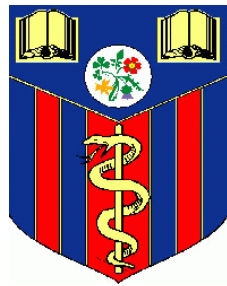


Identifying Subjects At Risk of Parkinson's Disease in the Community: PREDICT-PD



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Thesis submitted in fulfilment of the degree of
Doctor of Philosophy

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Dedicated to my newborn son, Oliver Aria John Noyce,
and in loving memory of my grandfather, John William White.

Declaration

I, Alastair John Noyce confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Alastair John Noyce
February 2016

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Details of collaborative work

Collaborative work was undertaken as indicated below by chapter:

- Chapter 2 - AJN performed the literature review; AJN and AS extracted data; JB performed the statistical analysis; CK guided the question selection for constipation.
- Chapter 4 - JB performed multilevel mixed effects linear regression and calculated coefficients of variation; AJN performed the remainder of the statistical analysis.
- Chapter 5 - JB derived the equation for the effect of age on PD; statistical analysis was performed by AJN and JB.
- Chapter 6 - AJN and NM performed the experiments together.
- Chapter 7 - JD calculated binding ratios and undertook analysis between PREDICT-PD participants and reference controls; AJN performed the remainder of the statistical analysis.
- Chapter 8 - AJN and JB performed the statistical analysis.

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1. PREDICT-PD: Identifying risk of Parkinson's disease in the community: methods and baseline results. Noyce AJ, Bestwick JP, Silveira-Moriyama L, et al. *J Neurol Neurosurg Psychiatry* 2014;85(1):31-37.
2. Bradykinesia-akinesia incoordination test: validating an online keyboard test of upper limb function. Noyce AJ, Nagy A, Acharya S, et al. *PLoS One* Apr 29;9(8):e105488.
3. Web-based assessment of Parkinson's prodromal markers identifies GBA variants. Noyce AJ, Mencacci NE, Schrag A et al. *Mov Disord* 2015;30(7):1002-1003.

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1. Bone health in chronic neurological disease: a focus on Multiple sclerosis and Parkinsonian syndromes. Dobson R, Yarnall A, Noyce AJ, Giovannoni G. *Pract Neurol* 2013;13(2):70-79.
2. Dopamine agonists rather than deep brain stimulation cause reflection impulsivity in Parkinson's disease. Djamshidian A, O'Sullivan SS, Foltynie T, et al. *J Parkinsons Dis* 2013;3(2):139-144.
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Abstract

There has been great interest in a definable prodromal period of Parkinson's disease (PD), which is thought to be characterised by non-motor manifestations. In preparatory work, an extensive review of early non-motor features and risk factors was undertaken to develop a preliminary algorithm to identify subjects at increased risk of PD. A website was configured and keyboard-tapping test developed to aid in risk-stratifying subjects for future PD.

This thesis first documents the validation of the keyboard-tapping test in PD patients and healthy controls, before its use alongside objective smell testing and a questionnaire formulated to assess early non-motor features and risk factors, all of which were delivered via the internet. The thesis describes the recruitment at baseline of over 1,300 healthy older people and annual follow-up assessments with the questionnaire, smell test and tapping test, which comprise the preliminary screening algorithm. Each year those estimated to be at higher risk were compared to lower risk subjects in terms of intermediate markers (smell loss, sleep disturbance and finger tapping speed) and differences between extremes of risk have been observed, consistent with the notion that higher risk subjects possess early features of PD. Selected higher and lower risk subjects were further investigated to determine whether there were differences in the frequency of genes associated with PD (*GBA* and *LRRK2*), and a proportion of subjects have been scanned using transcranial sonography and ^{123}I -FP-CIT SPECT to determine whether there were imaging differences between extremes of risk. The thesis concludes by demonstrating that higher risk subjects were more likely to be diagnosed with PD during follow-up over 3 years and proposes further lines of enquiry that can be followed, building on the work undertaken to-date.

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List of abbreviations

ASCII - American Standard Code for Information Interchange

AUC - Area Under Curve

BR - Binding Ratio

BRAIN test - BRadykinesia Akinesia INcoordination test

CCBs - Calcium Channel Blockers

CI - Confidence Interval

cm - centimetre

DaT - Dopamine Transporter

DLB - Dementia with Lewy Bodies

DMV - Dorsal Motor Nuclear Complex of Vagus

ED - Erectile Dysfunction

EDS - Excessive Daytime Somnolence

GBA - Glucosidase Beta Acid (also known as Glucocerebrosidase)

GWAS - Genome Wide Association Study

HAAS - Honolulu Asia Aging Study

HHP - Honolulu Heart Programme

HPFS - Health Professionals Follow-up Study

ICH - Intracranial Haemorrhage

ILB - Incidental Lewy Bodies

IQR - Interquartile Range

LRKK2 - Leucine Rich Repeat Kinase 2

List of abbreviations

MDS - Movement Disorders Society

MPTP - 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

msec - millisecond(s)

MSA - Multiple System Atrophy

NHNN - National Hospital for Neurology and Neurosurgery

NSAIDs - Non-Steroidal Anti-Inflammatory Drugs

OR - Odds Ratio

PARS - Parkinson's At-Risk Study

PD - Parkinson's disease

PPMI - Parkinson's Progression Markers Initiative

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

PPV - Positive Predictive Value

PSG - Polysomnography

RBD - Rapid Eye Movement (REM) sleep Behaviour Disorder

RCT - Randomised Controlled Trial

ROC - Receiver Operator Characteristic

RR - Relative Risk

SBR - Striatal Binding Ratio

SD - Standard Deviation

SN - Substantia Nigra

SPECT - Single Photon Emission Computed Tomography

SSL - Secure Sockets Layer

TCS - Transcranial Sonography

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

VOI - Volume Of Interest

Chapter 1

Introduction

Parkinson's disease (PD) is the most common degenerative movement disorder. It has a lifetime prevalence of 0.2%, which increases significantly with age.^{1,2} It is diagnosed clinically, upon an appropriate history and observing bradykinesia, which must be present along with either rigidity or tremor.³ Bradykinesia describes the sequence effect of reducing rate and/or amplitude with repetitive movements such as finger tapping. The classic PD tremor occurs at rest, rigidity occurs through the full range of movement, and the motor features persist asymmetrically in almost all cases.

Motor features of PD may emerge relatively late in the disease process when at least 50-60% of dopaminergic neurons have been lost in the substantia nigra (Figure 1.1).⁴ However, there is substantial heterogeneity in the clinical manifestations, even soon after diagnosis.⁵ Symptomatic treatment is efficacious, but there are currently no drugs that demonstrably slow the disease course and there is no cure. Progressive cell loss leads to increasing physical disability, and often cognitive impairment, with treatment failing to provide adequate control in the advanced stages. It is believed, albeit not proven, that disease may be too far advanced at the point of clinical diagnosis to be affected by potentially neuroprotective treatments (assuming that these were available).⁶ In recent years, there has been great interest in the prodromes of PD, which may precede overt motor features.^{7,8} Associations between a number of clinical entities and a subsequent diagnosis of PD have been established through observational study. Infrequent bowel opening or constipation, loss of smell sense, anxiety and depression, sleep disorders, not smoking and not drinking coffee, have all been consistently associated with PD. Most of these, in isolation, are also found commonly in the older adult population, but in combination could perhaps 'predict' individuals at risk of PD. However, the temporality and magnitude of effect of association with PD differs for each of these early features.

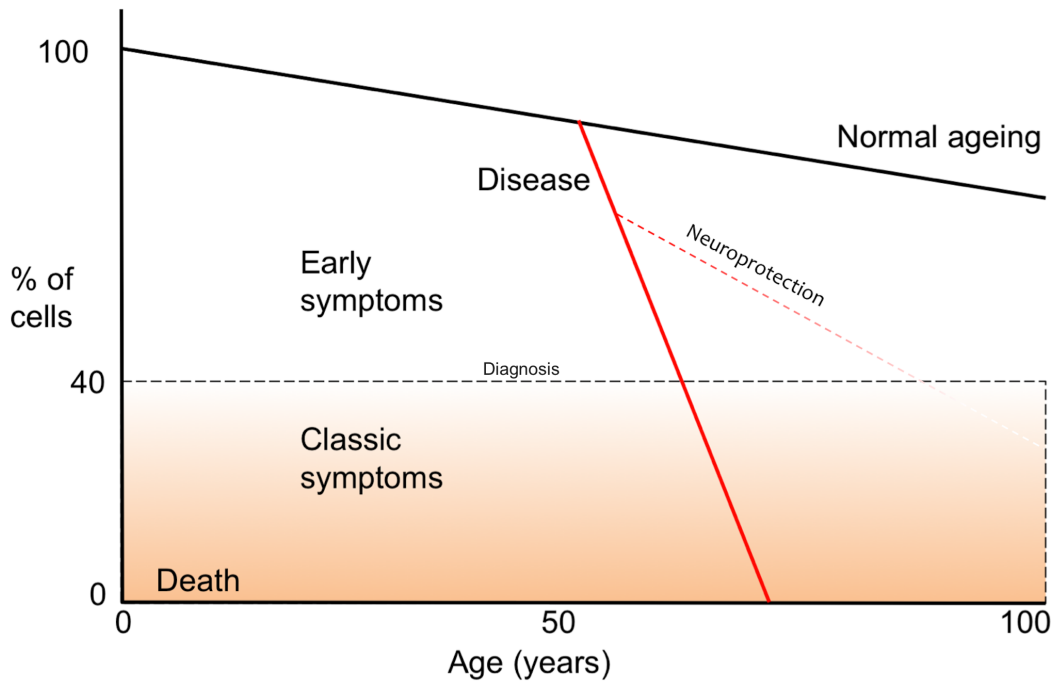


Fig. 1.1: A schematic depicting normal (black solid line) and PD-related (red solid line) nigral cell loss over time, including the point at which diagnosis typically occurs (hashed black line) and the potential for modifying the trajectory of degeneration, if identified earlier (hashed red line).

The pathological hallmark of PD is intra-neuronal inclusions (Lewy bodies) or linear deposits (Lewy neurites), which stain positive for alpha-synuclein.³ Braak and colleagues proposed the now widely accepted pathological staging system for PD, which suggested that Lewy pathology occurs in discrete areas of the brain before progressing to involve the basal ganglia.⁹ The progression of PD can be approximately clinically and pathologically correlated to the six stages described by Braak and colleagues, although there are many exceptions.

Stage 1 marks the onset of the disease process and involves the anterior olfactory nucleus, the olfactory bulbs and the dorsal motor nuclear complex of cranial nerves IX and X (DMV).⁹ Olfactory and autonomic dysfunction have frequently been reported as early non-motor features of PD. Separately, alpha-synuclein deposits have also been found in gastrointestinal neuronal tissue in patients with established PD and in subjects that underwent bowel biopsy pre-morbidly and were subsequently diagnosed with PD.^{10,11,12}

Braak stage 2 involves the locus coeruleus and subcoeruleus complex, the magnocellular area of the reticular formation and posterior raphe nucleus.⁹ Disease involvement in these areas could account for recognised sleep and mood disorders, including anxiety and depression, which have been reported to antedate motor disease. Stage 3 involves structures including the substantia nigra and amygdala, which may correspond with the onset of the classical motor features of PD. Stages

4 to 6 are defined by progressive involvement of cortical structures, perhaps accounting for the later features such as memory impairment, visual hallucinations and other visual disturbances, change in personality, and overt dementia.

Identifying individuals at the earliest stages of disease may enable clinical trials of emerging and repurposed drugs with the aim of preventing/delaying progression to clinically overt PD at a time when neuronal loss is not too far advanced (Figure 1.1). However, modifying risk in those that do not yet have a diagnosis represents a challenge. The terms ‘early disease’ or ‘at-risk’ may be used synonymously due to uncertainty about the point at which the pathological process starts, and there remains a lack of sufficiently validated biomarkers that may distinguish the two. Clarification is important since it will determine whether prevention is attempted on a primary or secondary basis, and the factors that initiate the pathological process may not necessarily be the same as those that subsequently drive progression.

1.1 Identification of individuals ‘at-risk’ of PD

1.1.1 Genetic risk factors

Understanding of the role that genes play in the pathogenesis of PD is growing. Approximately 11% of patients with PD have a first degree relative with PD, which is significantly higher than the frequency in age-matched control populations (approximately 5%).¹³ Mutations in a number of genes have been identified as causal for PD including those in alpha-synuclein (*SNCA*), leucine-rich repeat kinase 2 (*LRRK2*), parkin (*PARK2*), oncogene DJ1 (*DJ1*) and PTEN-induced putative kinase 1 (*PINK1*). Most result in early-onset disease and follow Mendelian inheritance patterns. *PARK2*, *DJ1* and *PINK1* are inherited in a recessive fashion, and *SNCA* and *LRRK2* are dominant.^{14,15} Study into the role of these genes implicates lysosomal and mitochondrial dysfunction, as well as inflammation in pathogenesis.^{16,17} Of the confirmed monogenic forms of PD, most result in abnormalities of one or more of these processes, but are rare and do not account for elevated risk at a population level (Figure 1.2).¹⁴ A key factor is alpha-synuclein, both in sporadic disease and monogenic forms due to mutations in *SNCA* (and potentially other genes). As well as forming the basis of Lewy pathology in an aggregated form, mounting evidence suggests that oligomeric forms of alpha-synuclein may be neurotoxic.^{3,18,19} The full picture of how these complex processes combine to result in neurodegeneration remains incomplete, but in addition to the above mechanisms, current theories include the possibility of prion-like cell-to-cell propagation.²⁰

LRRK2

Mutations in the *LRRK2* gene are the commonest known genetic cause for PD and the G2019S mutation occurs in 4% of hereditary and 1% of sporadic PD.²¹ *LRRK2*-related PD demonstrates age-dependent penetrance (28% have PD at 59 years, 51% at 69 years and 74% at 79 years), meaning that only a proportion of carriers will develop PD during life.²¹ The function of *LRRK2* (and its protein product Dardarin) is yet to be fully determined. It exists predominantly in the cytosol, but approximately 10% associates with the mitochondrial membrane, leading to the notion that pathogenicity may arise through mitochondrial dysfunction.^{22,23} *LRRK2* mutations result in heterogeneous pathology. The type of pathology is somewhat dependent on the actual mutation that has occurred, and includes alpha synuclein-containing Lewy bodies or neurites, neuronal degeneration and tauopathy.²⁴

The *LRRK2* consortium reported that incidence varies according to ethnicity (highest in north African Arabs and Ashkenazi Jews), and that the phenotype found in *LRRK2*-positive PD can be clinically indistinguishable from sporadic PD.²¹ *LRRK2* mutation carriers have been shown to have subclinical dopaminergic abnormalities, measured with functional imaging.²⁵ Parkinsonian carriers of the G2019S mutation have olfactory impairment comparable to that of PD in some, but not all series.^{26,27}

The relatively high frequency of *LRRK2* mutations makes it attractive to consider for pre-symptomatic case detection and recruitment to neuroprotective clinical trials. The high frequency of G2019S mutations in the UK is fortunate because *LRRK2* is a large gene, making it difficult and costly to screen for all known mutations. Genetic testing for *LRRK2* in the clinical setting is an area of controversy. In the absence of effective therapy, the main benefit currently is in improving diagnostic accuracy in those manifesting symptoms.²⁸

GBA

Gaucher's disease (GD) is the most common lysosomal storage disorder and results from a deficiency in the enzyme glucocerebrosidase. It follows Mendelian recessive inheritance patterns and is most commonly found in Ashkenazi Jews. Phenotypically, GD is divided into three types according to degree of neurological involvement. Type 1 is a mild form, whilst types 3 and 2 (especially) are more severe and are typically associated with neurological sequelae in early life. All result from mutations in the *GBA* gene, which is located on the long arm of chromosome 1.

1.1 Identification of individuals ‘at-risk’ of PD

An association between type 1 (typically non-neuronopathic) GD and early-onset parkinsonism was noted in reports of GD patients who began to manifest clinical features of PD.²⁹ In light of these observations, studies were initiated to identify whether an excess frequency of *GBA* mutations could be found in PD patients.³⁰ Heterozygous mutations in the *GBA* gene were associated with an increased odds of PD (odds ratio (OR) 5.43; 95% confidence interval (CI) 3.89 to 7.57) in a large multi-centre study.³¹ The same study showed that *GBA* mutations were particularly common in Ashkenazi Jews with PD, occurring in 15% of patients and 3% of controls. Whereas in unselected PD patients, 3.5% carry disease-associated *GBA* mutations compared to <1% of controls.^{31,32} Patients with *GBA* mutations tend to have earlier onset of parkinsonism and higher rates of cognitive impairment, but generally respond well to levodopa.^{32,33}

The study of manifesting and non-manifesting carriers of *LRRK2* and *GBA* mutations is important for understanding the prodromal phase of PD and for studies of drugs targeting specific pathways in the disease. Cohorts of these subjects have been (or are being) assembled to fulfil these aims.³⁴ The other causes of monogenic PD listed previously, are probably too rare to base predictive studies on, but continue to give important insight into disease mechanisms and therapeutic targets.

Lower risk genetic variants

Mutations in single genes do not account for all the heritable risk apparent in complex diseases, and genome-wide association studies (GWAS), in which large numbers of unrelated cases are compared to unrelated controls, have yielded informative results.³⁵ There are at least 28 independent risk genetic variants associated with PD that increase or decrease risk in a small but potentially additive way.³⁶ Many of these can be linked to putative disease mechanisms or are supported by the findings of candidate gene studies in PD and other neurodegenerative diseases, which increases confidence that the identified associations are real. The effect size estimates for individual gene variants are too small to power predictive studies, but recently polygenic risk profiles have been constructed by pooling the combined effect of multiple variants to estimate risk of PD, or indeed age of disease onset.³⁷

The heritable component of PD is estimated to be greater still, at around 30%, and identified risk loci and monogenic forms account for only about 5-10%.^{14,38} Over time, with increasing numbers of studied cases and controls, along with deep re-sequencing and precision phenotyping, a greater proportion of the heritability of PD will be uncovered. The influence that genetic variation has on PD is not limited to risk of getting disease alone, and specific variants are likely to contribute

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additionally to age of disease onset, progression and phenotype, with a number of indicative studies reported thus far.^{32,39,40} Furthermore, these and additional genetic factors may dictate therapeutic choices in the future both in the clinical setting and in recruitment to clinical trials. The genetic architecture of PD is continually expanding and increasingly complex. It has implications for multiple aspects of the disease, but other factors are also important in determining risk.

