 registered patients. Therefore, the total of practice/population coverage was 95.3% (989,064/1,037,363). There is no reason to suppose that the patients in Waltham Forest practices differ from the London

average by any important characteristic, which would influence the generality of the study results – though east London is overall a highly ethnically and socially diverse population and whilst representative of inner-city populations, it is not representative of England.

Table A2.1. Diagnostic codes for neurological exclusions

Cases	
Diagnosis	**EMIS Read Codes**
Atypical Parkinson's code	F24y0, F24y2, F11y2, F174
Dementia QOF* code	Eu02%, E00%, Eu01%, E02y1, E012%, Eu00%, E041, Eu041, F110, F111, F112, F116, F118, F21y2, A411%, A410, Eu107, F11x7
Multiple sclerosis code	F20%
Motor Neurone Disease/ALS code	F152%
Controls	
Diagnosis	**EMIS Read Codes**
Parkinson's code	F12%, F1303, F11x9,147F
Atypical Parkinson's code	F24y0, F24y2, F11y2, F174
Dementia QOF* code	Eu02%, E00%, Eu01%, E02y1, E012%, Eu00%, E041, Eu041, F110, F111, F112, F116, F118, F21y2, A411%, A410, Eu107, F11x7
Multiple sclerosis code	F20%
Motor Neurone Disease/ALS code	F152%

Table A2.2. Diagnostic codes for exposures

Exposure	EMIS Read Codes	UK Biobank ICD 10 codes
Overweight	22K5, 22K7, 22KC, 22KD, 22KE	
Smoking (ever)	Current: 1372.-1376. , 137C.-137D. , 137G.-137H. , 137J. , 137M. , 137P.-137R. , 137V. , 137X.-137f. , 137h. , 137m. , 137o. 137.. Ex-smoker: 1377.-137B. , 137F. , 137K. , 137N.-137O. , 137S.-137T. , 137j. , 137l. Never: 1371	
Alcohol (ever)	136%	
Type 2 diabetes	C10.. , C109J , C109K , C10C. , C10D. , C10E.% , C10F.%, C10G.% , C10H.% , C10M.% , C10N.% , PKyP. , C10P.% , C10Q. Excluded: C10F8	
Hypertension	G2... , G20..% , G24..-G2z.., Gyu2. , Gyu20 Excluded: G24z1 , G2400 , G2410 , G27..	
High cholesterol	44P	
Epilepsy	F25..%, F1321 , SC200 Exclusion: F2501 , F2504 , F2511 , F2516 , F256.% , F258.-F25A. , F25y4 , F25G. , F25H.	First-occurrence outcomes: date_g40_first_reported_epilepsy_f131048_0_0 date_g41_first_reported_status_epilepticus_f131050_0_0 ICD10: diagnoses_icd10_f41270_0_0 through diagnoses_icd10_f41270_0_222 G40 G40 Epilepsy G400 G40.0 Localisation-related (focal) (partial) idiopathic epilepsy and epileptic syndromes with seizures of localised onset

	G401 G40.1 Localisation-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures
	G402 G40.2 Localisation-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with complex partial seizures
	G403 G40.3 Generalised idiopathic epilepsy and epileptic syndromes
	G404 G40.4 Other generalised epilepsy and epileptic syndromes
	G405 G40.5 Special epileptic syndromes
	G406 G40.6 Grand mal seizures, unspecified (with or without petit mal)
	G407 G40.7 Petit mal, unspecified, without grand mal seizures
	G408 G40.8 Other epilepsy
	G409 G40.9 Epilepsy, unspecified
	G41 G41 Status epilepticus
	G410 G41.0 Grand mal status epilepticus
	G411 G41.1 Petit mal status epilepticus
	G412 G41.2 Complex partial status epilepticus
	G418 G41.8 Other status epilepticus
	G419 G41.9 Status epilepticus, unspecified
Head injury	S0%, S64%, F281, 700%, 701%, 703, S642, S62%
Underweight	1623%, 1625%, 1627%, 162Z%, 1D1A%, R032%, R0341%, R0348%, 22A8%, 22AZ%
Constipation	19C, 19C2 , 19CZ , J5200, J5201 , J5202 , J5204 , J520z
Hypotension	14AS , G87, G870 , G871 , G872 , G87z , Gyu90, R1y3 , 1B55
Erectile dysfunction	1598, 1ABB , 1ABC , 1D1B , 7C25E, E2273

Insomnia	1B1B%, 1B1Q , 1BX0 , E2741, E2742, E274D, Fy00 , R005	
Dizziness	1B5, 1B53 , 1B54 , R004 , R0040 , R0041 , R0042 , R004z,	
Anxiety	1B1, 1B12, 1B13, 1B1V, 1BK, E200%, Eu41, Eu410, Eu411, Eu412 , Eu41z, R2y2	
Fatigue	168, 1682, 1683, 1684, 168Z , E205, Eu460, R0071, R0073 , R0075 , R007z	
Depression	E0013 , E0021 , E112.% , E113.% , E118. , E11y2 , E11z2 , E130. , E135. , E2003 , E291. , E2B.. , E2B1. , Eu204 , Eu251 , Eu32.%, Eu33.% , Eu341 , Eu412 Exclusion: Eu32A , Eu32B , Eu329	
Memory symptoms	1B1A, 1B1Y , 1B1a , 1S21 , 28G, 3A10 , 3A20 , 3A30 , 3A40 , 3A50 , 3A60 , 3A70 , 3A80 , 3A91 , 3AA1 , 8BIk , 8HTY , 9Nk1 , E2A10, E2A11, R00z0	
Hearing loss	Deafness code: F59%, 2BL%, SJ15%, P40z%, A5602, 1C13% Audiology referrals: 8HT3 Referral to audiology clinic, 9N0W Seen in audiology clinic, 7P12% Diagnostic audiology	Combined Hearing problems (with/without noise and ICD10 diagnoses of hearing deficits) Self-reported hearing difficulty: hearing_difficultyproblems_f2247_0_0 (baseline only) (deafness is captured in this variable) hearing_difficultyproblems_with_background_noise_f2257_0_0 (baseline only) First-occurrence outcomes: date_h90_first_reported_conductive_and_sensorineural_hearing_loss_f131258_0_0 date_h91_first_reported_other_hearing_loss_f131260_0_0

date_h93_first_reported_other_disorders_of_ear_not_elsewhere_classified_f131264_0_0

date_h94_first_reported_other_disorders_of_ear_in_diseases_classified_elsewhere_f131266_0_0

date_h95_first_reported_postprocedural_disorders_of_ear_and_mastoid_process_not_elsewhere_classified_f131268_0_0

ICD10: diagnoses_icd10_f41270_0_0 through diagnoses_icd10_f41270_0_222

H90 H90 Conductive and sensorineural hearing loss

H900 H90.0 Conductive hearing loss, bilateral

H901 H90.1 Conductive hearing loss, unilateral with unrestricted hearing on the contralateral side

H902 H90.2 Conductive hearing loss, unspecified

H903 H90.3 Sensorineural hearing loss, bilateral

H904 H90.4 Sensorineural hearing loss, unilateral with unrestricted hearing on the contralateral side

H905 H90.5 Sensorineural hearing loss, unspecified

H906 H90.6 Mixed conductive and sensorineural hearing loss, bilateral

H907 H90.7 Mixed conductive and sensorineural hearing loss, unilateral with unrestricted hearing on the contralateral side

H908 H90.8 Mixed conductive and sensorineural hearing loss, unspecified

H91 H91 Other hearing loss

H910 H91.0 Ototoxic hearing loss

H911 H91.1 Presbycusis

	H912 H91.2 Sudden idiopathic hearing loss
	H913 H91.3 Deaf mutism, not elsewhere classified
	H918 H91.8 Other specified hearing loss
	H919 H91.9 Hearing loss, unspecified
	H930 H93.0 Degenerative and vascular disorders of ear
	H931 H93.1 Tinnitus
	H932 H93.2 Other abnormal auditory perceptions
	H933 H93.3 Disorders of acoustic nerve
	H940 H94.0 Acoustic neuritis in infectious and parasitic diseases classified elsewhere
Neck pain	16A% (exclude 16A1), N131 , N135z, N138
Shoulder pain	N0941, N0942, N094A, N0951, N210, N2457
Rigidity	1D12 , 294-1 (syn), 2942, 2944, F13z3,
Balance difficulties	1B5, 1B52 , 29L8 , 29LB , 29LD , 29LF , 2994, 2987
Tremor	1B22 , 297A , 297B , R0103, R20-1 (syn)

Table A2.3 Matched case-control analysis for comorbidities and risk factors according to time of presentation

Exposures	Category	<2 years % (PD: Controls)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	2-<5 years % (PD: Controls)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	5-<10 years % (PD: Controls)	Unadjusted OR (95%CI)	Adjusted OR (95% CI)	All pre-diagnostic % (PD: Controls)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Overweight		57 (5.4%): 469 (4.4%)	1.22 (0.92 to 1.63)	1.23 (0.92 to 1.63)	70 (6.6%): 777 (7.4%)	0.89 (0.69 to 1.15)	0.89 (0.69 to 1.15)	146 (13.8%): 1455 (13.8%)	1 (0.83 to 1.2)	1 (0.83 to 1.21)	527 (50%): 527 (50%)	1.05 (0.92 to 1.2)	1.06 (0.92 to 1.21)
Smoking (ever)*	Lifestyle	NA	NA	NA	NA	NA	NA	NA	NA	NA	361 (34.2%): 4239 (40.2%)	0.76 (0.66 to 0.87)	**0.74 (0.64 to 0.85)**
Alcohol (ever)		32 (3%): 338 (3.2%)	0.93 (0.64 to 1.35)	0.95 (0.65 to 1.37)	39 (3.7%): 490 (4.6%)	0.77 (0.55 to 1.08)	0.79 (0.57 to 1.11)	58 (5.5%): 824 (7.8%)	0.67 (0.51 to 0.88)	**0.68 (0.51 to 0.9)**	284 (26.9%): 3236 (30.7%)	0.79 (0.68 to 0.92)	**0.8 (0.68 to 0.93)**
Type 2 diabetes		27 (2.6%): 270 (2.6%)	1 (0.67 to 1.5)	0.99 (0.66 to 1.48)	34 (3.2%): 413 (3.9%)	0.82 (0.57 to 1.17)	0.81 (0.57 to 1.16)	74 (7.0%): 539 (5.1%)	1.41 (1.09 to 1.82)	**1.39 (1.08 to 1.79)**	250 (23.7%): 1949 (18.5%)	1.39 (1.19 to 1.62)	**1.4 (1.19 to 1.64)**
HBP	Vascular risk factors	48 (4.5%): 491 (4.7%)	0.97 (0.72 to 1.32)	0.98 (0.72 to 1.33)	69 (6.5%): 815 (7.7%)	0.83 (0.64 to 1.07)	0.84 (0.65 to 1.08)	143 (13.6%): 1141 (10.8%)	1.29 (1.07 to 1.56)	**1.29 (1.07 to 1.56)**	501 (47.5%): 4260 (40.4%)	1.36 (1.19 to 1.55)	**1.38 (1.21 to 1.58)**
High cholesterol		65 (6.2%): 536 (5.1%)	1.2 (0.92 to 1.57)	1.21 (0.92 to 1.59)	82 (7.8%): 966 (9.2%)	0.8 (0.63 to 1.02)	0.8 (0.63 to 1.02)	143 (13.6%): 1629 (15.4%)	0.82 (0.68 to 0.99)	**0.81 (0.67 to 0.99)**	468 (44.4%): 4645 (44%)	0.96 (0.83 to 1.1)	0.96 (0.83 to 1.1)
Epilepsy	Other	2 (0.2%): 2 (0%)	10 (1.41 to 70.99)	**8.97 (1.24 to 65.05)**	3 (0.3%): 6 (0.1%)	5 (1.25 to 19.99)	**4.8 (1.19 to 19.36)**	6 (0.6%):11 (0.1%)	5.46 (2.02 to 14.76)	**5.69 (2.1 to 15.41)**	27 (2.6%): 111 (1.1%)	2.5 (1.63 to 3.83)	**2.48 (1.62 to 3.81)**
Head injury		9 (0.9%): 23 (0.2%)	3.97 (1.83 to 8.65)	**3.95 (1.81 to 8.62)**	2 (0.2%): 37 (0.4%)	0.54 (0.13 to 2.24)	0.53 (0.13 to 2.2)	7 (0.7%): 40 (0.4%)	1.76 (0.78 to 3.93)	1.75 (0.78 to 3.91)	34 (3.2%): 221 (2.1%)	1.56 (1.08 to 2.26)	**1.56 (1.08 to 2.26)**

Matched case-control analysis: matching 10 controls for each case according to age and sex (unadjusted) and adjusted for ethnicity and IMD. HBP: high blood pressure. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date. OR: Odds Ratio. CI: Confidence Interval. PD: Parkinson's disease patients (n=1055), controls (n=10,550). *Data covers all pre-diagnostic period. It was not possible to classify data into 3 periods given that smoking initiation in later life is rare, therefore time before PD diagnosis was not considered. In bold: significant association (CI without including 1)

Table A2.4 Matched case-control analysis for non-motor prodromes according to time of presentation