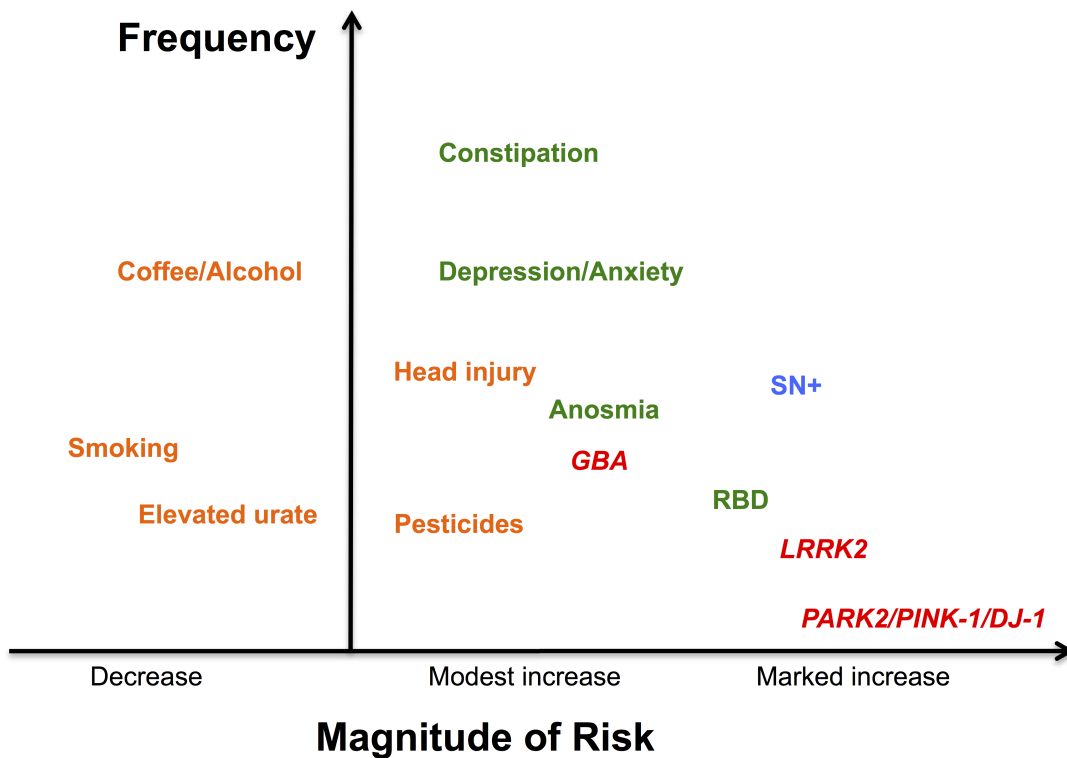


Fig. 1.2: Risk factors and early features of PD associated with increased (or decreased) risk of subsequent diagnosis. In the diagram, estimated magnitude of effect is plotted against estimated frequency.

Legend: SN+ is hyperechogenicity in the region of the substantia nigra using transcranial sonography. Risk factors are shown in orange, early non-motor features in green, genetic risk factors in red and imaging risk factors in blue.

1.1.2 Environmental risk factors

There is a large body of evidence demonstrating small but significantly elevated risk of PD associated with a number of environmental risk factors (Figure 1.2). Cohorts have been assembled to study the role of environmental factors in PD, as well as their interaction with genetic factors, including the Parkinson's Environment and Gene (PEG) study, the Agricultural Health Study, the PAQUID study, and the Geoparkinson Study.^{41,42,43,44} Environmental factors and associations with PD have been studied in larger community-based cohorts not exclusively focussed on

1.1 Identification of individuals ‘at-risk’ of PD

PD such as the Honolulu Asia Aging Study (HAAS) and the Cancer Prevention Study II Nutrition Cohort.^{45,46}

Some of the strongest evidence exists for pesticide exposure and proxies for this including farming occupation, rural living and well water drinking.⁴⁷ Specific pesticides implicated include rotenone and paraquat (structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which has also been linked to parkinsonism in illicit drug users⁴⁸), and both of these chemicals are used to create animal models of PD. Other potential toxins include heavy metals such as manganese, with exposure arising through occupations such as welding and in recreational ephedrone users.⁴⁹ It seems unlikely that environmental toxins play more than a minor role in PD risk overall, with most observational studies suggesting odds ratios in the region of 1.2-2.0.

Observational study data implicate head injury as a minor but significant risk factor for PD.⁵⁰ There is increasing evidence that individuals who suffer recurrent head injury, particularly sportspersons such as boxers, jockeys, American football and rugby players, are at risk of developing a range of degenerative neurological conditions including parkinsonism, dementia and motor neurone disease, although pathological examination of these subjects tends to reveal alternative pathology to that typically associated with PD.⁵¹

In stark contrast to other common chronic diseases, there exist a number of intriguing but consistent negative associations with PD and lifestyle factors such as smoking, caffeine and alcohol. The inverse relationship between cigarette smoking and PD was first recognised over 50 years ago, with multiple studies since confirming this association.⁵² A meta-analysis of the inverse relationship between smoking and caffeine consumption and PD, analysed results from 44 case-control studies and 4 cohort studies of smoking and PD, and 8 case-control and 5 cohort studies of coffee and PD. It concluded that the pooled relative risk (RR) for ‘ever’ versus ‘never’ smokers was 0.59 (95% CI 0.54 to 0.63) and that the risk of PD decreased as number of pack years increased. Furthermore it found that, compared with non-coffee drinkers, the pooled RR for PD in coffee drinkers was 0.69 (95% CI 0.59 to 0.80) and that each additional cup of coffee per day was associated with a 10% risk reduction.⁵³ Since this meta-analysis was undertaken a large number of additional observational studies have been published.

Whether these exposures offer true neuroprotective properties or whether negative association, at least with lifestyle factors, arises due to a common feature (e.g. avoidance as part of an early PD personality change) is yet to be determined. A true neuroprotective effect is supported by clinical studies reporting improvement of motor function in a clinical trial of caffeine to treat excessive daytime somnolence (EDS) in PD, improvement of dyskinesia with nicotine, lack of smoking-related

decline in olfaction in PD subjects, and PD animal models that show protective effects of nicotine on nigrostriatal damage.^{54,55,56,57} Spurious negative associations may arise as a result of a common problem with observational studies, which is reverse causality or confounding by prevalent disease. This may be plausible even in prospective studies that exclude cases of incident PD in the first few years of follow-up, because the prodromes of PD are likely to be very long, during which time the disease may be active, but the classical features not yet evident.

There are a number of drugs for which negative associations with PD have been reported in observational studies, including calcium channel blockers, non-steroidal anti-inflammatories and statins, and clinical trials to explore repurposing of some of these agents are underway.^{58,59,60} Another consistent negative association exists between levels of serum urate and PD, with a number of studies demonstrating a protective effect of elevated serum urate against PD.^{61,62,63} Given the consistency of this relationship, therapeutic alteration of urate levels is also a strong target for clinical trials.

The emergence and further characterisation of risk factors for PD (both genetic and environmental) will continue, but viewing these in independent silos is likely to hinder progress. Increasing research activity in understanding the overlap between genes and the environment will enhance further understanding of the causal basis of disease. As for many diseases, the total picture of risk remains incomplete due to apparent and substantial randomness of onset, the obscuration of risk factors either because of rarity, ubiquity or poor measurement, or the fact that disease tends to strike those at moderate risk, simply because those at highest risk are far fewer.

1.1.3 Early clinical features

Recognition of the importance of non-motor features of PD has been increasing for several years.^{64,65} Some non-motor symptoms are experienced early and there is substantial evidence which suggests that they can predate diagnosis by several years (Figure 1.2).⁷ A number of studies have demonstrated the association of PD with earlier diagnoses such as depression, anxiety, constipation and erectile dysfunction (ED).⁶⁶ The best characterised early non-motor features of PD however are idiopathic anosmia and rapid eye movement (REM) sleep behaviour disorder (RBD).

Olfactory loss (anosmia)

Anosmia is relatively common in the ageing population but a proportion of subjects with unexplained smell loss, may go on to develop neurodegenerative disease.⁶⁷

Olfactory disturbance is a common finding in patients with PD, occurring in up to 80% of cases, which is similar to the frequency of tremor.⁶⁸ There is also evidence that hyposmia (which describes impaired smell as opposed to absent smell) may precede the onset of motor features of PD, as follows:

1. A study recruited subjects that were first-degree relatives of patients with sporadic PD and performed smell identification tests and dopamine transporter (DaT) imaging in some. Of the 40 hyposmic subjects at baseline, 4 had abnormal DaT binding at 2 years follow-up and clinically had PD. The remaining 36 had accelerated decline in DaT binding, compared to normosmic subjects.⁶⁹
2. Transcranial sonography (TCS) was performed on 26 patients with idiopathic anosmia. Of these, 10 that had abnormal TCS went on to have DaT imaging, which showed abnormalities in dopamine uptake in 5 subjects.⁷⁰
3. In the HAAS, over 2000 subjects were tested with the Brief Smell Identification Test (B-SIT) at baseline and followed up for 8 years. This prospective, population-based study demonstrated a relative odds of 5.2 (95% CI 1.5 to 25.6) for developing PD over 4 years if the lowest smell quartile was compared to the reference group (the highest two quartiles). The relationship weakened at over 4 years.⁷¹

These examples highlight the potential value in identifying cases with idiopathic anosmia to study the prodromal phase of PD and the potential for olfactory testing as a clinical marker for ‘at-risk’ individuals.

Sleep disorders

RBD is characterised by vigorous, and sometimes injurious, enactment of vivid, action-packed dreams, caused by loss of normal REM sleep atonia, which is diagnosed using polysomnography (PSG). An investigation of 29 patients with RBD found that 11 of these (38%) had developed PD at 4 years follow-up.⁷² Another group studied subjects with RBD (perhaps occurring at Braak stage 2) for the presence of anosmia (Braak stage 1), and clinical and imaging evidence of striatal neuronal loss (Braak stage 3). Compared with normative data, the 30 RBD patients had significantly higher olfactory thresholds and five had clinical features consistent with PD, four of which were supported by imaging.⁷³ Other observational studies support the high rate of ‘conversion’ to parkinsonism in subjects with idiopathic RBD.^{74,75} Subtle motor changes have also been demonstrated many years prior to a diagnosis of parkinsonism in patients with confirmed RBD.⁷⁶

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Despite high rates of conversion to a parkinsonian syndrome, RBD is clinically and pathologically heterogenous in that it can predate PD, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA).^{74,75} RBD is rare in the general population and the largest observational study has only studied just over 300 subjects despite international collaborative efforts.⁷⁷ Twenty-five percent of idiopathic RBD subjects will convert to neurodegenerative disease at 3 years (40% at 5 years) and this timeline is feasible when considering clinical trials.⁷⁷ The emergence of anosmia or subtle motor signs in those with RBD appears to further refine estimates of those that are likely to convert.^{78,79}

Excessive daytime somnolence (EDS) may also antedate PD and is a common non-motor feature in established PD.⁸⁰ Investigation of over 3000 subjects from the HAAS reported an adjusted OR of 2.8 (95% CI 1.1 to 6.4) for developing PD if there was a history of EDS at baseline.⁸¹ However EDS can occur in older people for a myriad of reasons and case-finding of pre-diagnostic PD on EDS alone is unlikely to be fruitful.

Anxiety and depression

Anxiety and depression have been observed to precede the diagnosis of PD in several observational studies. One US case-control study collected data using a self-administered questionnaire and observed that a history of depression was associated with subsequent PD with an OR of 2.74 (95% CI 1.07 to 7.57).⁸² Further evidence was provided from another case-control study that examined medical records of PD patients to identify previous mental health problems.⁸³ The frequency of depression and anxiety was higher in cases than controls, with ORs of 1.9 (95% CI 1.1 to 3.2) and 2.2 (95% CI 1.4 to 3.4) respectively, and an OR of 2.4 (95% CI 1.2 to 4.8) for coexistent depression and anxiety. The association with depression was lost at 5 years prior to onset of motor PD, but a relationship with anxiety was noted at 20 years prior to disease onset.⁸³ A systematic review of depression and mental illness preceding PD concluded that in 5 out of 6 case-control studies, premorbid depression was significantly more common in PD patients than controls, but the association between anxiety and PD appeared less profound.⁸⁴

Autonomic dysfunction

The Honolulu Heart Programme (HHP) collected baseline data on 6,790 male subjects (average age 60 years old) regarding bowel habit and laxative use. At an average of 12 years follow-up, the excess risk of PD was 2.7 (95% CI 1.3 to 5.5) in those who reported <1 bowel motion compared to those who reported 1

bowel motion per day.⁸⁵ The risk increased further if ‘constipated’ individuals were compared to those who reported 2 bowel motions per day (excess risk 4.5, 95% CI 1.2 to 16.9). Further support for this association comes from a pathological study of the brain stem in patients from the HHP. Those with <1 bowel motion per day had a 4-fold relative odds of incidental brain stem Lewy bodies (ILB) compared to those that had >1 bowel motion per day.⁸⁶ It has been proposed that ILB might be the pathological precursor of PD.^{4,87} Retrospective studies also support infrequency of bowel opening prior to motor disease manifestation.^{88,89}

Impaired erectile function is a common non-motor manifestation of PD. Analysis of 32,616 men enrolled in the Health Professionals Follow-up Study (HPFS) was undertaken to identify whether ED antedated PD. This study reported a RR of 3.8 (95% CI 2.4 to 6.0) of developing PD in subjects reporting ED up to 16 years earlier compared to those that reported good erectile function.⁹⁰

Whilst bladder complaints are well recognised in PD, there is no substantial observational study evidence to indicate that disturbances in bladder control precede PD. A cohort study examined surgical specimens of 100 patients with immuno-staining for alpha-synuclein in abdominopelvic organs. Organs of the urinary tract were found to be more likely to contain alpha-synuclein than organs of the digestive tract. A small number of study subjects were followed up. Whilst there were no cases of PD or dementia at 16 months follow-up, one subject had RBD and anosmia, and an abnormal motor score on the Unified Parkinson’s Disease Rating scale (UPDRS). Two other patients had accelerated decline in motor performance on the UPDRS between their 16-month and 30-month follow-up assessments.⁹¹

Relevance of early non-motor features

Recognition of early non-motor features is potentially valuable for early identification of PD. The research literature initially described the early phase of PD as being the ‘pre-motor’ phase, but more recently this has fallen out of favour with the appreciation that subtle motor features can be present before diagnosis.^{66,76,92,93} The clinical diagnosis of PD requires multiple motor features to be established and whilst subtle motor signs may be present, a clinical diagnosis of PD cannot be made until these become more definite.³ Given that subtle or single motor abnormalities occur prior to diagnosis and alongside early non-motor features, this period is better referred to as the pre-diagnostic phase.⁶⁶ The Movement Disorders Society (MDS) Task Force has proposed the following terminology:⁹⁴

1. Preclinical PD - presence of neurodegenerative synucleinopathy without clinical symptoms (this stage will be defined by disease biomarkers when available).
2. Prodromal PD - presence of early symptoms and signs before PD diagnosis is possible.
3. Clinical PD - diagnosis of PD has been made based on the presence of classical motor signs.

The emergence of large longitudinal primary care datasets has and will allow detailed exploration of the full range of early motor and non-motor symptoms that predate PD, whilst being free from the biases implicit in many traditional observational studies. Alongside this, advances in wearable technology and the availability of remote testing could aid objective measurement of emerging motor dysfunction in those ‘at risk’ of PD.

1.2 Imaging markers of pre-diagnostic PD

Radio-tracer imaging with single photon emission computer tomography (SPECT) or positron emission tomography (PET), or transcranial sonography (TCS) have repeatedly demonstrated the ability to differentiate patients with PD from healthy individuals, with adequate sensitivity and specificity.⁹⁵ SPECT and PET imaging are accepted as diagnostic imaging modalities for PD, with results of diagnostic accuracy for TCS tending to vary between centres.^{96,97} SPECT imaging of pre-synaptic dopamine transporters (DaT) using ¹²³I-ioflupane, is used in routine clinical practice for the investigation of tremor and secondary parkinsonian disorders, particularly in specialist centres. In established PD, diagnostic performance of DaT-SPECT is high, with strong inter-rater agreement, and good long-term safety data.^{98,99,100} However, some patients with clinically suspected PD, have normal appearances on DaT-SPECT imaging and have been described as SWEDDs (scans without evidence of dopaminergic deficit). In some of these patients, imaging subsequently becomes abnormal, consistent with the suspected neurodegenerative process, but in others it remains normal, and most of these subjects probably have alternative underlying diagnoses.¹⁰¹

A variety of imaging modalities may also have the potential to identify sub-clinical PD prior to diagnosis.¹⁰² In the pre-diagnostic phase, SPECT and TCS were shown to be abnormal in small studies of patients with anosmia and RBD that were subsequently diagnosed with PD, suggesting that these were good radiological markers of likely progression in selected subjects with early non-motor

features.^{69,70,73} Progressive change in SPECT imaging prior to diagnosis has now been further reported in a larger study of patients with RBD.¹⁰³ Most recently, in the Parkinson's At Risk Study (PARS), subjects with idiopathic anosmia (and other prodromal markers) have been shown to have greater risk of DaT deficit using ¹²³I β -CIT SPECT.¹⁰⁴

In contrast to SPECT, TCS demonstrating hyperechogenicity of the substantia nigra (SN) appears to be a static rather than changing marker.¹⁰⁵ Nonetheless, the finding of SN hyperechogenicity in otherwise healthy subjects over the age of 50 years, is believed to be a strong risk factor for PD, albeit with a low positive predictive value when used in isolation.¹⁰⁶ Improved standardisation and quantitative analysis for TCS and SPECT may increase their utility in the pre-diagnostic phase of disease, with SPECT more likely to demonstrate sensitivity to change. Further consideration of TCS and SPECT is given in chapter 7.

High-field and novel sequences of magnetic resonance imaging (MRI) may also provide opportunities to address the challenge of imaging disease progression in the pre-diagnostic phase. Recent studies in established PD have shown correlations of MRI micro-structural imaging abnormalities with post-mortem findings and quantitative differences between patients and healthy subjects in terms of iron deposition, loss of neuromelanin and alterations in nigrosome 1.^{107,108,109,110,111}

Two other imaging modalities used in the assessment of parkinsonian syndromes are ¹²³I-meta-iodobenzylguanidine (MIBG) myocardial scintigraphy and optical coherence tomography (OCT). MIBG scintigraphy has been largely studied in Japan, with multiple reports showing a reduction in heart to mediastinum ratio of MIBG uptake in PD patients, compared with healthy controls or other degenerative causes of parkinsonism.¹¹² Cardiac sympathetic nerve involvement is a feature of ILB pathology.^{113,114} Altered MIBG uptake has been reported in patients with a range of early non-motor features of PD, including autonomic dysfunction, mood disorders and sleep disorders, meaning that it may be a good prodromal imaging marker for PD, but further studies are required.¹¹⁵ OCT is a widely available imaging modality and studies have shown thinning of the retinal nerve fibre layer in patients with PD, as well as correlation with disease duration and severity.¹¹⁶ Meta-analysis of thirteen case-control studies was undertaken and showed clear differences between patients and controls, however, as far as it known, it has not been applied in subjects with early non-motor features of PD.¹¹⁷ A summary of imaging modalities and considerations for their use is shown in Table 1.1.

Modality	Example tracer/ sequence	Observation(s)	Analysis	Accessibility	Cost	Suitability for screening
TCS	2-3.5Hz transducer	Hyperechogenicity in the region of the substantia nigra	Visual inspection, quantification	++++	+	+++
SPECT	^{123}I β -CIT ^{123}I -FP-CIT	Loss of binding in striatum	Visual inspection, quantification	++	+++	+++
PET	^{18}F -dopa ^{18}F -FDG	Loss of binding in the striatum May help differentiate atypical PD	Visual inspection, quantification	++	++++	+
MRI	Traditional (T1 & T2), T2/T2*, DTI, spin echo	Numerous reported none established	Visual inspection, quantification	+++	++	++
OCT	-	Thinning of the peripapillary ret- inal nerve fibre layer	Quantification	++	++	++
MIBG	^{123}I -meta-iodo benzylguanidine	Low heart to mediastinum ratio	Visual inspection, quantification	++	++	++

Table 1.1: Imaging modalities for Parkinson's disease

Legend: TCS = transcranial sonography; SPECT = single photon emission computed tomography; PET = positron emission tomography; MRI = magnetic resonance imaging; OCT = optical coherence tomography; MIBG = meta-iodobenzylguanidine; Hz = hertz; FDG = fludeoxyglucose; DTI = diffusion tensor imaging; PD = Parkinson's disease. Accessibility, cost and suitability for screening are estimated semi-quantitatively on a four point scale from + lowest to ++++ highest.

1.3 Studies of pre- and peri-diagnostic PD

Several studies (Table 1.2) have been initiated to:

1. Identify those in the pre-diagnostic and prodromal phases of PD.
2. Identify clinical and biological markers to track progression of pathology before diagnosis.
3. Create platforms to identify subjects for inclusion in neuroprotective drug trials.

Some studies have recruited individuals with a single strong risk factor such as carrier status for *LRRK2* or *GBA* mutations, or idiopathic RBD or anosmia, in order that subjects may be followed prospectively, whereas other approaches employ large population-based cohorts or retrospective case-control methods to examine associations with PD and previous medical history. From the former much is learned about the emergence of PD in specific risk groups, which in turn may prove to be appropriate cohorts for recruitment to clinical trials. They are likely to be more homogeneous in terms of their disease mechanisms, pathology and clinical features, as well as being the simplest in which to determine time to conversion. However, they are perhaps not representative of the spectrum of PD as a whole. The latter studies are difficult and costly to conduct, with in-depth assessments and appropriate sample sizes, but allow the investigation of risk/protective factors and early symptoms and signs that precede emergence of established PD. This may in turn enable strategic combination of factors to try and delineate individuals at high risk, whilst also capturing the full spectrum of PD. Although the magnitude of risk associated with individual risk factors and early non-motor features has been reported, the best combination of risk factors for predicting PD remains unknown. Several studies are now seeking to combine risk factors for PD in order to improve predictive power with which those at increased risk of PD can be identified, with and without imaging markers.

The Prospective validation of Risk factors for the development of Parkinson Syndromes (PRIPS) study was a large study that sought to determine the magnitude of risk of PD that SN hyperechogenicity conveyed.¹⁰⁶ It showed that SN hyperechogenicity in healthy individuals over the age of 50 years was a risk factor for PD which carried a RR of 17.3 (95% CI 3.7 to 81.3) for development of parkinsonism at 3 years of follow-up. The aforementioned PARS study used objective smell testing to identify subjects with idiopathic anosmia at stage 1, followed by DaT-SPECT at stage 2 to identify subclinical presynaptic denervation.¹⁰⁴ The study has reported early results which demonstrated reductions in nigrostriatal

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DaT binding in subjects with hyposmia compared to those with normal smell, as well as associations between a number of prodromal features of PD within the study cohort. The Tübinger evaluation of Risk factors for the Early detection of NeuroDegeneration (TREND) study examines subjects over 50 years of age with a limited combination of risk factors (age plus anosmia or depression or RBD), using serial studies of movement, laboratory tests and imaging, with follow up to incident PD. Baseline data from this cohort have recently been reported showing associations between prodromal markers and other early associated features of PD.¹¹⁸

Two large multi-centre studies, one coordinated from the US (the Parkinson's Progression Markers Initiative, PPMI) and one based in the UK (the Tracking Parkinson's or PRoBaND study), recruit patients immediately after the clinical diagnosis of PD and undertake detailed clinical, imaging and biomarker studies longitudinally. Whilst not strictly looking at pre-diagnostic PD, the PPMI and PRoBaND studies will help define the role of clinical markers (motor and non-motor) in the early measurement of PD and the identification of novel imaging and laboratory biomarkers, as well as giving insight into what might be apparent through reverse extrapolation to the pre-diagnostic phase. The PPMI study also includes a prodromal arm (P-PPMI) which recruits subjects with RBD or anosmia, and a genetic arm for those with *LRRK2*, *GBA* or *SNCA* mutations, all of whom are assessed and followed in the same way as PD subjects, allowing for a seamless examination of the pre-diagnostic and early disease stages of PD. Similarly, the PRoBaND recruits first-degree relatives of patients for further assessment. Finally, as part of a large study aimed at understanding the biological basis of early PD in patients with established PD, the Oxford Parkinson's Disease Centre (OPDC) includes smaller high-risk groups of first-degree relatives or idiopathic RBD. Clinical assessments, laboratory and imaging biomarker studies are being undertaken and early results are emerging.¹¹⁹

1.3 Studies of pre- and peri-diagnostic PD

Acronym	Study name	Participants	Country	Number recruited	Tests/exposures	Outcome
HAAS	Honolulu Asia Aging Study	Middle-older age men of Japanese descent	US	8,006	Smell (B-SIT), EDS, constipation, reaction time	Clinical diagnosis of PD, pathological diagnosis of Lewy body disorder
PRIPS	Prospective validation of Risk factors for the development of Parkinson Syndromes	Subjects over 50 years old	Germany/Austria	1,847	TCS, smell (SS), UPDRS	Clinical diagnosis of PD
TREND	Tübinger evaluation of Risk factors for the Early detection of Neuro-Degeneration	Subjects over 50 years with anosmia, self-report RBD or depression	Germany	>1,200	TCS, smell (SS), UPDRS quantitative motor, blood biomarkers, psychometry	Clinical diagnosis of PD
PARS	Parkinson's At-Risk Syndrome study	Subjects over 50 years with hyposmia & DaT deficit	US	4,999 UPSIT smell test at baseline	UPSIT, DaT-SPECT, UPDRS, cognition blood biomarkers	Clinical diagnosis of PD/ DaT deficit on SPECT
P-PPMI	Prodromal Parkinson's Progression Markers Initiative	Subjects with prodromal features or gene mutations	International	Anosmia/RBD=65 Genetic =150	CSF & blood biomarkers, UPDRS, cognition, sleep and autonomic, UPSIT	Clinical diagnosis of PD
OPDC	Oxford Parkinson's Disease Centre study	Subjects with first-degree relative with PD or subjects with RBD	UK	190	UPDRS, non-motor assessments, UPSIT, blood and CSF biomarkers	Clinical diagnosis of PD

Table 1.2: Studies of the pre-diagnostic phase of Parkinson's disease

Legend: PD = Parkinson's disease; RBD = REM sleep behaviour disorder; UPSIT = University of Pennsylvania smell identification test; B-SIT = brief smell identification test; SS = Sniffin' sticks; DaT = dopamine transporter; SPECT = single photon emission computed tomography; UPDRS = unified Parkinson's disease rating scale; TCS = transcranial sonography; CSF = cerebrospinal fluid; EDS = excessive daytime somnolence.

1.4 Challenges and opportunities

The above studies aim to overcome the challenge of identifying ‘at-risk’ individuals that may develop PD with a view to initiating treatment to avoid or delay symptoms. It is also important to explore biomarkers that might be sensitive to progression and reflect the underlying disease process, at a time when clinical features are variable or not yet established (Figure 1.3). These studies aim to document the time immediately before, during and after the emergence of clinically recognisable PD, and aim to delineate the clinical and biomarker features of this phase that will be crucial to commencing clinical trials. However, the screening potential offered by approaches that rely on limited combinations of risk factors and early non-motor features, or have a restricted focus to gene carriers only, may limit the potential gain for the wider patient community. An approach that combines large numbers of risk factors for PD and that has the potential to screen a large, community-based population, in order to capture the full spectrum of PD, whilst remaining cost-effective, would be ideal.

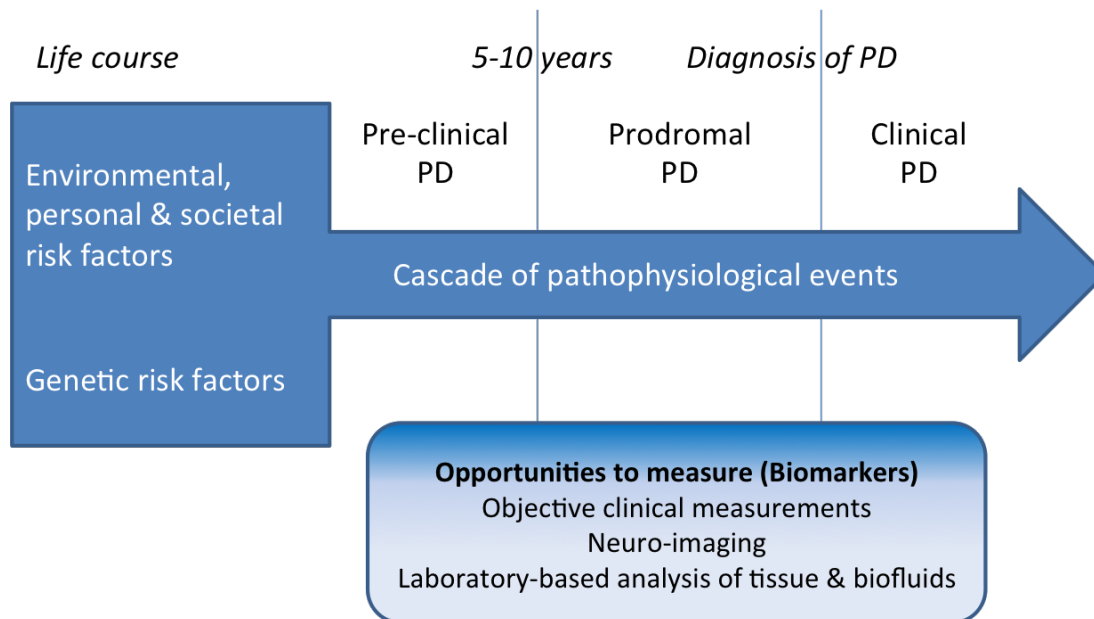


Fig. 1.3: A schematic showing determinants of risk, the pre-diagnostic phase (pre-clinical and prodromal phases) and clinical phase of PD, with the parallel application of risk and disease progression markers to measure disease activity across phases.

Chapter 2

Preliminary Work

2.1 Early non-motor features, risk and protective factors for PD - systematic review & meta-analysis

Observational studies have reported a variety of risk factors for PD and early non-motor features occurring before a diagnosis of PD (see chapter 1). There have been a small number of systematic reviews and meta-analyses seeking to pool risk estimates from these studies, including those considering associations with smoking, coffee consumption, pesticide exposure, use of non-steroidal anti-inflammatory drugs (NSAIDs), and subsequent PD.^{53,59,120,121} No study has comprehensively assessed the range of early non-motor features and risk factors that predate a diagnosis of PD. A systematic review of published literatureⁱ was initiated to determine the range of early non-motor features and risk factors that predate diagnosis of PD, restricted to those amenable to community screening.¹²²

2.1.1 Methods

Search strategy

The PRISMA 2009 guidelines for systematic review and meta-analysis and the Cochrane Collaboration definition of both terms were followed.^{123,124} A MEDLINE database search was undertaken using Pubmed from 1966 until March 2011 for studies reporting factors that could be used to screen for risk of future PD. The MeSH terms search used was: "Constipation" OR "Sleep Disorders" OR "Olfaction Disorders" OR "Smoking" OR "Colour Vision" OR "Coffee" OR "Erectile Dysfunction" OR "Depression" OR "Anxiety" OR "Mood Disorders"

ⁱThis was undertaken prior to commencing the work described in subsequent chapters but is crucial to the project and is therefore replicated in full here.

Preliminary Work

OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors" OR "Anti-Inflammatory Agents, Non-Steroidal" OR "Solvents" OR "Pesticides" OR "Body Mass Index" OR "Family" OR "Risk" OR "Risk Factors" AND "Parkinson Disease". Analysis was restricted to articles written in English. Reference lists of suitable retrieved articles were hand searched for any missed references, as were the reference lists of existing relevant meta-analyses identified in the original search. The final search was carried out on 31st March 2011.

Inclusion criteria

Published studies were included if they fulfilled the following criteria: a) assessed at least one risk factor or early non-motor symptom preceding a subsequent diagnosis of PD; b) reported original data on relative risks (RR) or odds ratios (OR) from cohorts representative of the general population or case-control studies with cases defined as patients diagnosed with PD; c) reported data that could be easily obtained in a community or primary care environment, i.e. those factors that could be determined through questionnaires or widely-available blood tests.

Exclusion criteria

Review articles, editorials and commentaries, hypothesis papers, letters that reported no new data, meta-analyses and abstracts were all excluded. Studies were also excluded if they: a) reported on treatment and management of PD, b) considered associations with established PD (i.e. not preceding PD), c) reported factors not easily ascertainable in the community setting (e.g. complicated questionnaires on food frequencies, life events, physical activity, environmental, solvent or toxin exposures and occupations), d) studied young-onset PD only, e) did not use a control group or provide adequate details of the control group (including prevalence studies), f) used blood relatives as the control group, g) were twin studies, h) genetic studies or laboratory studies not used widely, i) reported on the same risk factor in a common study population (where more than one paper reported on the same population, the larger or, where equal size, the most recent report was chosen), j) reported on a disease other than PD, k) reported measures other than OR/RR or an equivalent (such as proportional mortality rate and standardised hospitalisation rate) or from which an OR could not be calculated. If there was disagreement between investigators (AJN, JB, AS), the articles were discussed in further detail until an agreement was reached.

Data extraction

Study characteristics, risk estimate of the main study finding and secondary findings, were extracted for all eligible studies using a standardised template. Only factors for which a significant association was reported in at least one study were included. Risk factors were included according to binary measurements e.g. 'yes' versus 'no' for having a first degree relative with PD and 'ever' versus 'never' for alcohol. Data that reported exposures as quartiles or quintiles where the lowest exposure quartile was equal to zero were converted to binary terms. Associations reported with medical conditions, drugs or toxins known to cause symptomatic parkinsonism such as antipsychotics or carbon monoxide poisoning were excluded. Studies that reported associations with dementia occurring before onset of PD were not included, as these cases would not fulfil current criteria for PD and might include cases of Dementia with Lewy Bodies (DLB).¹²⁵ For cases, figures were used for PD instead of parkinsonism. In studies that reported data for both young-onset and typical-age-of-onset PD, the young-onset data were excluded where possible. If case-control studies made comparison with more than one control group, the control group most representative of the healthy general population was used. If studies did not report OR, RR or an equivalent measure, the raw data were reviewed to determine if ORs could be calculated. In studies that reported both crude ORs and adjusted ORs, the adjusted figures were used. After application of the above methods, no additional studies required exclusion for quality reasons. Length of time that any given factor preceded onset of PD was not included in the analysis due to inconsistent reporting of these data.

Statistical Analysis

Where a factor of interest was reported by two or more studies in a consistent manner, these were combined in a meta-analysis; first separately for case-control and cohort studies (given that cohort studies are less subject to bias), and secondly for all studies together (considering ORs from case-control studies to be estimates of RRs) to generate a pooled effect size and 95% confidence interval (CI) for each factor. Heterogeneity between studies was assessed using the I^2 statistic and, where statistically significant heterogeneity was found ($p < 0.05$), the random effects model was used to combine results.^{126,127} Publication bias was assessed using Egger's test and where statistically significant bias was found the 'trim and fill' method was used to adjust for it.^{128,129} Where data were not given in a way that could be used in the meta-analysis or where only one significant study was identified for a given risk factor, the findings of these studies were listed as part of the review. All analyses were performed using Stata.