Exposures	Category	Time period								
		<2 years			2-<5 years			5-<10 years		
		% (PD: Control)	Unadjusted OR	Adjusted OR	% (PD: Control)	Unadjusted OR	Adjusted OR	% (PD: Control)	Unadjusted OR	Adjusted OR
Underweight	Metabolic	7 (0.7%): 26 (0.2%)	2.73 (1.17 to 6.37)	**2.58 (1.1 to 6.02)**	4 (0.4%):49 (0.5%)	0.81 (0.29 to 2.25)	0.78 (0.28 to 2.18)	10 (0.9%): 69 (0.7%)	1.45 (0.74 to 2.83)	1.44 (0.74 to 2.82)
Hypotension		13 (1.2%): 19 (0.2%)	6.84 (3.38 to 13.85)	**6.81 (3.35 to 13.8)**	12 (1.1%): 25 (0.2%)	4.88 (2.44 to 9.77)	**4.73 (2.36 to 9.5)**	6 (0.6%): 30 (0.3%)	2.01 (0.83 to 4.85)	1.9 (0.79 to 4.6)
Constipation	Autonomic	44 (4.2%): 140 (1.3%)	3.29 (2.32 to 4.66)	**3.29 (2.32 to 4.67)**	53 (5%): 205 (1.9%)	2.68 (1.97 to 3.66)	**2.66 (1.95 to 3.63)**	53 (5%): 188 (1.8%)	2.96 (2.16 to 4.06)	**2.97 (2.16 to 4.07)**
Erectile dysfunction*		21 (3.3%): 185 (2.9%)	1.14 (0.72 to 1.80)	1.14 (0.72 to 1.8)	34 (5.4%): 302 (4.8%)	1.13 (0.79 to 1.64)	1.13 (0.78 to 1.63)	51 (8.1%): 350 (5.5%)	1.51 (1.11 to 2.05)	**1.52 (1.12 to 2.08)**
Depression		24 (2.3%): 52 (0.5%)	4.69 (2.88 to 7.63)	**4.61 (2.82 to 7.52)**	18 (1.7%): 111 (1.1%)	1.65 (0.99 to 2.73)	1.64 (0.98 to 2.72)	28 (2.7%): 144 (1.4%)	1.97 (1.31 to 2.97)	**1.94 (1.29 to 2.92)**
Anxiety		32 (3%): 106 (1%)	3.08 (2.06 to 4.60)	**3.01 (2.02 to 4.5)**	18 (1.7%): 137 (1.3%)	1.32 (0.8 to 2.18)	1.29 (0.78 to 2.13)	37 (3.5%): 243 (2.3%)	1.55 (1.09 to 2.21)	**1.53 (1.07 to 2.18)**
Insomnia	Neuro-psychiatric	21 (2%): 97 (0.9%)	2.18 (1.36 to 3.51)	**2.17 (1.35 to 3.48)**	25 (2.4%): 137 (1.3%)	1.85 (1.20 to 2.85)	**1.87 (1.21 to 2.88)**	24 (2.3%): 164 (1.6%)	1.48 (0.96 to 2.28)	1.47 (0.95 to 2.27)
Fatigue		26 (2.5%): 138 (1.3%)	1.91 (1.25 to 2.93)	**1.86 (1.21 to 2.85)**	27 (2.6%): 194 (1.8%)	1.41 (0.93 to 2.13)	1.4 (0.93 to 2.11)	26 (2.5%): 243 (2.3%)	1.08 (0.71 to 1.63)	1.06 (0.7 to 1.6)
Dizziness		36 (3.4%): 229 (2.2%)	1.59 (1.11 to 2.27)	**1.57 (1.09 to 2.24)**	54 (5.1%): 276 (2.6%)	2.01 (1.49 to 2.71)	**1.99 (1.47 to 2.68)**	60 (5.7%): 369 (3.5%)	1.68 (1.27 to 2.24)	**1.66 (1.25 to 2.21)**
Memory symptoms		52 (4.9%): 63 (0.6%)	8.6 (5.91 to 12.49)	**8.73 (6.0 to 12.7)**	18 (1.7%): 59 (0.6%)	3.08 (1.81 to 5.24)	**3.09 (1.81 to 5.26)**	8 (0.8%): 39 (0.4%)	2.06 (0.96 to 4.42)	2.01 (0.93 to 4.31)
Hearing loss	Sensory	23 (2.2%): 140 (1.3%)	1.66 (1.06 to 2.58)	**1.65 (1.06 to 2.58)**	29 (2.7%): 170 (1.6%)	1.73 (1.16 to 2.57)	**1.73 (1.16 to 2.57)**	24 (2.3%): 163 (1.5%)	1.48 (0.96 to 2.29)	1.46 (0.95 to 2.26)

Matched case-control analysis: matching 10 controls for each case according to age and sex (unadjusted) and adjusted for ethnicity and IMD. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date (PD diagnosis). OR: Odds Ratio. CI: Confidence Interval. PD: Parkinson's disease patients (n=1055), controls (n=10,550). *Male patients only.

Table A2.5 Unmatched analysis (adjusted for age and sex) for comorbidities and risk factors according to time of presentation

Exposures	Category	<2 years		2-<5 years		5-<10 years		All pre-diagnostic	
		% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95% CI)	% (PD: Controls)	Adjusted OR (95% CI)
Overweight	Lifestyle	57 (5.4%): 65,775 (6.5%)	1.12 (0.86 to 1.47)	70 (6.6%): 83,536 (8.3%)	0.82 (0.64 to 1.05)	146 (13.8%): 106,421 (10.5%)	1.15 (0.96 to 1.37)	527 (50%): 379275 (37.6%)	**1.31 (1.15 to 1.48)**
Smoking (ever)*		361 (34.2%): 33,9129 (33.6%)	**0.82 (0.72 to 0.93)**	361 (34.2%): 33,9129 (33.6%)	**0.82 (0.72 to 0.93)**	361 (34.2%): 33,9129 (33.6%)	**0.82 (0.72 to 0.93)**	361 (34.2%): 339129 (33.6%)	**0.82 (0.72 to 0.93)**
Alcohol (ever)		32 (3%): 67,994 (6.7%)	0.86 (0.60 to 1.23)	39 (3.7%): 89,842 (8.9%)	0.73 (0.53 to 1.01)	58 (5.5%): 88,696 (8.8%)	**0.75 (0.57 to 0.98)**	284 (26.9%): 336380 (33.3%)	0.95 (0.82 to 1.09)
Type 2 diabetes	Vascular risk factors	27 (2.6%): 9,964 (1.0%)	1.15 (0.78 to 1.69)	34 (3.2%): 13,685 (1.4%)	1.0 (0.71 to 1.41)	74 (7.0%): 16,627 (1.6%)	**1.7 (1.34 to 2.17)**	250 (23.7%): 60045 (5.9%)	**1.73 (1.5 to 1.99)**
Hypertension		48 (4.5%): 15,020 (1.5%)	1.02 (0.76 to 1.37)	69 (6.5%): 21,234 (2.1%)	0.99 (0.77 to 1.26)	143 (13.6%): 28,972 (2.9%)	**1.57 (1.31 to 1.87)**	501 (47.5%): 108135 (10.7%)	**1.77 (1.57 to 2)**
High cholesterol		65 (6.2%): 39,087 (3.9%)	1.13 (0.88 to 1.46)	82 (7.8%): 57,876 (5.7%)	0.87 (0.7 to 1.1)	143 (13.6%): 80,205 (7.9%)	1.0 (0.84 to 1.2)	468 (44.4%): 236538 (23.4%)	**1.24 (1.09 to 1.41)**
Epilepsy	Other	2 (0.2%): 603 (0.1%)	**5.14 (1.26 to 20.99)**	3 (0.3%): 875 (0.1%)	**3.92 (1.24 to 12.39)**	6 (0.6%):1,310 (0.1%)	**4.52 (1.99 to 10.24)**	27 (2.6%): 8831 (0.9%)	**2.62 (1.78 to 3.86)**
Head injury		9 (0.9%): 4,120 (0.4%)	**3.21 (1.65 to 6.24)**	2 (0.2%): 4,693 (0.5%)	0.62 (0.15 to 2.49)	7 (0.7%): 5,718 (0.6%)	1.82 (0.86 to 3.85)	34 (3.2%): 31485 (3.1%)	**1.55 (1.1 to 2.18)**

Standard: multivariable logistic model for PD with OR and 95% CI adjusted for age and sex. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date. OR: Odds Ratio. CI: Confidence Interval. PD: Parkinson's disease patients (n=1055), controls (n=1,009,523). *Data covers all pre-diagnostic period. It was not possible to classify data into 3 periods given that smoking initiation in later life is rare, therefore time before PD diagnosis was not considered. In bold: significant association (CI without including 1)

Table A2.6 Unmatched analysis (adjusted for age and sex) for non-motor prodromes according to time of presentation