2.1.2 Results

The literature search yielded 3,856 English-language articles, of which 202 were eligible for inclusion in the systematic review and 173 in the meta-analysis (Figure 2.1). The reasons for exclusion of other studies are provided. Full details of studies included in the meta-analysis are listed in Appendix A (Tables 1 and 2). Twenty-nine articles that could not be included in the meta-analysis, primarily due to inconsistencies in method of measurement of the studied risk factor or because only one study had reported a significant result on a given factor, are described and shown in Appendix A (Table 3).

Summary results for positive and negative associations with PD found in the meta-analysis and those where no association was found are shown (Figures 2.2, 2.3 and 2.4). Significant positive associations were found for family history of PD (including any relative and first degree relatives only), family history of tremor, preceding constipation, prior mood disorder, exposure to pesticides (or herbicides or insecticides), previous head injury, rural living, beta-blocker use, farming/agricultural occupation and well water drinking. Significant negative associations were found for smoking, coffee drinking, prior hypertension, use of NSAIDs, calcium channel-blocker (CCB) use and alcohol consumption. No significant association was found for oral contraceptive pill use, preceding oophorectomy, hormone replacement therapy, preceding diabetes mellitus, cancer or gastric ulcer, acetaminophen/paracetamol or aspirin, tea drinking and prior general anaesthetic. There was a trend towards a protective effect associated with the use of statins that fell just short of statistical significance.

In the systematic review additional associations that were reported but could not be categorised consistently included negative associations with raised serum urate, and conflicting results with total serum cholesterol, obesity, physical activity, any antihypertensive medication (without further sub-classification), education and various occupations. Single studies also reported negative associations with both parents having smoked and use of smokeless tobacco, and positive associations with family history of any neurological disease, hyposmia, erectile dysfunction and excessive daytime sleepiness (EDS), complaints of stiffness, imbalance or tremor, having a first degree relative with melanoma, having brown, blond and red hair relative to black hair, a variety of infectious diseases, immediate-type hypersensitivity, anaemia, duration of fertile life and cumulative length of pregnancies, having three or more children and having no children (see Appendix A, Table 3).

2.1 Early non-motor features, risk and protective factors for PD - systematic review & meta-analysis

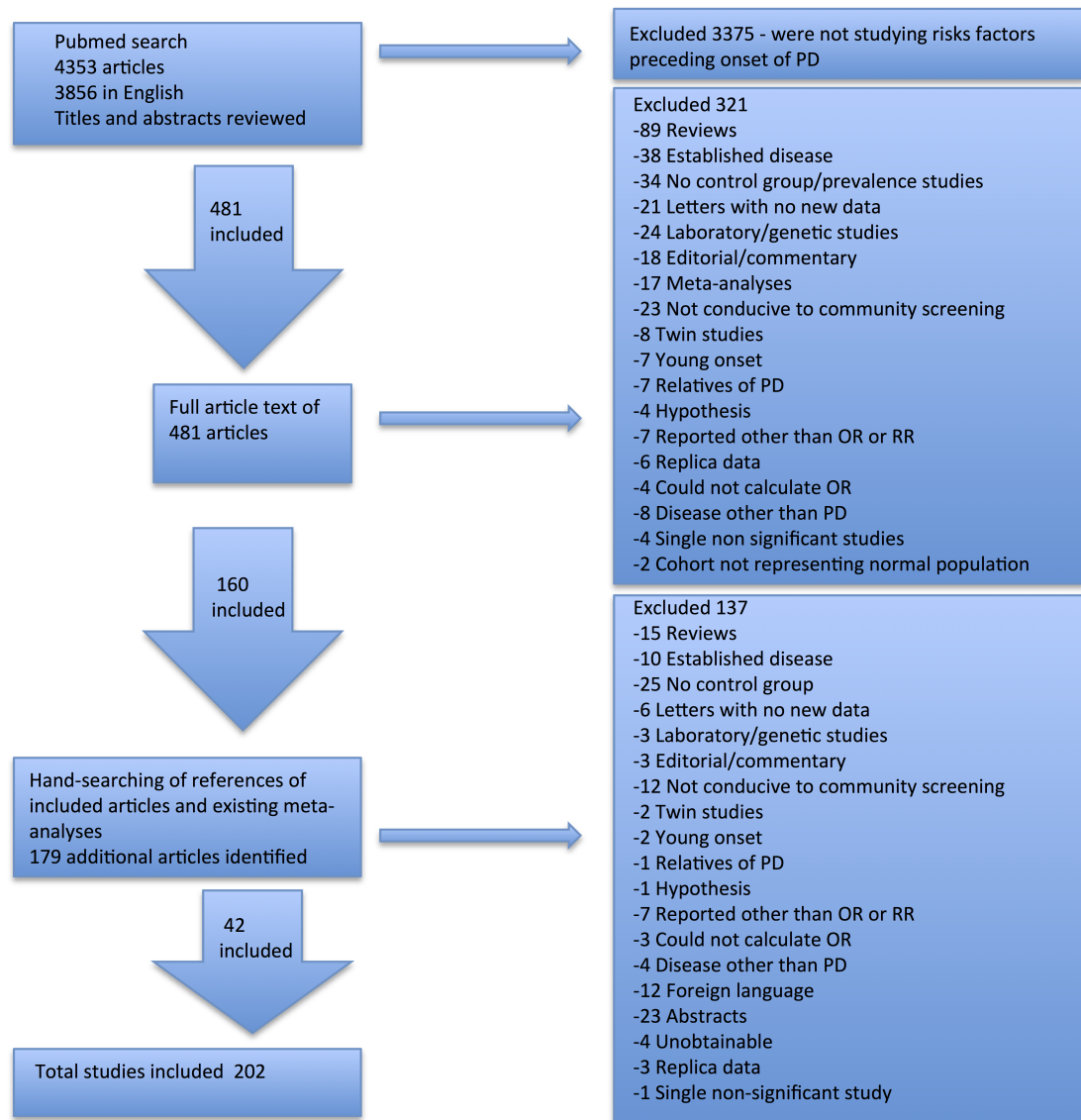


Fig. 2.1: Studies included and excluded from the systematic review and meta-analyses.

Legend: OR = odds ratio; PD = Parkinson disease; RR = relative risk.

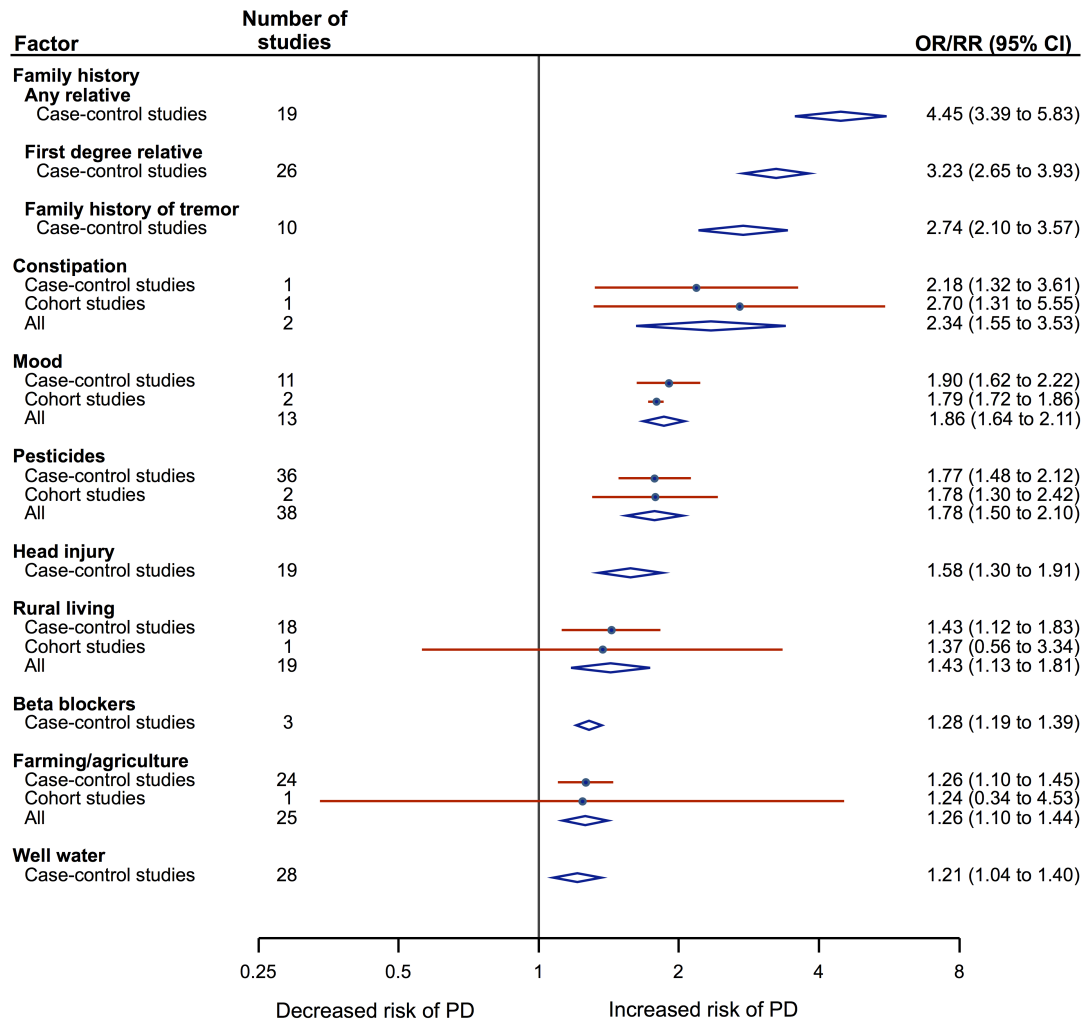


Fig. 2.2: Meta-analyses of significant positive associations with future PD.
 Legend: CI = confidence interval; RR = relative risk; OR = odds ratio.

2.1 Early non-motor features, risk and protective factors for PD - systematic review & meta-analysis

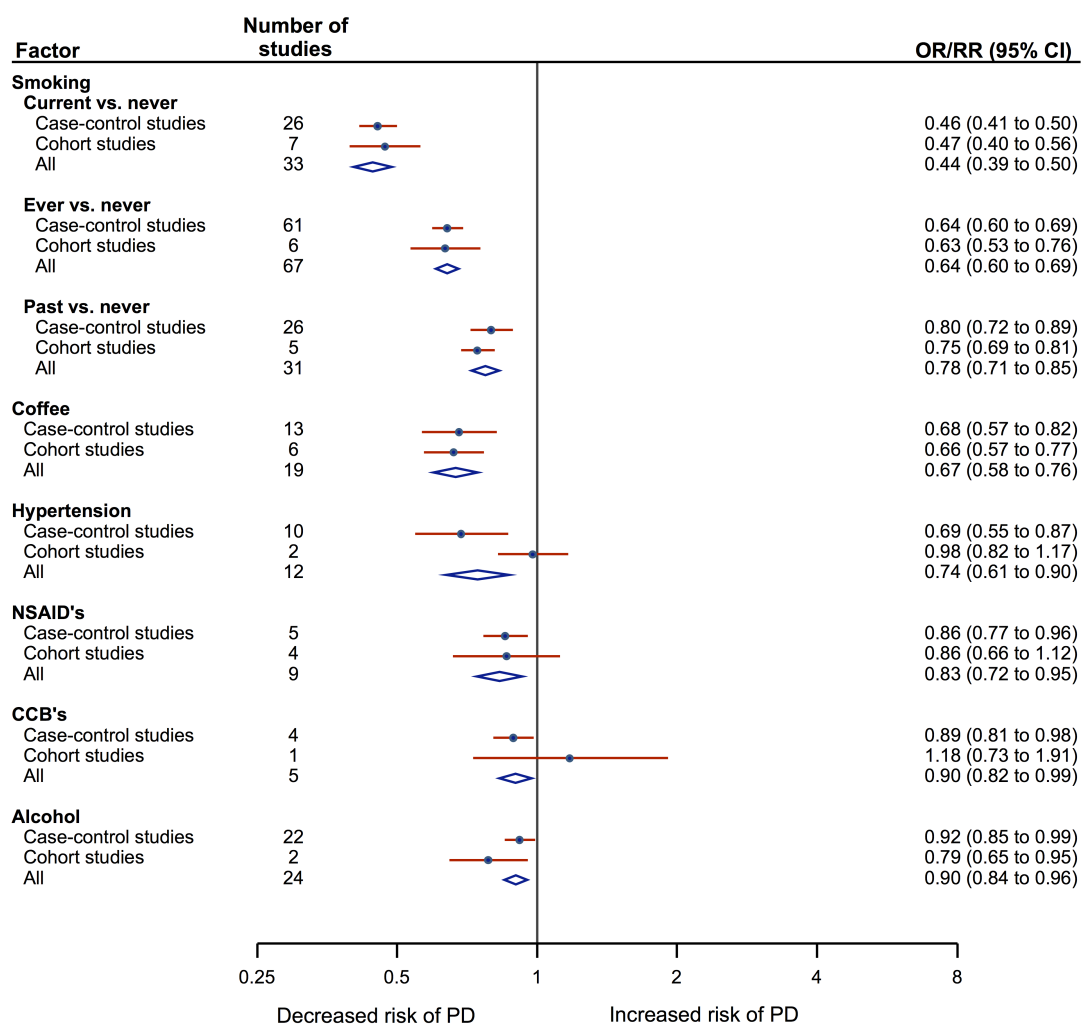


Fig. 2.3: Meta-analyses of significant negative associations with future PD. Legend: CCB's = calcium channel blockers; CI = confidence interval; NSAID's = non-steroidal anti-inflammatory drugs; RR = relative risk; OR = odds ratio.

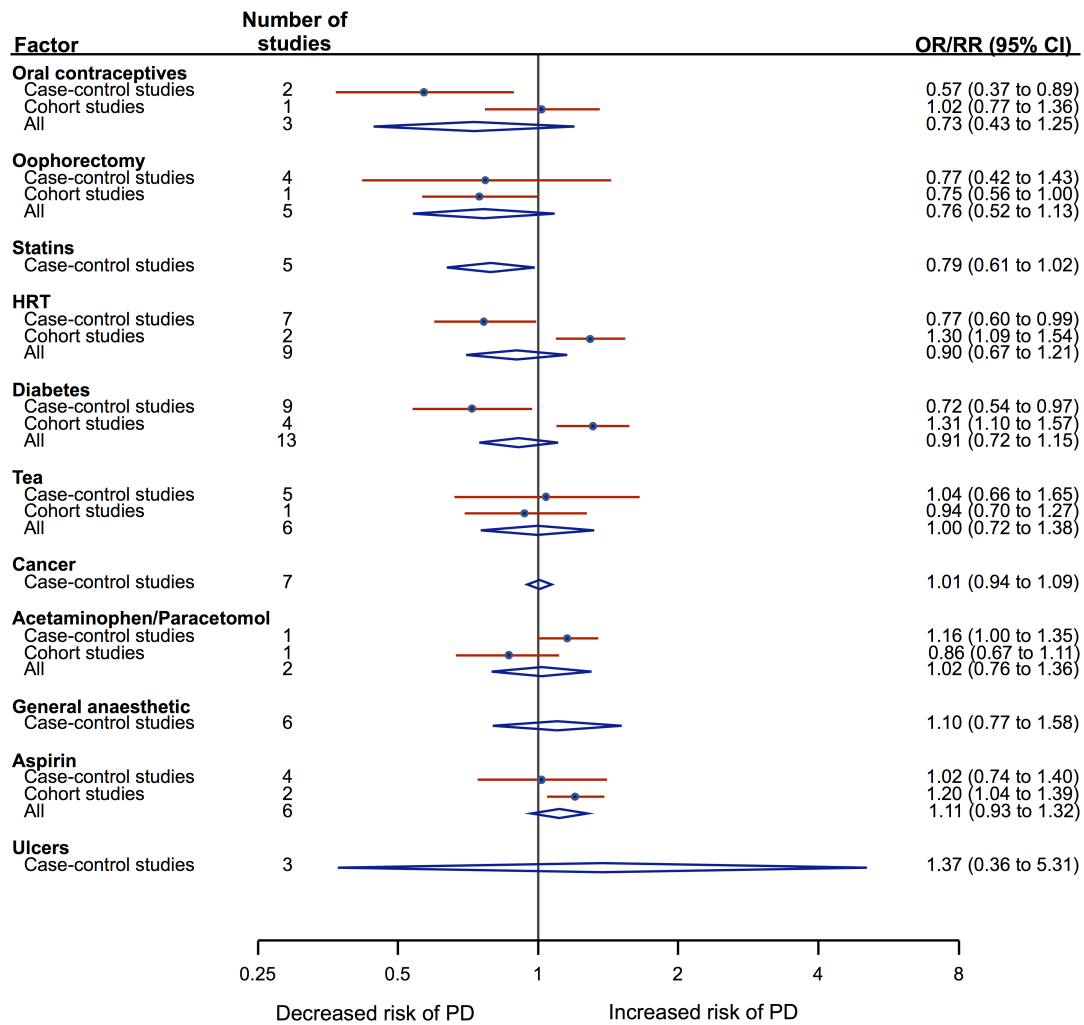


Fig. 2.4: Meta-analyses of non-significant associations with future PD.
 Legend: CI = confidence interval; HRT = hormone replacement therapy; RR = relative risk; OR = odds ratio.

Assessment of publication bias

According to Egger's test there was evidence of publication bias for the factors ever smoking ($p=0.017$), coffee ($p=0.002$), pesticides ($p<0.001$), oestrogen ($p=0.016$), statins ($p=0.038$), any family history of PD ($p=0.011$), family history of tremor ($p=0.009$) and well water drinking ($p=0.005$). Using the 'trim and fill' method to account for bias had no effect on the summary estimate for ever smoking, coffee, oestrogen or statins but did diminish the summary estimates for pesticides (RR from 1.78 to 1.53 (95% CI 1.29 to 1.80)), any family history of PD (from 4.45 to 3.25 (95% CI 2.43 to 4.35)), family history of tremor (from 2.74 to 2.51 (95% CI 1.96 to 3.22)) and well water drinking (from 1.21 to 1.20 (95% CI 1.04 to 1.39)) but the conclusion that these were statistically significant risk factors for PD was not altered.

2.1.3 Discussion

The systematic review identified over 40 individual risk factors of potential value for clinical screening. Identified factors include genetic and environmental risk factors, co-morbidities and medication exposures, as well as early non-motor features which may represent the earliest stages of PD. Whilst some of these have possible pathogenic importance and others may represent the earliest stages of PD, they serve as markers which may help identify subjects who are at high risk of a future diagnosis of PD. They also provide important information for the earlier diagnosis of PD, which is often delayed by several years.¹³⁰ Of the 30 factors that had data amenable to meta-analysis, 19 significantly altered risk of future PD and 11 did not reach statistical significance. This information may be used to form the basis for primary care or community-based screening as well as improve our understanding of contribution of risk to subsequent PD for each of these factors.

Family history

Having a family member with PD was the strongest risk factor for later diagnosis of PD. A number of monogenic causes of PD have been identified and multiple susceptibility loci described (see chapter 1).^{14,15,36} Whilst there is still a large genetic component of risk, as yet unexplained, familial aggregation may also occur through the effect of a shared environment e.g. concordance for not smoking and avoidance of coffee, which may influence the risk of PD, either independently or in conjunction with genetic susceptibility.

Lifestyle and environmental factors

A history of smoking reduced the risk of PD by about 36%, with coffee and alcohol (but not tea) consumption also reducing risk. For smoking the effect was strongest in current smokers and weakest in past smokers (56% for current smokers and 22% for past smokers), but the association remained significant in all. The nature of this association is still poorly understood, and cannot be explained simply by selection bias and confounding.^{131,132,133,134,135} The risk reduction from ‘ever’ versus ‘never’ coffee use and ‘ever’ versus ‘never’ alcohol use was approximately 33% and 10% respectively. A confounding effect of smoking, coffee and alcohol use in combination has been noted but other investigators suggest independence of these factors.^{136,137}

Pesticide exposure has been frequently implicated in PD causation.^{48,138} Despite differences in populations studied and study design, an overall positive association with ‘ever’ exposure to pesticides was identified. Some suggest there may be a greater risk associated with length of exposure to pesticides, but this was not evaluated in this analysis. The finding that farming was also associated with subsequent PD may be in part due to increased likelihood of exposure to pesticides and other chemicals. Inconsistencies in adjustment between studies made it impossible to determine the importance of this interaction. Rural living and well water exposure were also significant positive associations. Additional surrogates for pesticide or chemical exposures were not included such as gardening, plantation work and solvent exposures requiring more detailed exposure and lifestyle questionnaires. Other occupations that significantly increased the risk of PD included being a physician, clerk, carpenter, cleaner or having a legal occupation. Production workers, drivers, technicians, transport/communication workers, mechanical/factory workers, metal workers, sales occupations, service occupations and engineers were reported to have a significantly reduced risk of subsequent PD. Construction/extraction work had a significant positive effect in one study and a significant negative effect in another.

Head injury may carry increased risk of PD, particularly in those with repeated head injury.^{50,51} In this meta-analysis head injury with or without loss of consciousness had a significant but modest effect on the risk of subsequent diagnosis of PD.

Early symptoms

This review re-affirms the proposed early non-motor symptoms, which may predate the diagnosis of PD by several years. Constipation and mood disorders appeared to approximately double an individual’s risk of subsequent PD in the meta-analysis.

2.1 Early non-motor features, risk and protective factors for PD - systematic review & meta-analysis

Both constipation and mood disorders have been suggested to correlate with brainstem involvement that together with olfactory bulb involvement, occurs early in PD and later spreads to the substantia nigra and to the cortex.⁹ This notion would also support a role for hyposmia, erectile dysfunction and EDS as early features of PD, all of which have been reported to be associated with significantly increased risk in well-conducted cohort studies.^{71,81,90} For RBD there was no suitable observational study to allow the calculation of risk estimates for the association with PD. Further studies are required to replicate (for hyposmia, erectile dysfunction and EDS) or delineate (for RBD) the magnitude of risk each of these conveys and an appropriate method of measurement for each. The association of a preceding tremor, imbalance and stiffness with later PD (reported in single studies), may point to early motor manifestations of the disease before a diagnosis can be made.⁹²

Co-morbidities and medications

Hypertension was associated with reduced risk of PD, but the role of selective mortality in these individuals had not been studied. The combined analysis of studies of CCBs suggested a mild but significant overall reduction of PD risk. Beta-blockers, on the other hand, were associated with increased PD risk. This could potentially be explained by trials of beta-blockers in those with isolated tremor that later go on to be diagnosed with PD. A history of diabetes mellitus did not significantly alter risk; however further studies may be necessary given that the case-control studies showed a statistically significant decrease in risk while the cohort studies, which are less prone to bias, showed a statistically significant increase in risk. Statins showed a trend for reduction in risk of PD in the meta-analysis, which may be due to a protective role through reduction of oxidative stress. Hypercholesterolaemia, obesity and physical activity could not be included due to differences in assessment, and the systematic review found conflicting results.

NSAIDs were associated with risk reduction by approximately 17%. There is increasing evidence that inflammation may play a role in the pathogenesis of PD, which may underlie this finding.^{16,17,139,140} Aspirin and acetaminophen/paracetamol were not associated with significant alteration of risk. A number of studies suggested that raised plasma urate might protect against PD and this may also be related to inflammatory mechanisms since serum urate is a free radical scavenger and therefore protects against oxidative stress, which may be contributing to dopaminergic neuronal loss.¹⁴¹

There was no association of subsequent PD with the oral contraceptive pill, surgical menopause or hormone replacement therapy, although further studies

for the latter are needed, given that case-control studies showed a statistically significant decrease in risk, while cohort studies showed a statistically significant increase in risk. There was no significant association of PD and preceding cancer. Whilst melanoma was not included as a specific search term, a recent meta-analysis suggested a positive association of established PD with melanoma, but no association when melanoma precedes a diagnosis of PD.¹⁴²

Helicobacter pylori infection has been suggested as a risk factor for PD but there were no studies that specifically measured an effect size estimate between *H. pylori* and subsequent risk PD. A surrogate for this could be gastric ulceration, but in the meta-analysis there was no association with gastric ulcer and subsequent PD diagnosis.

Factors not included in analysis

Age and gender were not included in this review as they have mainly been reported in uncontrolled prevalence studies. Thus, whilst some of the studies included in the analysis reported risk associated with these variables, a combined risk estimate is unlikely to be representative of the full published literature on these factors. Nevertheless, the increased prevalence rate with increasing age and the higher prevalence in men is accepted and reporting is therefore usually stratified by age and gender.^{143,144}

Limitations

The search was restricted to articles written in English and reports written in other languages were not included. Due to the large number of studies used and the heterogeneity of methods and reported findings, variables were selected on the grounds that they could be screened for on a population basis. Risk factors and early symptoms not easily screened for would not have been included. Not all studies reported estimates of risk that were adjusted for confounders. Where adjustments had been made, these data were included in the analysis.

Risk factors were dichotomised, which ignored potential dose effects. However, an essential feature of the analysis was to define factors that could be defined in binary terms, to allow them to be employed in combination for large-scale screening.

Statistically significant heterogeneity was found in the majority (24 out of 30) of meta-analyses performed. In 16 of these there was moderate heterogeneity (I^2 50-75%) and in 4 there was high heterogeneity ($I^2 > 75\%$). This was expected because of differences in case ascertainment, study population characteristics, exposure measurement, and whether crude or adjusted risk estimates were reported. For

this reason, the pooled estimates should be interpreted cautiously, in particular for those estimates with high heterogeneity (hypertension, statin use, previous gastric ulceration and rural living).

2.2 Selection and measurement of factors for a PD risk algorithm

This was the first comprehensive systematic review and meta-analysis to take account of all risk factors for PD that might be suitable for screening in the community, and it provided the strength of evidence and magnitude of effect for each. Despite the fact that some factors may not operate independently of one another, the potential to develop a community-level, combined screening algorithm for PD was apparent. However, in selecting components for an algorithm that could screen for early non-motor features and risk factors, the following factors were considered to be important:

1. Was there an appropriate and available screening test or question(naire) for the exposure/factor/feature of interest?
2. Had the identified test or question(naire) been validated in PD?
3. Could it be administered in a self-report fashion?
4. If not, was there a surrogate?

These four questions were applied to each of the identified factors above to determine how they could be ascertained and the magnitude of risk/protective effect that would be appropriate to model in the algorithm.

- Age - Age-specific risk of PD was determined in a prospective study of approximately 22,000 subjects.¹⁴³ The 10-year risks were 0.11 (95% CI 0.04 to 0.17) at age 45 years, 0.51 (95% CI 0.41 to 0.63) at age 55 years, 1.51 (95% CI 1.30 to 1.71) at age 65 years, 2.63 (95% CI 2.24 to 3.01) at age 75 years, and 3.91 (95% CI 2.96 to 4.86) at age 85 years. Self-report of age was felt sufficiently likely to be reliable.
- Family history - Having a first, second and third-degree relative with PD conveyed different magnitudes of risk. The results of the meta-analysis were used to estimate odds of PD. Given the self-report nature of the questionnaire and potential for reporting error, only having a first-degree relative (father, mother, sister, brother, son, daughter) was included in the algorithm.

- Gender - Male gender was associated with increased risk of PD in two studies from the UK that were based in Cambridge (population size = 700 000, ratio male:female 1.36 (95% CI 1.11 to 1.65)) and Aberdeen (population size = 148 600, ratio male:female 2.30 (95% CI 1.55 to 3.28)).¹⁴⁵ Self-report of gender was felt sufficiently likely to be reliable.
- Smoking - The results of the meta-analysis were used to estimate odds of PD according to smoking status ('ever' (current and past smokers) versus 'never'). Self-report of smoking status was felt to be moderately reliable and additional questions were included to verify information, such as number of years smoked and number of cigarettes smoked per day.
- Coffee and alcohol - The results of the meta-analysis were used to estimate odds of PD according to coffee and alcohol drinking status ('ever' versus 'never' for each). Self reporting of coffee and alcohol consumption was felt to be moderately reliable and an additional question related to quantity.
- Bowel opening - From the systematic review, there was only one prospective cohort study (HHP) that provided data on bowel habit and laxative use.⁸⁵ A criticism of this study is that it did not use a validated questionnaire for constipation. Questionnaires do exist, and have been highly correlated with clinical observations and investigations.¹⁴⁶ Another criticism is that Abbott and colleagues demonstrated that individuals who open their bowels less than once a day are more likely to develop PD compared with those that do so once a day or more.⁸⁵ This finding does not fully support constipation as a predictor, since the definition of constipation requires a combination of features, one of which is fewer than three defecations per week.¹⁴⁷ Validated questionnaires exist and of those available, the Bowel Disease Questionnaire from the Mayo Clinic, closely aligned with the information gathered from the HHP.¹⁴⁸ Questions were selected from this questionnaire (under the guidance of CK) to ascertain presence or absence of constipation including frequency of bowel opening, laxative use, stool hardness and effort to defecate.
- Anxiety and depression - Numerous studies have assessed risk of PD given a past history of anxiety or depression and pooled estimates were calculated in the meta-analysis. The questionnaires used to identify anxiety and depression are often inadequate because the answers to many questions overlap between mood disorders and PD. Of the questionnaires that are validated in PD, the 14-point Hospital Anxiety Depression Score (HADS) to identify mood disorders was thought most appropriate for use in the algorithm.¹⁴⁹ It has been shown to have moderate suitability for screening

2.2 Selection and measurement of factors for a PD risk algorithm

for depression, provides details on anxiety, and can be self-completed in about 5 minutes.¹⁵⁰ The HADS has been suggested as a screening test for anxiety in PD since it has satisfactory internal consistency and test-retest reliability.¹⁵¹ Permission was sought and granted to use the HADS in the risk algorithm.

- Erectile dysfunction (ED) - ED was shown to antedate PD in a large cohort study (HPFS).⁹⁰ Self-report of ED was felt to be moderately reliable and the question used in the HPFS was selected. Subjects were asked to rate their ability in the previous 3 months, to have and maintain an erection adequate for intercourse. In the original study, responses were categorised as very good, good/fair, poor/very poor.
- Excessive Daytime Somnolence (EDS) - EDS was reported to be associated with future PD in a large cohort study (HAAS). However, it was ascertained via a questionnaire administered by a technician. Screening questionnaires used for EDS were felt to be unreliable as self-report tools (personal communication with Paul Reading, Neurologist and Sleep Expert) and so EDS was not selected.
- RBD - The evidence for RBD as an early feature of PD was convincing and derived from a variety of sources and study designs. RBD is diagnosed in the clinical setting using polysomnography (PSG) and electromyography. In recognition that RBD can predate diagnosis of PD, a group designed and validated a five-minute RBD screening questionnaire (the RBDSQ) to screen for RBD.¹⁵² A cut-off score of 5 discriminated RBD subjects from controls with a sensitivity of 96% and a specificity of 56% compared with gold-standard PSG. Permission was sought and granted to use the RBDSQ in the screening algorithm.
- Olfactory loss - The HAAS demonstrated the ability of the 12-item B-SIT to detect olfactory dysfunction preceding diagnosis of PD. The B-SIT is not as well validated as the full-length 40-item UPSIT in testing smell identification, and subjective reporting of smell is inaccurate. It was decided that subjects could receive the US version of the UPSIT via the post, self-complete the booklets and return them by post. The US version was selected over the UK version in light of previous extensive validation, including its use with UK research participants.
- Head injury - Self-report of head injury was felt to be moderately reliable but there was concern over what subjects might interpret as being sufficient

to warrant reporting. Therefore the selected question attempted to capture significant head injuries only with the following wording - 'Have you ever hit your head so strongly that you nearly fainted or lost consciousness, or sustained significant trauma to the face or nose?'. This question was not previously validated.

- Hypertension - Self-report of hypertension was thought to be unreliable given that some participants would not know whether their blood pressure was elevated and others might interpret treated hypertension as being normotensive. To improve reliability, self-reported medication lists were also referred to in order to ascertain presence of hypertension.
- NSAIDs, CCBs and beta blockers - These classes of drug were shown to have associations with PD in the meta-analysis and information regarding their use was collected using a free text medication box. Manual checking and categorisation of medication was required prior to analysis.
- Pesticides - Self report of pesticides was thought to be unreliable in terms of amount and duration of exposure, and the agents exposed to. It was decided that even if some subjects were able to report this information accurately, the numbers that did so would be small and there would be no means of confirming reports. Rural living, farming, and well water consumption were assumed to be proxies for pesticide exposure in the systematic review, and only information on farming was gathered via self-report of occupation.
- Other factors - Determinants that did not reach significance in the systematic review and meta-analysis but could be ascertained through survey included height and weight (for calculation of BMI). Various hormonal factors (HRT, OCP), diabetes, cancer, hypercholesterolaemia, peptic ulcer disease and statin use could be ascertained through medication and co-morbidity lists. Education and occupation could be determined through specific questions. Diet and physical activity are very difficult factors on which to collect information and were not felt to be appropriate or in keeping with the necessary brevity of the tests. Serum urate could not be collected and factors identified in single studies (apart from those listed above) were not sought.

In addition to selecting the factors that would comprise the risk algorithm, the method of delivery was a key consideration. Cost-effectiveness and the ability to 'scale up' the approach were important considerations, and it was decided that the algorithm should test subjects via the internet using a customised portal.

Screenshots of the screening questionnaire in the web portal are shown in Appendix B.

2.3 Design and development of the BRAIN-tap test

The BRadykinesia Akinesia INcoordination (BRAIN) test was developed as a software tool for detecting signs of neurological disease, including PD and cerebellar dysfunction.^{153,154} Sequential finger tapping is part of the routine neurological examination for the detection of bradykinesia, defined as slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive action.

The traditional alternate finger tap test, in which the patient is observed tapping two mounted counters 15cm apart as fast as possible, has been used for many years to measure response to symptomatic drug treatment. The BRAIN test replicated this using a computer screen and keyboard. The user alternately tapped the S and the ; (semicolon) keys as rapidly and as accurately as possible over a 60-second time period.

The original version of the BRAIN test was programmed to run in MS-DOS mode on an IBM-compatible personal computer.¹⁵³ Here, a modified version was developed that could run in all standard internet browsers, called the BRAIN-tap test.

2.3.1 BRAIN-tap test parameters

A software developer (DD) was commissioned to produce a 60-second test that would record alternate key taps and calculate appropriate parameters to measure speed and accuracy of tapping. 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)

Preliminary Work

Data from key presses were used in calculations that attempted to replicate the parameters developed and validated in the original version of the BRAIN test:

- Kinesia score (KS) - a measure of rate of movement. The total number of keystrokes per 60-second time period. In the original version it was the sum of 'key pressed' values.
- Akinesia time (AT) - a measure of dwell time on the keys. In the original version, it was the cumulative time over the test period that any key was pressed for longer than 17 millisecond (msec; the repeat rate for the keyboard). For example, if the fastest time that a key could be pressed and released ('Time Up' minus 'Time down') was 46msec, the AT for that key would be 29msec ($46-17 = 29$ msec). If 120 keys were pressed during a 60-second test, the AT would be 3.6 seconds.
- Dysmetria score (DS) - a measure of coordination. It was a weighted count of all incorrectly hit keys, with correction for speed. In the original version, incorrectly hit keys were weighted according to their distance from the target key. Keys immediately adjacent to the target key were given a score of 1, those adjacent to these scored 2, and all other keys scored 3. The sum of incorrectly hit keys was divided by KS to correct for rate-related inaccuracies.
- Arrhythmia score (AS) - a measure of rhythmicity. It was the variance of time in between keystrokes (variance of traveling time). In the original version, it used the time difference values derived from 'Time up' to next 'Time down'.