Exposure	Category	<2 years		2-<5 years		5-<10 years	
		% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95%CI)	% (PD: Controls)	Adjusted OR (95%CI)
Underweight*	Metabolic	7 (0.7%): 18,656 (1.8%)	**2.14 (1.01 to 4.54)**	4 (0.4%): 24,270 (2.4%)	0.73 (0.27 to 1.96)	10 (0.9%): 27,686 (2.7%)	1.18 (0.63 to 2.22)
Constipation		44 (4.2%): 9,589 (0.9%)	**3.85 (2.84 to 5.24)**	53 (5%): 13,415 (1.3%)	**3.58 (2.71 to 4.74)**	53 (5%): 17655 (1.7%)	**3.27 (2.47 to 4.33)**
Hypotension	Autonomic	13 (1.2): 811 (0.1%)	**7.36 (4.2 to 12.93)**	12 (1.1%): 837 (0.1%)	**6.22 (3.47 to 11.17)**	6 (0.6%): 959 (0.1%)	**3.33 (1.48 to 7.5)**
Erectile dysfunction		21 (2.0%): 6,464 (0.6%)	1.34 (0.86 to 2.07)	34 (3.2%): 11,108 (1.1%)	1.38 (0.97 to 1.95)	51 (4.8%): 11,270 (1.1%)	**1.95 (1.46 to 2.61)**
Insomnia		21 (2.0%): 9,786 (1.0%)	**2.29 (1.48 to 3.54)**	25 (2.4%): 13,001 (1.3%)	**2.05 (1.38 to 3.06)**	24 (2.3%): 16,180 (1.6%)	**1.59 (1.06 to 2.39)**
Dizziness		36 (3.4%): 13,651 (1.4%)	**2.17 (1.55 to 3.04)**	54 (5.1%): 17,801 (1.8%)	**2.55 (1.93 to 3.37)**	60 (5.7%): 23,654 (2.3%)	**2.31 (1.78 to 3.01)**
Anxiety	Neuro-psychiatric	32 (3.0%): 23,149 (2.3%)	**3.13 (2.19 to 4.48)**	18 (1.7%): 25,535 (2.5%)	1.42 (0.89 to 2.28)	37 (3.5%): 26,950 (2.7%)	**1.92 (1.38 to 2.68)**
Fatigue		26 (2.5%): 22,428 (2.2%)	**2.14 (1.44 to 3.17)**	27 (2.6%): 27,183 (2.7%)	**1.7 (1.15 to 2.5)**	26 (2.5%): 30,917 (3.1%)	1.27 (0.86 to 1.89)
Depression		24 (2.3%): 8,491 (0.8%)	**4.47 (2.96 to 6.75)**	18 (1.7%): 11,817 (1.2%)	**2.05 (1.28 to 3.27)**	28 (2.7%): 17,428 (1.7%)	**1.83 (1.25 to 2.67)**
Memory symptoms		52 (4.9%): 2,832 (0.3%)	**9.84 (7.39 to 13.11)**	18 (1.7%): 3115 (0.3%)	**3.41 (2.13 to 5.46)**	8 (0.8%): 1,949 (0.2%)	**2.78 (1.38 to 5.61)**
Hearing loss	Sensory	23 (2.2%): 6,386 (0.6%)	**1.84 (1.21 to 2.79)**	29 (2.7%): 6,942 (0.7%)	**2.24 (1.54 to 3.26)**	24 (2.3%): 7,073 (0.7%)	**1.75 (1.17 to 2.64)**

Standard: multivariable logistic model for PD with OR and 95% CI adjusted for age and sex. Time span: <2 years, 2-<5 years and 5-<10 years before PD diagnosis or index date. OR: odds ratio. CI: Confidence Interval, *Unlike overweight, underweight is not linked to a coexistence vascular disease. It could be considered as part of the neurodegenerative process. For that reason, we decided to include it in the metabolic prodrome category. In bold: significant association (CI without including 1)

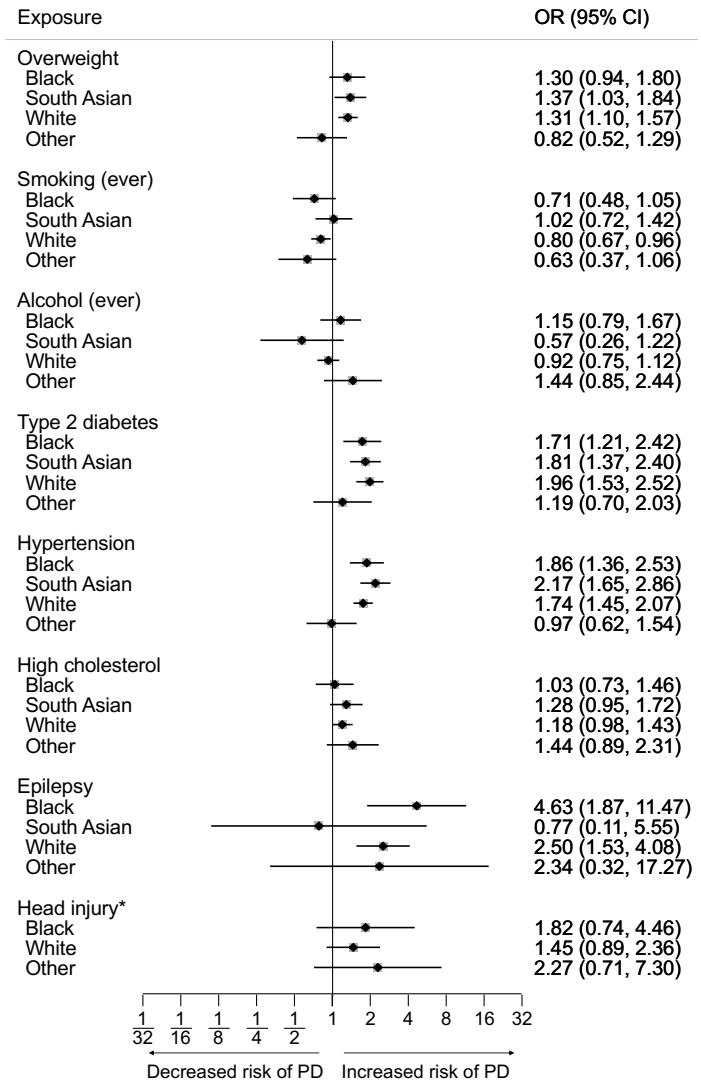

Figure A2.1 Forest plot depicting comorbidities and risk factors with Parkinson's disease across ethnic groups in the East London population. Data points represent odds ratios +/− 95% confidence intervals. Ethnic groups: White (British, Irish, Other White), Black (African, Caribbean, Other Black), South Asian (Bangladeshi, Indian, Pakistani), other (Chinese and mixed groups). *There was no reported head injury in the South Asian group