Once the software produced a calculation for each parameter, a period of testing started to compare calculations embedded in the software with hand-calculations using the raw key-press data. This was to ensure that software calculations were measuring exactly how they were expected to. Ten full tests were undertaken in each step of analysis using the 60-second version of the test and a modified 10-second version of the test for brevity in some instances. A summary of observations from this comparative analysis and changes to software calculations were as follows:

- KS was inversely related to DS for both software calculations and hand calculations - this was expected since faster test performance may result in decreased accuracy.

2.3 Design and development of the BRAIN-tap test

- Differences were observed between software-calculated AS and hand-calculated AS but the two scores were clearly related - it transpired that the software-calculated scores included a travelling time of 0msec before the first key press, resulting in high variance compared with hand-calculated AS. The variance equation was adjusted to calculate sample variance (as opposed to population variance) by making the denominator $n-1$. These steps resolved the differences observed in the software-calculated AS.
- AT was strongly correlated with KS - this was an undesirable association because it meant that if a subject achieved high KS scores, their cumulative dwell time could be higher than a subject who achieved very low KS but substantially longer AT per key-press. Furthermore the 17msec keyboard repeat rate used in the calculation of AT in the original version of the test could not be replicated on modern keyboards (and also varied between keyboard and operating system settings). The previous calculation of cumulative AT was therefore revised for a calculation of mean AT (total dwell time divided by number of key presses), without adjustment for keyboard repeat rate.
- DS was revised so that the target key would generate a score of 1, adjacent keys scored 2 and all other keys scored 3.

2.3.2 Preliminary validation

The BRAIN-tap test was embedded in a web-portal to enable further testing and validation with the support of web-developers (AC and CT). An email communication was sent to staff at the Blizzard Institute, Queen Mary University of London, inviting them to complete the test remotely. The test was undertaken by 177 subjects and results are shown in Table 2.1.

	Kinesia score (KS)	Akinesia time (AT)	Arrhythmia score (AS)	Dysmetria score (DS)
Mean	137.9	98.8	36365	1.054
(95% CI)	134.1 to 141.7	89.5 to 108.0	21267 to 51464	1.042 to 1.065
Median	137.0	81	12565	1.029
(IQR)	123.5 to 150.5	68.0 to 99.0	7051 to 21873	1.007 to 1.070
SD	25.8	62.4	97006	0.077

Table 2.1: Preliminary tapping data collected from 177 controls remotely. Legend: CI = confidence interval; IQR = interquartile range; SD = standard deviation.

Further investigation was then undertaken in patients with PD, recruited from the movement disorders clinic at Barts and the London NHS Trust.¹⁵⁵ Patients were diagnosed according to the Queen Square Brain Bank Criteria.³ Both hands were tested for each patient and KS was correlated against total motor UPDRS scores. This preliminary work demonstrated significant correlations between KS and UPDRS, and established that there was no difference in the mean number of taps in the first 30-seconds and the last 30-seconds of the 60-second test.¹⁵⁵ The test was shortened to 30-seconds prior to full validation which was undertaken as part of the work contributing to this degree (see chapter 4).

2.4 Design & development of an internet portal for PD risk screening

A custom-built website was commissioned to deliver the predictive algorithm in a user-friendly format and store the results securely in line with data protection laws. The domain names www.predictpd.com and www.predictpd.org were purchased. Development was undertaken by a commercial organisation (AC and CT at Grass Roots) who customised proprietary software called People Portal.

Specific considerations in designing the website were that user age, level of education and experience with computers and the internet may vary greatly. The aim was to develop a website where simplicity, brevity and data security were of paramount importance. It had to engage users through a variety of media and evolve through user feedback, and be largely automated, with minimal administrator support needed. Users had to be able to access study information, self-register and consent, and a facility for requesting forgotten passwords was necessary. The developers designed a logo for PREDICT-PD to capture the essence of the project (a crystal ball and bold orange text).

On the administration side of the portal, it was necessary to have a survey tool that could administer a modifiable questionnaire and that would gather responses in a secure and reliable manner. The ability to view anonymised data and export these data in standard spreadsheets was also required. The ability to access user information that might change during the study period was necessary, including postal address, phone number and email address. This information was important to enable communication with study participants.

The ‘user-journey’ was considered carefully throughout development. The study homepage explained the purpose of the study and what was required of participants (Figure 2.5). The registration process followed standard procedures used by other secure websites storing personal information. Registration required

2.4 Design & development of an internet portal for PD risk screening

a first name and surname, date of birth, postal address, email address and telephone number. The email address served as the username for login to the website. Registration was completed by choosing a password, comprising letters and numbers, which was used to access the website in the future alongside the username.



The screenshot shows a web browser window displaying the Predict PD login page. The browser's address bar shows the URL <https://www.predictpd.com/pd/predictpd/login.xhtml>. The page has a light blue header with the Predict PD logo on the left and a login prompt on the right: "You aren't logged in yet. [Login](#)". Below the header, there are links for "Smaller text" and "Larger text". The main content area is white and contains the following sections:

- Welcome to Predict PD**: A heading followed by two paragraphs of introductory text about the project's aim to identify people at risk of Parkinson's disease.
- Recruitment Information**: A paragraph stating that the project is currently recruiting for a pilot study of 1,000 individuals aged 60-80.
- Thank you**: A simple acknowledgment.
- Login**: A section with instructions to enter an email address and password, followed by a "Login" button and links for "Forgotten password?" and "Not registered?".
- Contact Us**: A section featuring a photograph of red tulips and contact details for Dr. Alastair Noyce, including his phone number (0208 123 8873), email (a.noyce@qmul.ac.uk), and website (www.predictpd.com).

The footer of the page is dark blue and includes social media icons for Facebook, Twitter, YouTube, and Google+, along with copyright information: "Copyright 2010 Predict PD. Powered by **Grass Roots** technology. Supported by **Parkinson's UK. Join Us**".

Fig. 2.5: Study login and registration page.

After registering, participants were shown the online consent form and full participant information. Questions about the study were directed to the investigators via email, telephone or post. After completing the check boxes on the consent form and clicking the 'submit' button, participants were taken to the participant homepage (Figure 2.6). Participants were shown a brief summary of the tests that would follow. The first test was the screening questionnaire, which covered the early features and risk factors discussed in section 2.2 and took approximately 20 minutes to complete. The survey had a user-friendly interface (drop-down boxes and radio buttons) and navigation options away from the survey were removed to

Preliminary Work

improve completion rates. In addition, the screening questionnaire covered level of education, occupation, height and weight (for BMI). Free text input was used to record co-morbidities and current medication. Participants were then taken to the BRAIN-tap test page (section 2.3) and completed a test for each hand. There was then the opportunity to provide feedback on the website or the study itself. After completing the test elements, a smell test survey option became automatically available within the website. This questionnaire enabled the answers of the UPSIT smell test to be completed online after smell tests had been received in the post.

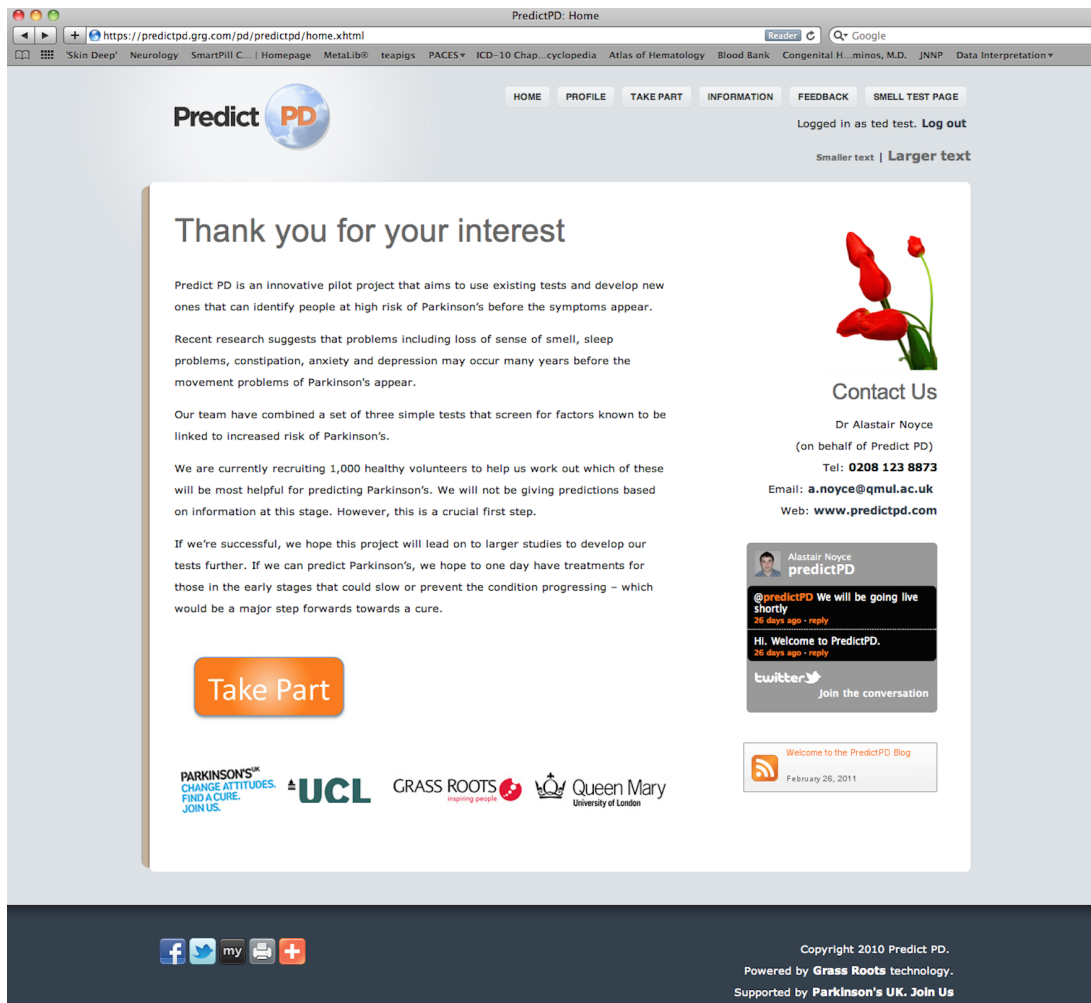


Fig. 2.6: Participant homepage.

The website was secured by a dedicated Secure Sockets Layer (SSL) certificate. This was renewed on an annual basis and provided 128-bit (up to 256-bit) data encryption. SSL certification is designed for websites that deal with sensitive information such as bank account data and medical health records. The software and databases hosted and stored by the commercial organisation (Grass Roots) were ISO 27001 accredited, which is the international data handling standard. Investigators required access to both subject identifiable information (for commu-

2.4 Design & development of an internet portal for PD risk screening

nications and dispatch of additional tests) and test results. Both datasets were held separately to preserve the integrity of identifiable information.

The web-portal configuration, development of the BRAIN-tap test and selection of factors for the screening algorithm were completed before the work pertaining to the research degree began.

Chapter 3

Project aims and objectives

- To recruit a group of participants from the community via the internet that would form the basis of a longitudinal cohort to determine those at risk of PD.
 1. To use a web-based platform for subject recruitment and stratification with a preliminary algorithm for PD risk, comprising questions on risk factors and early non-motor features, and objective smell and finger-tapping tests.
 2. To validate the computer keyboard finger-tapping test (the BRAIN-tap test) in PD patients and healthy controls, and integrate this into the above web-platform.
- To demonstrate that subjects estimated as being higher risk have an excess of features associated with the PD prodrome when compared to subjects estimated to be at lower risk, including:
 1. Differences in intermediate markers (smell loss, sleep disturbance and finger tapping speed).
 2. Differences in the frequency of gene mutations.
 3. Differences in imaging appearances on transcranial sonography and ^{123}I -FP-CIT SPECT.
- To explore whether subjects estimated as being at higher risk have a greater chance of diagnosis with PD during follow-up.

Chapter 4

The BRadykinesia Akinesia INcoordination (BRAIN)-tap test

4.1 Introduction

The Bradykinesia Akinesia Incoordination (BRAIN) test was a software tool for detecting signs of neurological disease. The original version of the BRAIN test was programmed to run in MS-DOS mode on an IBM-compatible personal computer.¹⁵³ A modified version (the BRAIN-tap test) that can run in all standard internet browsers was developed, in which the patient or research volunteer completed the test online and the results were uploaded to a secure database for storage and analysis (see chapter 2.3). Here the validation of this test is described.

4.2 Methods

Participants gave informed consent via the BRAIN-tap test website. The web-based consent form listed relevant consent statements and each statement was associated with a check box. Participants checked each box before clicking the ‘submit’ button on screen. The Queen Square Research Ethics Committee approved the study (reference 09/H0716/48) and this specific method of obtaining consent. Patients who fulfilled the Queen Square Brain Bank criteria for the clinical diagnosis of PD and age-matched non-neurological controls were recruited from the outpatient department at the Royal London Hospital and the National Hospital for Neurology and Neurosurgery.³

Participants undertook the test seated at a desktop computer and keyboard. They followed on-screen test instructions and received no assistance during the test. Participants were allowed to choose which hand was tested first in acknowledgement of the fact that use may frequently be unobserved. Each participant undertook

two tests (one for each hand). Preliminary study established that a 30 second time period gave adequate information and would lead to greater compliance than testing for one minute (see chapter 2.3). Demographic data were recorded for all participants including gender, year of birth, level of education and self-reported hand dominance. For controls, additional co-morbidity information was recorded. For patients with PD, current medication, time of last dose of levodopa, number of years since diagnosis and Hoehn-Yahr stage were recorded. Patients with PD were examined using the motor section of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS). The MDS-UPDRS includes a question about the clinical state of patients on medication. 'On' is the typical functional state when patients are receiving medication and have a good response and 'Off' is the typical functional state when patients have a poor response in spite of taking medication. These definitions of 'On' and 'Off' were recorded for each patient.

For reliability testing, 17 of the controls were asked to repeat the test multiple times for each hand. This had the secondary advantage of being able to investigate the possibility of a learning effect. Due to the tendency of motor features of PD to change in relation to medication and potentially time of day, PD patients were not included in tests measuring reliability. However, six patients with PD and known motor fluctuations were invited to undertake the test on several occasions during the day, before and after medication in order to evaluate the BRAIN-tap test in monitoring motor fluctuations.

The BRAIN-tap test calculates four variables from the raw key-press data:

- kinesia score (KS), the number of key taps in 30 seconds.
- akinesia time (AT), the mean dwell time on each key in milliseconds (msec).
- dysmetria score (DS), a weighted index using the number of incorrectly hit keys scored in a target fashion (1 point for the correct key, 2 points for immediately adjacent keys and 3 for other keys) then divided by the total number of key taps (i.e. if all keys are hit correctly, the score should be 1.0).
- incoordination (or arrhythmia) score (IS), the variance of the time interval in msec between keystrokes.ⁱ

Statistical methods

Descriptive statistics were calculated for all four variables. For continuous variables, means were reported if the data were normally distributed (assessed using

ⁱIS was called AS in chapter 2 but was changed to IS to avoid confusion with AT.

the Shapiro-Wilks test) and medians were reported if not normally distributed. Within-group (PD or control) comparisons were performed using the paired t-test for normal distributed data or Wilcoxon-signed rank test for non-normally distributed data. Between-group comparisons were made using the unpaired t-test or Wilcoxon rank sum test. The sensitivity and specificity of test parameters separately were determined using receiver operated characteristic (ROC) curves. The combined effect of parameters to identify cases versus controls was assessed using logistic regression. Associations between UPDRS and BRAIN-tap test parameters were estimated using Spearman's rank correlation coefficient for non-normally distributed data. Coefficients of variation were calculated to determine reliability of test parameters in control subjects undertaking the test multiple times and p-values were derived from using multilevel mixed effects linear regression.ⁱⁱ The pre-determined significance level for all calculations was $p=0.05$. All analyses were performed using Stata and GraphPad Prism for Mac.

4.3 Results

There were 58 PD patients and 93 non-neurological controls included in the main analysis (for group characteristics see Table 4.1). One PD patient and one control subject were ambidextrous and were excluded from analyses that compared dominant and non-dominant hands. One PD patient tested in the clinic had incomplete UPDRS data and was excluded from those specific analyses.

Associations of KS, AT, IS and DS with age, gender, education, occupation and co-morbidities were undertaken in controls (see Table 4.2). KS, AT and IS correlated with age of control subjects ($r = -0.47, 0.31$ & 0.34 respectively; $p < 0.001, 0.002$ & < 0.001 respectively). KS decreased by 0.66 points, AT increased by 1.35% and IS increased by 4.7% per year of age (all $p = 0.001$). No significant correlation between DS and age was seen ($r = 0.16$; $p = 0.12$). Lower levels of education tended to give poorer scores for KS and AT, and gave significantly poorer scores for IS and DS. Analyses considering occupation showed that having a professional occupation gave significantly better KS scores, but there were no significant differences in the other parameters for different occupations. The presence of comorbidity did little to affect test results except for the finding that having any comorbidity worsened IS compared to those with no comorbidity and KS was non-significantly lower in those with depression. There was no significant effect of handedness and parameters were similar between males and females, except that females were significantly more accurate than males (improved DS).

ⁱⁱThis specific analysis was done by Jonathan Bestwick. All other analysis was done by Alastair Noyce.

	PD	Controls
Number	58	93
Mean age (SD)	63.0 (10.6)	60.5 (13.1)
Gender		
· Male	37 (64%)	32 (34%)
· Female	21 (36%)	61 (66%)
Education		
· Primary	2 (4%)	4 (4%)
· Secondary	35 (60%)	46 (50%)
· Higher	7 (12%)	19 (20%)
· Further	14 (24%)	24 (26%)
Occupation		
· Professional	10 (17%)	26 (28%)
· Non-professional skilled	23 (40%)	37 (40%)
· Non-professional non-skilled	10 (17%)	22 (24%)
· Retired with no additional information	15 (26%)	8 (8%)
Handedness		
· Right	54 (93%)	81 (87%)
· Left	3 (5%)	11 (12%)
· Ambidextrous	1 (2%)	1 (1%)
Mean years since diagnosis (SD)	8.4 (6.6)	-
On/Off at time of test*		
· On	38 (66%)	-
· Off	20 (34%)	-
Levodopa		
· Yes	52 (90%)	-
· No	6 (10%)	-
Mean minutes since levodopa dose (SD)	186 (133)	-
Hoehn-Yahr stage		
· Stage 1	11 (19%)	-
· Stage 2	34 (59%)	-
· Stage 3	13 (22%)	-

Table 4.1: Demographic information for cases and controls.