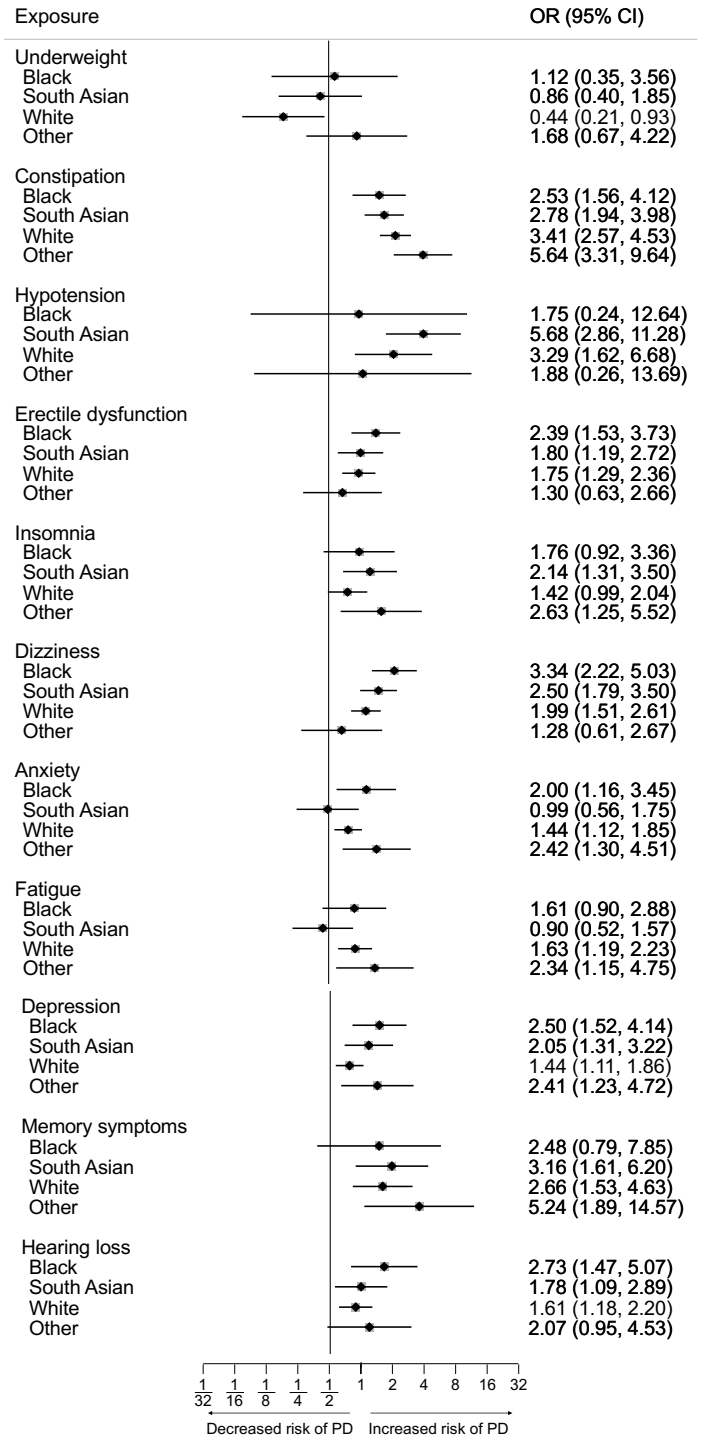

Figure A2.2 Forest plot depicting non-motor pre-diagnostic manifestations of Parkinson's disease across ethnic groups in the East London population. Data points represent odds ratios +/− 95% confidence intervals. Ethnic groups: White (British, Irish, Other White), Black (African, Caribbean, Other Black), South Asian (Bangladeshi, Indian, Pakistani), other (Chinese and mixed groups)

Appendix B

Replication analysis in Bruneck Study Cohort

Distribution of baseline enhanced PREDICT-PD risk scores and association with incident PS at follow-up (Buchman definition) without including motor performance as per UPDRS at baseline (and without SN echogenicity)

Follow-up	**N**	**Enhanced PREDICT-PD risk scores of PS-free subjects (n=312)**	**Enhanced PREDICT-PD risk scores of incident PS cases (n=63)**	**p-value [b]**	**OR with log10 risk scores (95% CI) [c] p-value**
0-5 years	375	1:394.4 (1:133.5 to 1:803.0)	1:129.4 (1:50.0 to 1:277.2)	< 0.001	2.46 (1.74 to 3.47); < 0.001

Abbreviations:
CI = confidence interval; OR = odds ratio; PD = Parkinson's Disease.
[a] Distribution of enhanced PREDICT-PD scores is given in median with 25th and 75th confidence interval.
[b] Significance level for distribution of risk score were calculated using Mann Whitney U Test, as data was not normally distributed.
[c] Binary logistic regression analysis of log 10 odds was used to calculate OR and 95% CI. OR are given for a 1unit change in log 10 risk scores.

Appendix C

Details of the studies in Chapter 4

Monitoring motor fluctuations

Methods

We evaluated the use of the DFT test in assessing motor fluctuations. To capture real-life motor fluctuations, assessments were carried out in patients' home. I invited patients to perform the DFT and BRAIN test and examined them following the MDS-UPDRS-III instructions. I organised assessments to coincide with the timings of patients' usual motor fluctuations and clinical impressions during home visits further confirmed patients' fluctuation states. I made the adjustments to the schedule where necessary (i.e. waiting for patients' medication effect to wear off in order to test the 'Off' state). I invited four patients to complete the DFT test asynchronously on further occasions at home, for longitudinal monitoring of motor fluctuations. As it was not possible for in-person corroboration of functional states, I invited patients to complete the test according to their subjective impressions – patients recognised 'On' state as when levodopa was effective and motor symptoms were controlled, and 'Off' state as when levodopa was ineffective and motor symptoms re-emerged.

I conducted paired t-tests and Wilcoxon matched-pairs signed rank tests using the best recorded 'On'/'Off' state to investigate whether the DFT test and MDS-UPDRS-III could differentiate between fluctuations. Further, I used mixed effect models to define the effects of 'On' and 'Off' state on each outcome measure (MDS-UDPRS-III finger tapping sub-score, KS20, AT20, IS20, KS30, AT30 and IS30). Each model included therapy state (2-levels: 'On' and 'Off' state) as a fixed effect and subject number and trial number as random effects. I set the significance level for all calculations as $p<0.0025$ (derived by Bonferroni calculation to reduce type 1 error). All data were analysed using GraphPad Prism version 8.0.2, IBM SPSS version 27 and Stata version 15.

Results

I recruited nine PD patients for monitoring motor complications (mean age in years ± SD: 62.78 ± 7.10, mean disease duration in years ± SD: 9.00 ± 5.52 and gender distribution: 5 male and 4 female patients). I excluded one patient from daytime monitoring with MDS-UPDRS-III analysis due to unexpected lack of fluctuations on the day of assessment. During the home visits, all patients had taken levodopa ≤12 hours (4.03 hours since their last levodopa dose on average) and achieved an 'On' state 1.17 hours on average after taking levodopa. Four patients with fluctuations agreed to carry out independent remote testing to monitor their daytime motor fluctuations.

The DFT test provided suggestive evidence for a difference between patients' subjective 'On' and 'Off' states using KS20 and IS20, although neither difference was statistically significant (KS20 'Off' – 62.78 (95% CI 50.06-75.50) vs KS20 'On' – 71.78 (95% CI (61.49-82.07), p=0.05; IS20 'Off' – 3452 (95% CI (1833-20178) vs IS20 'On' – 1232 (95% CI (845.3-10017), p=0.04. Table C4.1). Contrastingly, the finger tapping sub-score of the MDS-UPDRS-III showed no significant differences between their 'On' and 'Off' states (mean FT sub-score 'Off': 2 (95% CI 1.37-2.63) vs mean FT sub-score 'On': 1.5 (95% CI 0.73-2.27), p=0.10; Table C4.1).Further, mixed effect models were used to measure the effects of therapy (2-level fixed effect: 'On' and 'Off' state) on each motor outcome (test parameters and finger tapping sub-score). The effect of therapy was defined based on the variability of parameters across all trials (random effects). KS20 was found to have the strongest correlation with 'On' and 'Off' states, being almost 7 times higher in 'On' state compared with 'Off' state (coefficient=6.7, 95% CI 2.42- 11.09; Table C4.2). This result was in agreement with what is represented in Figure C4.1: amongst the four patients who completed the tapping tests more than twice, KS20 was found to be the most consistent parameter with subjective motor fluctuations (Figure C4.1). In patient 1, KS and AT scores from the DFT and BRAIN test fluctuated during the day and were in agreement with subjective 'On-Off' motor states. In patient 2, KS20 scores performed during 'On' states progressively decreased throughout the day, whilst remaining relatively constant across 'Off' periods. However, this pattern was not reflected in BRAIN test parameters. Of note, in patient 4, the KS20 score did not improve following the third levodopa dose, possibly reflecting an additional 'No On' or 'Delayed On', which again was not detected by the BRAIN test.

Table C4.1. Comparison DFT kinematic parameters between 'On' and 'Off' states

Parameter	PD 'Off'	PD 'On'	p-value
Mean KS20 in	62.78	71.78	0.05[a]
taps (95% CI)	(50.06, 75.50)	(61.49, 82.07)	
Mean AT20 in	155.0	153.1	0.88[a]
msec (95% CI)	(118.3, 191.7)	(120.6, 185.7)	
Median IS20 in	3452	1232	0.04[b]
msec² (IQR)	(1833, 20178)	(845.3, 10017)	
Mean MDS-UPDRS-FT	2	1.5	0.10[a]
(95% CI)	(1.37, 2.63)	(0.73, 2.27)	

KS20, kinesia score; AT20, akinesia time; IS20, incoordination score; CI, confidence interval; IQR, interquartile range. [a]Two-tailed paired t-test, [b]Wilcoxon matched-pairs signed rank test

Table C4.2 Mixed effect models examining the effect of motor fluctuation on outcome measures

Outcome measure	Coefficient	95% CI
KS20	6.76	2.42 – 11.09
KS30	2.28	-2.19 – 6.76
AT20	-7.54	-20.24 – 5.16
AT30	-5.78	-16.92 – 5.36
IT20	-3376.83	-7267.09 – 513.45
IT30	5801.40	-7809.68 – 19412.48
MDS-UPDRS-FT	-0.76	-1.07 – -0.46

KS20/30, kinesia score; AT20/30, akinesia time; IS20/30, incoordination score; CI, confidence interval. Mixed effect model coefficient. Fixed effect: 'On' and 'Off' state. Random effect: number of trials.

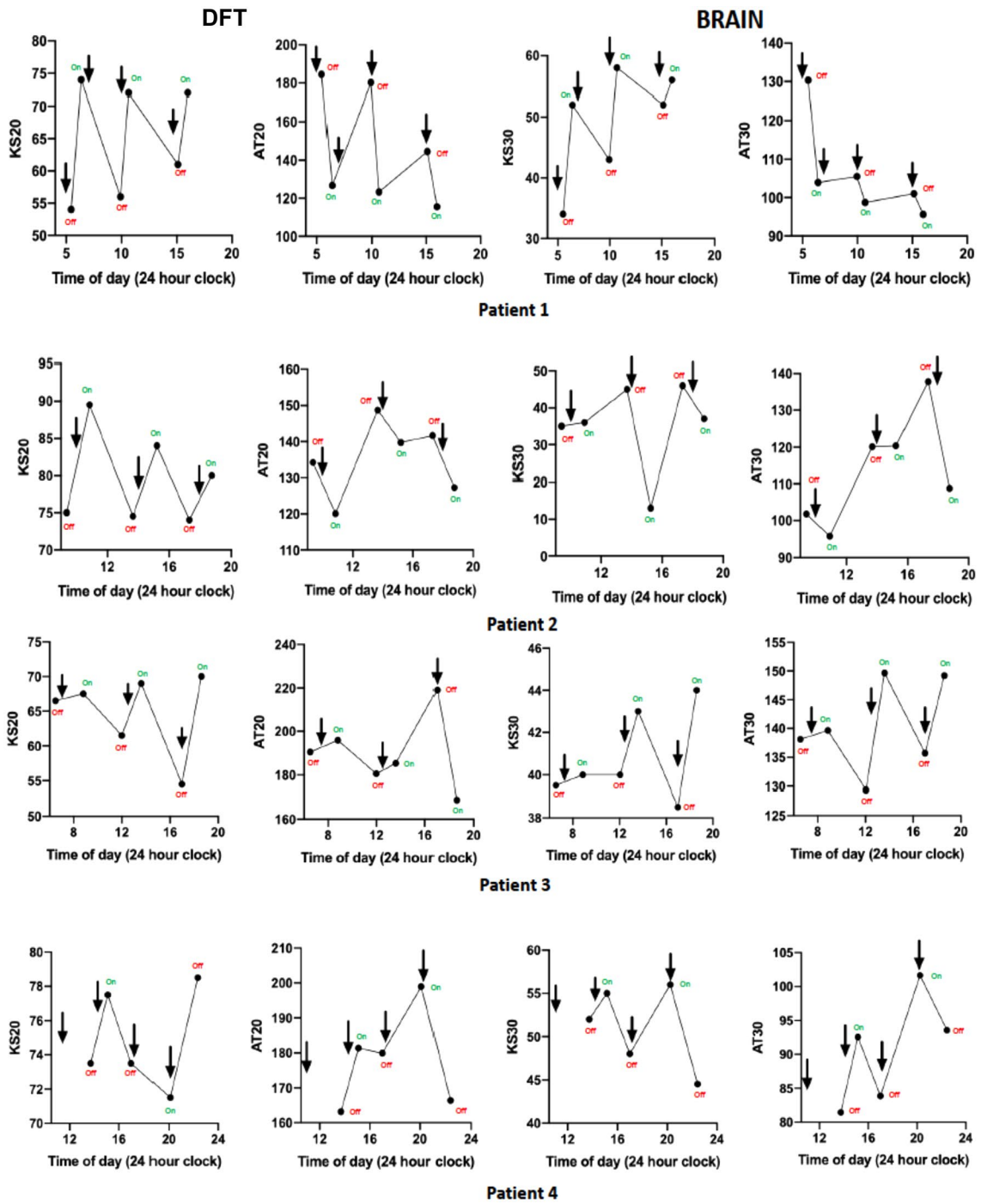

Figure C4.1 Repeat testing in 4 PD patients with predictable motor fluctuations using the DFT and BRAIN test. Dots represent when the test was completed, and arrows denote the time when levodopa was taken. KS20 (DFT test) and KS30 (BRAIN test) scores are expected to increase in the 'On' state, whereas AT20 and AT30 scores are expected to decrease in the 'On' state.

Discussion

In contrast to MDS-UPDRS finger tapping sub-scores, the DFT test showed promise in detecting motor fluctuations. The DFT demonstrated a better correlation with subjective motor fluctuations than the BRAIN test, suggesting that distal motor impairment might have a stronger impact on patients' quality of life. This also reinforces the idea that distal and proximal movements may be differentially affected in PD.[1-4] Whilst these findings are exploratory, both tapping tests show potential in objectively capturing daily symptom oscillations. They may provide clinicians with a clearer understanding of patients' subjective interpretations of fluctuation states, enabling individualised tailoring of management plans. These findings warrant further analysis, it may be worth examining how well patients' subjective impressions correlate with these objective measures.

In terms of limitations, 12-hours washout of levodopa was not implemented due to ethical considerations of disabling 'Off' state complications. Existing literature also notes that patients can experience prolonged motor improvement following levodopa, due to 'long-duration response to levodopa (LDR)', thus rendering overnight withdrawal unreliable.[5] A further limitation faced by the DFT as a longitudinal monitoring tool, is the potential for confounding factors such as mood and alertness to influence patients' subjective interpretations of 'On' and 'Off' states, as opposed to it being based solely on motor function. Although it was not possible to control these external factors, the results aimed to represent the 'real-life' situation of remote monitoring in patients.

Future directions for the DFT test include assessing it in combination with the BRAIN test as a form of remote longitudinal monitoring of patients' upper-limb function, which may help to facilitate treatment adjustments.

To conclude, the DFT test offers a remote and objective method of capturing distal upper-limb function. Further work is warranted to validate the DFT test as a supplementary clinical tool for diagnosis and remote monitoring of PD motor complications.