Legend: PD = Parkinson's disease; SD = standard deviation. *On/Off in this table refers to the question in the MDS-UPDRS, which asks whether participants could feel the effects of medication at the time of testing.

	No. of subjects	KS (mean)	p-value	AT (median)	p-value	IS (median)	p-value	DS (median)	p-value
Gender									
· Male	32	59.6	0.71	90.6	0.31	6887	0.73	1.062	0.03
· Female	61	60.7		99.2		6758		1.034	
Education									
· Primary	4	54.4	0.19	133.1	0.14	36913	0.03	1.232	0.02
· Secondary	46	58.0		99.9		7417		1.054	
· Higher	19	64.2		88.5		9496		1.038	
· Further	24	62.8		92.0		5446		1.027	
Occupation									
· Professional	26	65.7	0.05	92.0	0.48	5416	0.10	1.041	0.57
· NPS	37	58.5		102.9		10645		1.042	
· NPNS	22	59.8		92.9		7402		1.063	
· Retired	8	52.9		110.6		8939		1.030	
Co-morbidity									
· No additional	40	63.4	0.06	93.8	0.23	5072	0.02	1.034	0.37
· Affecting limbs	7	55.3		125.2		13419		1.076	
· Depression	3	45.3		102.9		9310		1.000	
· Medical conditions	43	59.4		91.9		10645		1.044	
Handedness									
· Right	81	59.8	0.44*	99.2	0.65*	7093	0.46*	1.035	0.13*
· Left	11	63.1		91.6		4955		1.080	
· Ambidextrous	1	75		60.2		5384		1.109	

Table 4.2: Analysis of factors that influence KS, AT, IS, DS in control subjects.

Legend: NPS = non-professional skilled; NPNS = non-professional non-skilled.

* p-values for comparisons between right and left handedness only.

	Mean KS (95% CI)	Median AT (IQR)	Median IS (IQR)	Median DS (IQR)
PD	44.2 (40.9 to 47.5)	138.7 (100.5 to 221.0)	13813 (8744 to 29857)	1.042 (1.014 to 1.147)
Controls	60.3 (57.6 to 63.0)	97.3 (80.7 to 131.2)	6758 (4030 to 16664)	1.044 (1.013 to 1.110)
p-value	<0.001	<0.001	<0.001	0.45

	Sensitivity (cut-off)	Sensitivity (cut-off)	Sensitivity (cut-off)
Specificity	90%	31.0%* (183)	24.1%* (29954)
	85%	39.7% (156)	29.3% (23527)
	80%	43.1% (149)	36.2% (18527)

Table 4.3: Comparison of KS, AT, IS, DS in all PD patients versus controls.

Legend: PD = Parkinson's disease; CI = confidence interval; IQR = interquartile range.
 * If analyses were limited to include only patients that were 'Off' (n=20) the sensitivities (and cut-offs) for 90% specificity for KS, AT and IS were 65% (43), 50% (175) and 55% (29373) respectively.

When PD patients ($n=58$) and controls ($n=93$) were compared using averages of the scores from each hand; KS, AT and IS discriminated between groups, but DS did not (see Table 4.3 and Figure 4.1). KS showed the best discrimination between PD and controls with sensitivities of 45%, 50% and 57% for specificities of 90%, 85%, and 80% respectively. Corresponding sensitivities were 31%, 40% and 43% for AT and 24%, 29% and 36% for IS. The addition of AT or IS to KS did not improve discrimination compared with KS alone (assessed by multivariate logistic regression). When patients that were ‘On’ were excluded and patients that were ‘Off’ ($n=20$) were compared to controls, the sensitivities for 90% specificity were 65%, 50% and 55% for KS, AT and IS respectively. Subjects tested whilst ‘On’ had better KS scores than subjects who were tested whilst ‘Off’ (47.8 and 37.3 respectively, $p=0.002$). IS scores were also significantly better in those that were ‘On’ (11682 and 29568 respectively, $p=0.007$) and there was trend for improvement in AT (125.1msec and 172.6msec respectively, $p=0.27$).

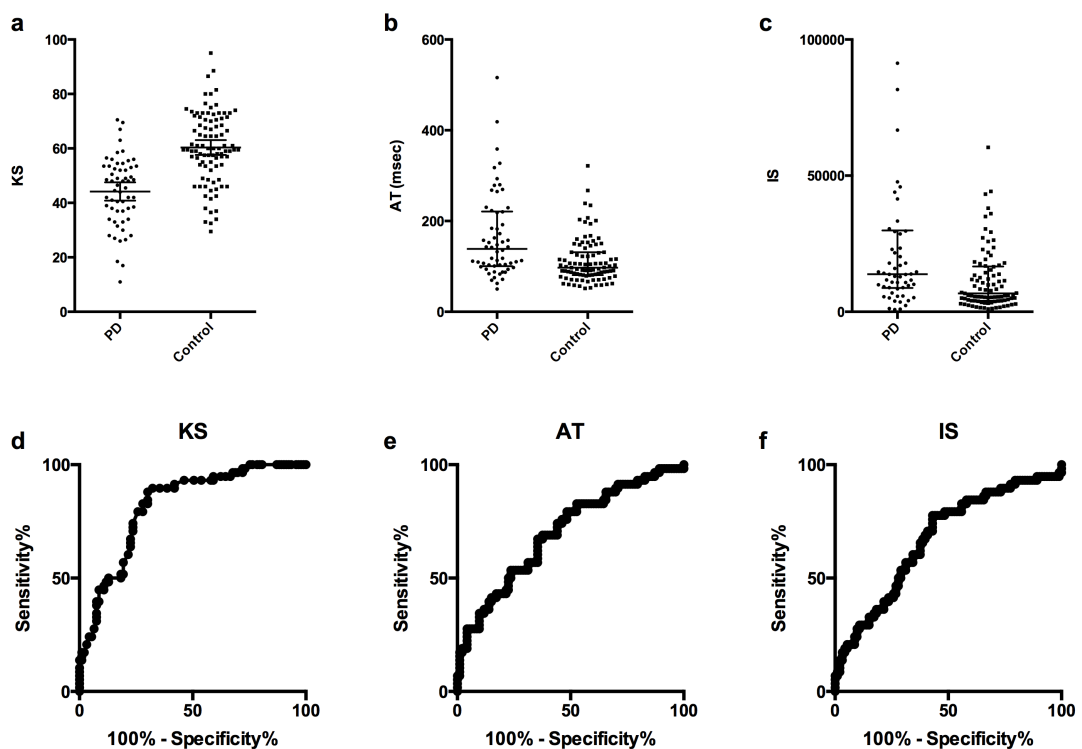


Fig. 4.1: Comparisons between cases and controls are made for: (a) mean KS with 95% confidence intervals, (b) median AT with interquartile range, and (c) median IS with interquartile range. Corresponding receiver-operating characteristic (ROC) curves are also shown for these three parameters (d-f).

Hands were compared in patients and controls (see Table 4.4). In both the dominant and non-dominant hands tests mean KS was significantly lower in PD patients than controls, and AT and IS were significantly higher. In patients and

controls the dominant hand significantly out-performed the non-dominant hand for KS and AT, but not for IS or DS.

BRAIN-tap test scores in PD patients only were compared to total motor UPDRS scores and sub-scores (see Figure 4.2) using averages of the scores from each hand. KS showed a moderate inverse correlation with total motor UPDRS (Spearman's $r = -0.53$, $p < 0.001$). AT and IS showed weak but significant positive correlations with total motor UPDRS (Spearman's $r = 0.27$, $p = 0.03$ and $r = 0.28$, $p = 0.03$ respectively). DS showed no correlation. Further correlations were undertaken with sub-sections of the UPDRS including upper limb tone, finger tapping, hand opening and closing, and pronation-supination (see Table 4.5).

In PD patients there was a difference of borderline significance with lower KS in the more affected hand when compared to the less affected hand (mean KS 42.5 v 44.8, $p = 0.053$). There was no difference in AT, IS and DS between the two hands (median AT 134 v 128, $p = 0.350$; median IS 12604 v 12048, $p = 0.421$, median DS 1.034 v 1.051, $p = 0.569$). Duration of PD in years did not correlate with any of the four parameters (data not shown).

Seventeen of the controls repeated the BRAIN test five times for each hand to estimate the reliability of KS, AT, IS and DS. The coefficient of variation for KS was 6.0%, for AT was 7.3%, and for DS was 3.4%. IS had a high coefficient of variation reflecting the fact that pauses in the test (even in control subjects) magnify the variance of travelling time significantly, decreasing the reliability of IS overall. There was a mild learning effect that saw KS increase by 1.2 taps per attempt ($p = 0.002$) but no learning effect for AT (decrease of 1.0msec per attempt, $p = 0.203$) or for IS and DS (p values derived from multilevel mixed effects linear regression).ⁱⁱⁱ

Finally, using KS and AT, the effect of medication was assessed in a small number of PD patients with predictable motor fluctuations (Figure 4.3) and also in patients with unpredictable fluctuations (Figure 4.4).

ⁱⁱⁱPerformed by Jonathan Bestwick.

	Mean KS (95% CI)			Median AT (IQR)		
	<u>PD</u>	<u>Control</u>	p-value	<u>PD</u>	<u>Control</u>	p-value
Dominant hand	46.5 (42.8 to 50.2)	63.1 (60.1 to 66.1)	<0.001	121 (83 to 201)	87 (68 to 114)	<0.001
Non-dominant hand	42.1 (38.8 to 45.5)	57.3 (54.6 to 59.9)	<0.001	147 (112 to 232)	111 (87 to 155)	<0.001
p-value	<0.001	<0.001		<0.001	<0.001	
	Median IS (IQR)			Median DS (IQR)		
	<u>PD</u>	<u>Control</u>	p-value	<u>PD</u>	<u>Control</u>	p-value
Dominant hand	10315 (4830 to 15562)	5969 (2631 to 14505)	0.014	1.036 (1.00 to 1.14)	1.037 (1.00 to 1.08)	0.60
Non-dominant hand	12762 (5508 to 33489)	6162 (2966 to 14040)	<0.001	1.042 (1.00 to 1.16)	1.043 (1.00 to 1.11)	0.90
p-value	0.74	0.78		0.91	0.12	

Table 4.4: BRAIN-tap test parameters in PD patients and controls according to hand.
Legend: CI = confidence interval; IQR = interquartile range.
Ambidextrous PD patient and control excluded.

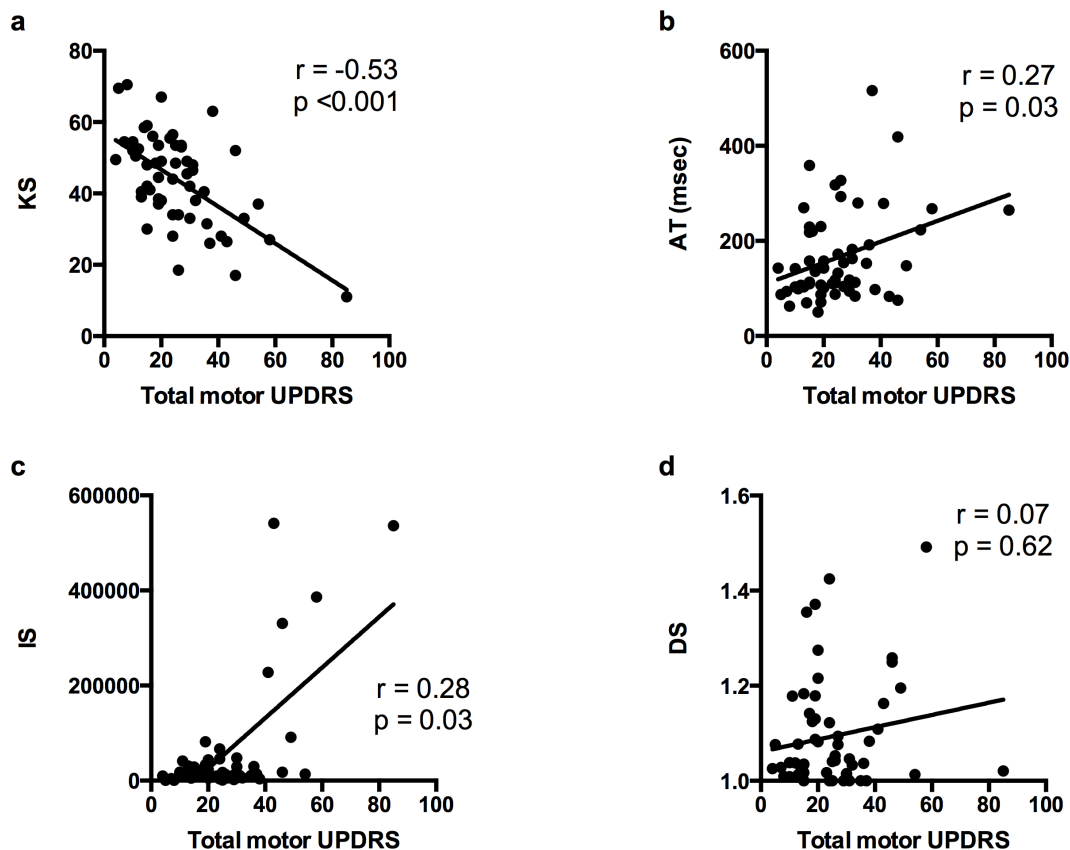


Fig. 4.2: Total motor UPDRS correlated against BRAIN-tap test parameters.
Legend: r = Spearman's rho.

	KS		AT		IS		DS	
	r	p-value	r	p-value	r	p-value	r	p-value
Tone	-0.21	0.03	0.17	0.08	0.10	0.31	-0.02	0.80
Finger tapping	-0.44	<0.001	0.20	0.03	0.33	<0.001	0.03	0.79
Hand move	-0.57	<0.001	0.35	<0.001	0.39	<0.001	0.06	0.55
Pro-supination	-0.34	<0.001	0.31	0.001	0.21	0.02	-0.01	0.91

Table 4.5: BRAIN-tap test parameters and UPDRS sub-scores.
Legend: r = Spearman's rho.

4.4 Discussion

The BRAIN test has previously been shown to differentiate individuals with PD from healthy controls and also to correlate with PD severity measured by disease-specific rating scales.^{153,154} The results of these two studies, in which the original version of the test was performed on a single laptop computer under defined conditions, have been replicated here with this new online version of the

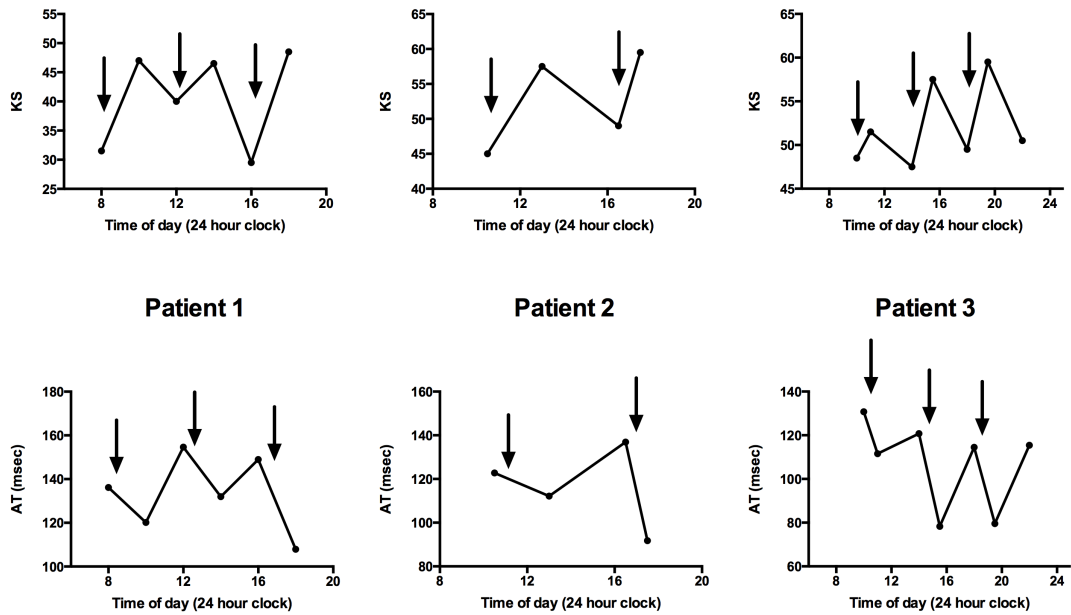


Fig. 4.3: Predictable motor fluctuations in KS and AT. Arrows indicate times at which a dose of levodopa-containing medication was taken.