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Table C4.3 Analysis of characteristics that influence SMART test amplitude, frequency and velocity in controls

		Amplitude			Frequency			Velocity		
		Mean	**CV**	**Slope**	**Mean**	**CV**	**Slope**	**Mean**	**CV**	**Slope**
Mean Age	63.81 years	0.05	-0.18	0.18	-0.26	0.04	-0.27	-0.06	-0.03	-0.04
	70.0 years	0.03	-0.01	0.13	-0.40*	0.09	-0.11	-0.45*	-0.12	-0.02
Gender										
- Female	19	31.22	0.19	-0.31	2.95	0.11	0.0002	1.54	0.33	-0.02
- Male	11	30.90	0.18	-0.61	3.59	0.12	-0.0065	1.77	0.27	-0.03
Handedness										
- Dominant	30	31.80	0.18	-0.33	3.12	0.14	0.001	1.66	-0.06	-0.03
- Nondominant	30	30.25	0.17	-0.46	3.10	0.11	-0.015*	1.50	-0.05	-0.02

Mean and medians given except for associations with age where Spearman correlation coefficient (r) is given. Comparison between gender and handedness were analysed using Welch's t-tests (two-tailed). All p-value were not significant ($p>0.005$) except for slope frequency* between dominant and non-dominant hand ($p=0.001$)

Table C4.4 Correlation between SMART test parameters and MDS-UPDRS-III FT sub-scores

PD cases (n=24) *	**Spearman correlation with FT sub-score**	**p-value**
Amplitude		
Mean	-0.49 (-0.81 to -0.17)	0.003
CV	-0.07 (-0.36 to 0.49)	0.758
Slope	0.44 (0.07 to 0.81)	0.018
Frequency		
Mean	0.175 (-0.24 to 0.59)	0.414
CV	0.25 (-0.16 to 0.66)	0.239
Slope	-0.16 (-0.58 to 0.25)	0.443
Velocity		
Mean	-0.43 (-0.78 to -0.08)	0.016
CV	0.44 (0.09 to 0.79)	0.013
Slope	0.34 (0.05 to 0.74)	0.087

All parameters presented with 95% coefficient interval (CI). FT: Finger tapping UPDRS: Unified Parkinson's disease rating scale, CV: coefficient variation. * 2 cases scored 0 and 3. They were excluded since they might influence the regression.

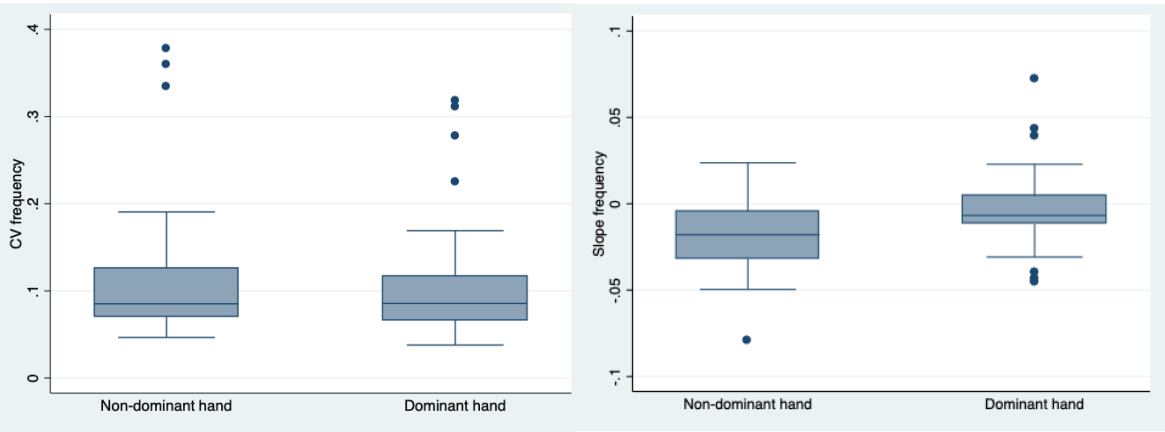

Figure C4.2 Non-dominant vs dominant hand comparison in CV frequency (left boxplot) and slope frequency (right boxplot)

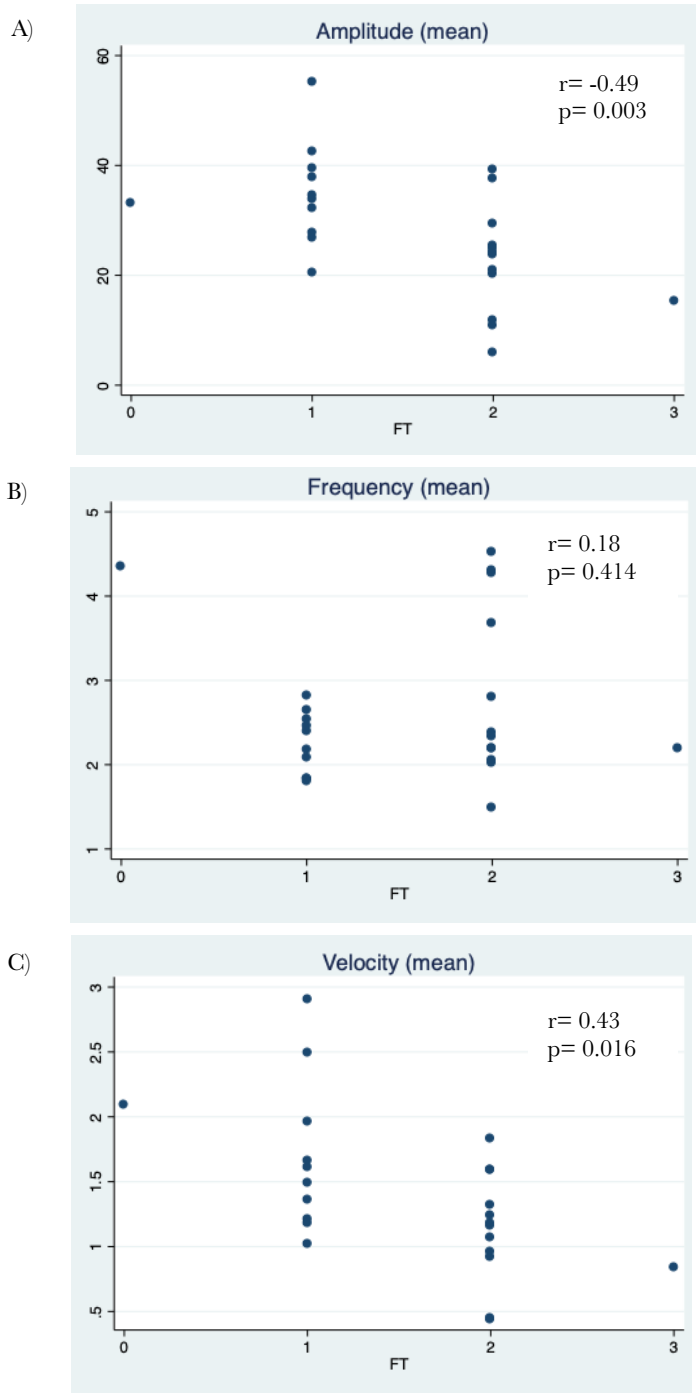

Figure C4.3 Correlation between SMART test parameters and finger-tapping sub-score from MDS-UPDRS-III: 0 (normal), 1 (slight), 2 (mild), 3 (moderate). There were no PD cases scoring 4 (severe). Evidence of floor effect (wide range of SMART test performance) between score 1 and 2.

Appendix D

Details of the study presented in Chapter 5

Table D5.1 Dominant vs non-dominant iRBD hand performance

	Dominant hand (n=33)	Non-dominant hand (n=33)	p-value
BRAIN-KS (taps/30sec), mean (SD)	56.61 (14.29)	49.45 (15.19)	<0.001
DFT-KS (taps/20sec), mean (SD)	87.33 (23.39)	83.26 (15.76)	0.364
BRAIN-AT (msec/30sec), mean (SD)	98.13 (37.65)	131.43 (50.56)	<0.001
DFT-AT (msec/20sec), mean (SD)	99.60 (24.94)	110.70 (27.56)	0.111
BRAIN-IS ($msec^2$/30sec), mean (SD)	8575.80 (7525.54)	9282.65 (9513.64)	0.711
DFT-IS ($msec^2$/20sec), mean (SD)	2802.17 (5135.73)	4477.08 (6574.36)	0.285

SD: standard deviation, KS: kinesia score (number of taps per 30/20 seconds), AT: average dwell time (msec) that keys are depressed reflecting akinesia, IS: variance ($msec^2$) of travelling time between keystrokes reflecting rhythm.

Table D5.2 Dominant vs non-dominant control hand performance

	Dominant hand (n=29)	Non-dominant hand (n=29)	p-value
BRAIN-KS (taps/30sec), mean (SD)	68.34 (9.66)	61.03 (9.98)	<0.001
DFT-KS (taps/20sec), mean (SD)	99.79 (10.33)	90.59 (11.62)	<0.001
BRAIN-AT (msec/30sec), mean (SD)	80.03 (16.71)	109.86 (25.89)	<0.001
DFT-AT (msec/20sec), mean (SD)	88.82 (22.09)	102.64 (23.03)	<0.001
BRAIN-IS ($msec^2$/30sec), mean (SD)	3200.86 (2939.28)	3072.42 (2126.76)	0.833
DFT-IS ($msec^2$/20sec), mean (SD)	853.61 (1319.54)	1367.52 (1602.68)	0.109

SD: standard deviation, KS: kinesia score (number of taps per 30/20 seconds), AT: average dwell time (msec) that keys are depressed reflecting akinesia, IS: variance ($msec^2$) of travelling time between keystrokes reflecting rhythm.

Table D5.3 Comparison of DFT kinetic parameters between dominant hand and corresponding ROC analysis

	Mean KS-DFT (SD)	**Mean AT-DFT (SD)**	**Median IS-DFT (SD)**
iRBD (n=33)	87.33 (23.39)	99.60 (24.94)	1095.33 (370.98 to 2951.68)
Controls	99.79 (10.33)	88.82 (22.09)	361.66 (256.84 to 853.01)
p-value	0.020[a]	0.017[a]	0.021[b]
	KS-DFT Sensitivity	**AT-DFT Sensitivity**	**IS-DFT Sensitivity**
Specificity 90% (cut-off)	33.3% (85)	9.1% (125.7)	48.5% (1244.7)
Specificity 85% (cut-off)	36.4% (88)	33.3% (111.1)	48.5% (1184.0)
Specificity 80% (cut-off)	39.4% (90)	41.4% (103.8)	54.5% (894.8)
Best combination (cut-off)	60.6% sensitivity for 55.2% specificity (96)	69.7% sensitivity for 62.1 specificity (88.3)	72.7% sensitivity for 51.7% specificity (393.8)
Area under curve the ROC curve (95% CI)	0.65 (0.51 to 0.80)	0.66 (0.52 to 0.80)	0.65 (0.50 to 0.79)

KS, kinesia score; AT, akinesia time; IS, incoordination score; CI, confidence interval; IQR, interquartile range; SD, standard deviation; a) Welch's test for unequal variances; b) Mann-Whitney test. ROC, Receiver Operating Characteristic.

Table D5.4 Comparison of BRAIN kinetic parameters dominant hands and corresponding ROC analysis

	Mean KS-BRAIN (SD)	**Mean AT-BRAIN (SD)**	**Median IS-BRAIN (IQR)**
iRBD (n=33)	56.61 (14.30)	98.13 (37.65)	6262.03 (3945.31 to 11549.02)
Controls	68.34 (9.66)	80.03 (16.71)	2049.33 (1250.30 to 3945.9)
p-value	<0.001[a]	0.008[a]	<0.001[b]
	KS-BRAIN Sensitivity	**AT-BRAIN Sensitivity**	**IS-BRAIN Sensitivity**
Specificity 90% (cut-off)	57.6% (57)	27.3% (110.5)	33.3% (8005.0)
Specificity 85% (cut-off)	57.6% (58)	33.3% (101.2)	51.5% (6207)
Specificity 80% (cut-off)	60.6% (60)	39.4% (95.7)	60.6% (4616.1)
Best combination (cut-off)	69.7% sensitivity for 62.1% specificity (65)	66.7% sensitivity for 62.1% specificity (79.60)	75.8% sensitivity for 72.4% specificity (3945)
Area under curve the ROC curve (95% CI)	0.75 (0.62 to 0.88)	0.65 (0.51 to 0.79)	0.78 (0.66 to 0.89)

KS, kinesia score; AT, akinesia time; IS, incoordination score; CI, confidence interval; IQR, interquartile range; SD, standard deviation; a) Welch's test for unequal variances; b) Mann-Whitney test. ROC,

Appendix E

Risk disclosure questionnaire

"What, when and how should information on iRBD and risk of Parkinson's and related conditions be communicated?"

We would like to ask you some questions about what you were told at the time of your diagnosis and how you were told.

1. Did your doctor give you information about the link of iRBD with other conditions, including developing a neurodegenerative condition (such as Parkinson's disease) in the future?
   - Yes
   - No
   - I don't know/remember
2. If yes, did your doctor ask what you wanted to know prior to giving this information?
   - Yes
   - No
   - I don't know/remember
3. Were you provided with patient information leaflets, websites or other sources of information to know more about the links between iRBD and neurodegenerative conditions?
   - Yes
   - No
   - I don't know/remember
4. Apart from the information provided (if this was given), did you search for information about iRBD on the internet?
   - Yes
   - No
   - I don't know/remember

Now we would like to ask you some questions about whether your preference would have been different to what happened (i.e. what, when and how would you have liked to be told?)

5. Did you want to have information regarding the potential future implications of having iRBD at that time?
   - Yes
   - No
   - I don't know

If the answer is "No", please go to question 6.

If the answer is "Yes", please go to questions 7, 8 and 9.

6. What were the reasons why you did NOT want to know about the potential future implications of iRBD at that time? (mark all that apply)

- It is my right to NOT know, as I am the patient
- If there is nothing that I can do about what may happen in the future, I'd rather not know
- I would have liked to know, but at a later date - please give detail
- I would have liked to know, but be told differently - please give detail
- Other reasons [free text]

7. What were the reasons that you did WANT to know about the potential future implications of iRBD? (mark all that apply)

- It is my right to know, as I am the patient
- I wanted to have all the information so that I could make informed decisions for the future
- Even if there is nothing that I can do about what may happen in the future, I would be interested in participating in research related to iRBD
- Other reasons [free text]

8. From where would you have liked to receive the information about the potential future implications of iRBD? (mark all that apply)

- By the doctor diagnosing and treating my iRBD
- By my GP
- With information in a patient information leaflet
- With information online from scientific and patients' associations websites

9. Did you have a preference regarding when to be told about the potential future implications of iRBD?

- No.
- Yes, from the moment I received the diagnosis of iRBD.
- Yes, when my doctor detected initial signs of parkinsonism.
- Yes, but later in life, if treatments to reduce or stop the risk to develop Parkinson's and related conditions became available.