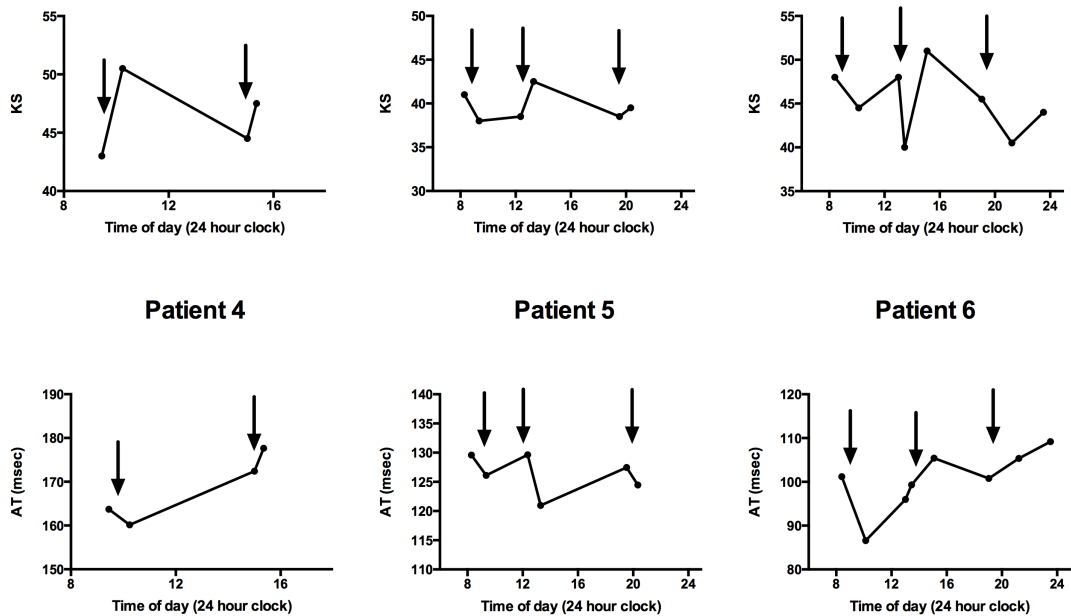


Fig. 4.4: Unpredictable motor fluctuations in KS and AT. Arrows indicate times at which a dose of levodopa-containing medication was taken.

test, allowing it to be administered without direct observation or investigator input. It can be accessed remotely from wherever there is an internet connection and a computer (laptop or desktop) keyboard.

In PD patients, KS correlated significantly with total motor UPDRS score, and limb specific sub-scores, as clinical indicators of motor disease severity. AT and IS also correlated significantly, albeit less strongly than KS, with the total motor UPDRS score and some of the limb specific sub-scores.

Data for PD and non-neurological controls showed wide intra-group variability and yet the differences between KS, AT and IS are highly significant and of clinically relevant magnitude, enabling cut-offs for sensitivity and specificity to be determined. Results are presented to optimise specificity, with resulting moderate to low sensitivity, thereby reducing the false-positive rate in acknowledgment that the test will often be performed remotely. Of course sensitivity and specificity operate on a continuum and much higher sensitivity can be achieved if the cut-offs are altered to accept a higher false positive rate.

Age clearly affected most of BRAIN-tap test parameters and should be taken into consideration in future studies using the test. Education also influenced parameters perhaps reflecting an effect of computer literacy. However, this was not further reflected when examining occupation, with which strong relationships could not be found. This perhaps reflects widespread uptake of computers regardless of whether use is work-related or recreational. Patients that were tested whilst ‘On’ performed better than those that were ‘Off’. When PD patients that were ‘Off’ were compared to controls, the discriminative ability of KS, AT and IS improved significantly. Comparison of most and least affected sides in PD patients only showed trends for poorer scores on the most affected side, perhaps reflecting bilateral involvement in 80% of the patient group (Hoehn and Yahr score 2 & 3).

Whilst not seemingly useful for differentiating PD from healthy controls, the dysmetria score (DS) does provide a useful reference for judging whether tests have been completed properly, which is particularly valuable for remote testing. For example, the mean KS in controls is 60.3 and has a standard deviation of 13.1. Using three standard deviations as a cut-off, it is unlikely that an individual can exceed 100 alternate taps in 30 seconds without a dramatic loss of accuracy as reflected by the DS. Occasionally very high scores have been seen in remote tests, with perfect or near-perfect accuracy (e.g. KS of 200 and DS of 1.0). This suggests that the subject is using two hands to hit the keys and is not alternating between keys with a single hand, and such results should be excluded from analyses.

Some highly specialised tools have been developed that can accurately measure the specific motor deficit that occurs in PD and some have the capacity to differentiate the sequential tapping abnormalities in PD (true bradykinesia) from

that seen in progressive supranuclear palsy and atypical tremors.^{156,157,158} When compared to such tools, the BRAIN-tap test appears fairly crude, but these specialist tests, whilst fulfilling a valuable role in research, are not currently applicable in routine clinical practice. Further study using the BRAIN-tap test could assess whether a sequence effect can be demonstrated in patients that are ‘Off’ medication following a period of drug withdrawal and also study greater numbers of patients with motor fluctuations in their ‘On’ and ‘Off’ phases, since the current study does not address these important aspects fully.

A criticism levelled at the BRAIN test during preliminary testing was that not all keyboards have identical characters, particularly outside the US and UK. Furthermore, the position of the S and ; keys on the keyboard can vary between countries. These keys were originally chosen because they are 15 cm apart on a US/UK English standard desktop computer keyboard and most laptop computers. If one considers a standard keyboard divided in two halves by an imaginary line down the centre, then these keys occupy a central position on their respective sides. In countries where US/UK English keyboards are not standard, the test can still be used with the keys that correspond to the position of S and ; and has been implemented successfully by groups in Italy, Norway and the Netherlands (personal communications).

During testing no evidence was found that use of different keyboards resulted in significant differences to results between subjects. However, use on tablet computers may be limited by the availability of the ; key and the different nature of touching a screen rather than pressing a key. As such it is not advisable to use the BRAIN-tap test on tablet computers or smart phones (but again this is a focus of further work). In addition, use of sterile covers for keyboards in clinical settings may impair key presses. This might conceivably result in loss of accuracy (DS30) but is unlikely to affect the other three parameters (KS, AT and IS).

Undoubtedly the greatest value of the BRAIN-tap test in established PD may come in the longitudinal monitoring of individual patients throughout the duration of their disease, including response to treatment and monitoring motor fluctuations. Repeat testing in controls suggests good reliability for three of the four parameters and only a minimal learning effect. In this analysis, control subjects repeated the tapping tests back-to-back and the fact that only a minimal learning effect was noted makes it unlikely that improvements due to learning would be seen were serial tests separated by days or weeks.

In the Honolulu Asia Ageing Study (HAAS) it was demonstrated that men in the slowest tertile of a reaction time test were significantly more likely to have Lewy body pathology at post mortem.¹⁵⁹ Postuma and colleagues have followed up a large cohort of patients with RBD, which is a strong risk factor for PD. They

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demonstrated that motor deterioration could be measured for approximately 4-8 years across a number of motor domains (including alternate finger tapping) prior to the diagnosis of PD. The premotor period has been estimated to last between 5-15 years prior to the diagnosis of PD.⁷⁶ The term pre-diagnostic PD is preferred in this context; given the stringent motor criteria that must be met for a clinical diagnosis (including demonstration of a sequence effect), it seems likely that subtle motor dysfunction must be present at an earlier stage.^{92,93}

The online BRAIN-tap test is a simple, validated, objective tool to longitudinally monitor motor function not only in established PD but also in studies seeking to identify those at higher risk of future PD. The BRAIN-tap test can be accessed at www.braintaptest.com. Tokens for individual use can be requested by clinicians and researchers via the website.

Chapter 5

Risk of PD in the community: baseline data from PREDICT-PD

5.1 Introduction

A range of risk factors and early non-motor features of PD have been reported from observational studies and were reviewed in chapter 1. Chapter 2 described preliminary work, including a systematic review and meta-analysis to determine the magnitude of risk associated with each factor, the selection of factors to ascertain risk in the community using a preliminary algorithm, and the development of an online portal to recruit and test research participants. In chapter 5, the initiation and baseline line data analysis of the PREDICT-PD study is described, which incorporated the work of previous chapters, including the validated BRAIN-tap test from chapter 4.

At baseline, the performance of the preliminary algorithm was assessed by comparing the occurrence of a combination of ‘intermediate markers’ for future PD, including three of the strongest individual markers of increased PD risk (smell loss, self-reported RBD and finger-tapping speed), in those estimated to be at higher risk of PD compared with those estimated to be at lower risk. Confirmation of validity will be tested in longitudinal follow-up of study participants, using imaging, genetics and incident PD diagnosis as additional outcomes.

5.2 Methods

The study was approved by Queen Square Research Ethics Committee (reference 10/H0716/85). Participants were recruited via the study website following a limited advertising campaign on local radio and in magazines with an older readership, and by email to members of the Parkinson’s UK charity. Participants

submitted an online consent form before passing to the secure test area. Inclusion criteria were residency in the UK and age 60-80 years. Exclusion criteria were pre-existing PD, movement disorder, stroke, motor neurone disease, dementia, or drug usage known to be associated with iatrogenic parkinsonism.

Participants completed a survey with demographic questions and items related to early non-motor features and risk factors for PD, which incorporated validated questionnaires, i.e. the Hospital Anxiety Depression Scale (HADS),¹⁴⁹ the RBD Screening Questionnaire (RBDSQ),¹⁵² and a number of individual questions that had been used in good quality observational studies that previously reported risk factors for PD (see chapter 2). For the RBDSQ, a cut-off score of ≥ 5 was used, which has previously been shown to have a sensitivity of 96% and specificity of 56% for diagnosis of RBD confirmed by polysomnography (PSG).¹⁵² The overall survey length was 56 items and it took approximately 20 minutes to complete, based on prior testing by members of the research team and 10 independent healthy volunteers aged 60-80 years, whose data were not included in the results. Screen shots of the questionnaire embedded in the website can be seen in Appendix B.

Immediately after the survey, participants were invited to undertake a keyboard tapping task, the BRAIN-tap test, used to assess upper limb motor function (see chapters 2 and 4). Participants were also sent the US version of the University of Pennsylvania smell identification test (UPSIT) via post. The UPSIT is a 40-item scratch and sniff smell test (composed of 4 booklets, each with 10 test pages) that has been used extensively in the investigation of smell disturbance in neurological disease. Based on methods used by the Parkinson's At-Risk Study (PARS), in which impaired smell was used to identify individuals at risk of future PD, the lower 15th centile of UPSIT scores was used as a cut-off to denote hyposmia (scores were not adjusted for age and gender as they were in PARS).¹⁶⁰ Answers to the UPSIT were entered on the study website by most participants. A minority (n=155) returned results completed in the test booklets only.

Analysis

Based on the results of systematic review (see chapter 2), which provided risk estimates for each early non-motor feature or risk factor significantly associated with altered risk of PD, a preliminary algorithm was developed to provide PD risk estimates for each participant. The algorithm included age, gender, smoking status, first degree relative with PD, coffee use, alcohol use, hypertension, NSAID use, calcium channel blocker use, beta blocker use, constipation, previous head injury, anxiety or depression and erectile dysfunction (in males only). Most factors were sought in binary terms (i.e. presence or absence) except for bowel movement frequency (7 possible answers for frequency with a cut off of less than 1 movement

per day denoting low frequency or laxative use), erectile dysfunction (3 options with ‘poor’ indicating dysfunction) and mood (a cut off score of 11 or above in either the anxiety or depression components of the HADS questionnaire denoting moderate forms of these disorders or antidepressant use). In order to keep the survey simple, pesticide exposures, proxies for organo-chemical exposure, and more complicated factors were not included. Any subject that reported a neurological diagnosis listed in the exclusion criteria was removed from the analysis.

Smell loss and RBD, which are reported to be two of the strongest estimated risk factors for future PD (along with finger tapping in selected risk groups⁷⁶), were not included in the algorithm. These were instead used as ‘intermediate markers’ or proxies for preliminary validation of the basic algorithm (see below). For each individual, the age-related risk (expressed as an odds) of developing PD was determined based on results from the Physicians Health Study.¹⁴³ The equation determined from these data was:¹

$$\text{Odds of PD} = 1 : 28.53049 + 73.67057e^{(-0.165308(\text{age}-60))}$$

As these data were from an all-male cohort, and prevalence of PD is approximately 1.5 times greater in men, the age-related odds for women was reduced accordingly.¹⁴⁴ An individual’s risk was increased or decreased by each of the above factors according to the strength of association with PD reported in the systematic review. For example a male, current smoker with a 1:100 age-related odds of developing PD was calculated to have an odds of $0.44 \times 1:100 = 1:227$. If that individual also had a family history of PD their odds was calculated as $4.45 \times 1:227 = 1:51$. The odds for all participants were ranked and the 15% with the highest risk and 15% with the lowest risk scores were identified.

The preliminary validity of this recruitment and risk estimation method was tested by comparing three of the potentially strongest individual markers of increased PD risk (smell loss, RBD and finger-tapping speed) between the 15% of participants calculated to be at highest and lowest risk. Several lines of evidence (including pathology and imaging) suggest that loss of smell may be a sensitive (albeit not specific) predictor of PD.^{68,69,70,71} RBD is rare in the general population but its presence carries a high risk of conversion to PD in longitudinal cohort studies making it relatively specific, albeit not sensitive.^{72,75,77} Bradykinesia is a core feature of PD and subtle motor signs can precede the diagnosis of PD by several years, suggesting that finger tapping may be a sensitive early feature for those at higher risk of PD.^{3,76,92,93}

¹Derived by Jonathan Bestwick.

The central hypothesis at baseline was that if the preliminary algorithm was successful, those in the higher risk group would have significantly lower UPSIT scores, higher rates of RBD and slower tapping speeds (KS) than the lower-risk group. Confirmatory findings would suggest that the algorithm was enriching a population for increased risk of PD.

Statistical methods

UPSIT, RBDSQ and BRAIN-tap test scores between the 15% highest and 15% lowest risk individuals according to the algorithm were compared using t-tests and described using means and 95% confidence intervals, where data were normally distributed. Where data did not follow a normal distribution, medians (with interquartile ranges) and the Wilcoxon Rank Sum test were used. Comparisons using categorical data were made using the Chi-squared test. The relationships between UPSIT, BRAIN and RBDSQ scores with estimated risk of PD (transformed to log odds) in the whole dataset were also examined using median, linear and Poisson regression respectively.ⁱⁱ All analyses were performed using Stata.

5.3 Results

Figure 5.1 shows the flow of participants in the study. Of the 1463 individuals that registered, consented and completed the survey, 140 of these met exclusion criteria in light of their past medical history (including existing PD and other neurological diagnoses), country of residence or age. This left 1323 eligible survey responders, of whom 1146 undertook the BRAIN-tap test and submitted results for at least one hand. Upon analysing these data, 67 BRAIN-tap test results were deemed unsuitable for inclusion leaving 1079 results for the final analysis. Most excluded BRAIN-tap test results were because of implausibly low (<20) or implausibly high (>110) KS scores since they indicated that the test instructions had been misunderstood or, that the test was performed using two hands together rather than separately. Other cases were excluded if their dysmetria score (DS) was greater than or equal to 1.5, indicating at least half the keystrokes were to the wrong key. These cut-offs were assigned based on previous BRAIN-tap test data (see chapter 4). The funding enabled 1065 participants to be sent smell tests. Results were received for 908 of these either via the website or as a hard copy, of which 20 were incomplete and 888 were suitable for inclusion in final analysis. Thus 81.6% of eligible individuals who successfully undertook the survey

ⁱⁱThis part of the analysis was performed by Jonathan Bestwick.

completed a suitable BRAIN-tap test, and 83.4% of people that were sent a smell test completed it and submitted results that could be used in the analysis.

The prevalence of factors that contributed to individuals' predicted risk of PD is presented for the whole group, for the 15% of participants with the highest and for the 15% of participants with the lowest estimated risks in Table 5.1. The results for the three proxies (UPSIT, RBDSQ and BRAIN-tap test scores) were also determined in these three groups (Table 5.2).

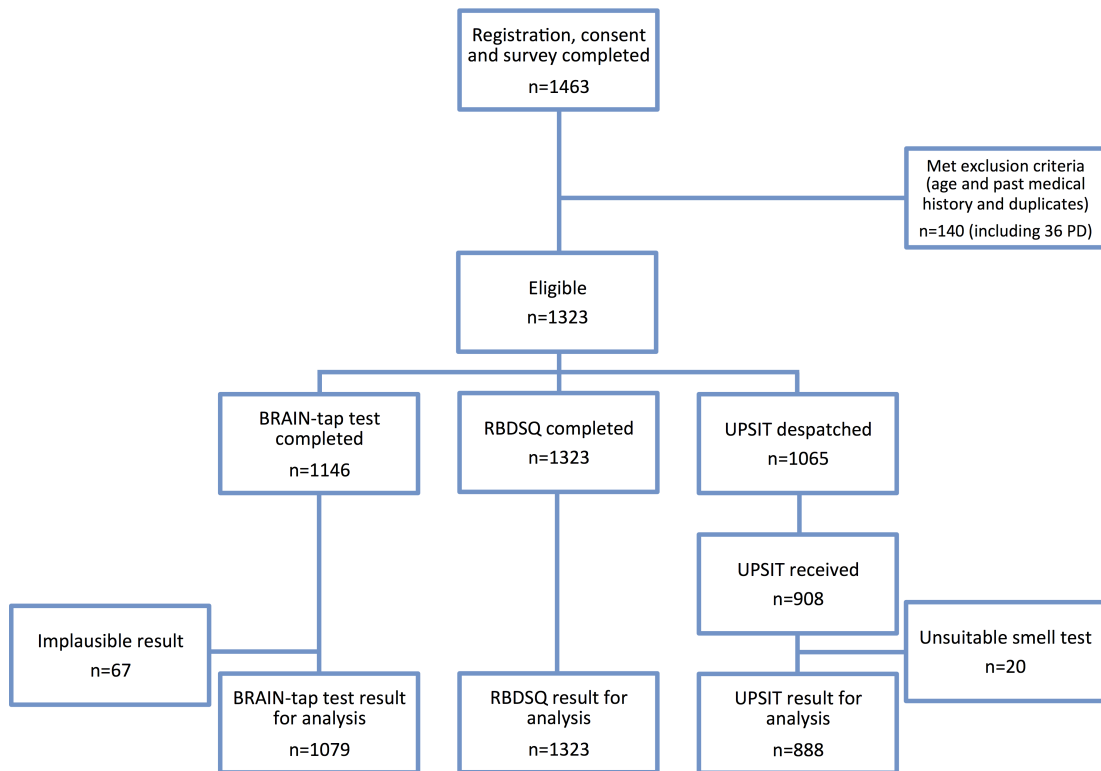


Fig. 5.1: Number of participants that were recruited, were eligible and that were sent and completed acceptable smell tests (UPSIT), REM-sleep behaviour disorder questionnaires (RBDSQ) and finger tapping (BRAIN-tap) tests.

	All subjects n=1,323	Higher risk n=198	Lower risk n=198
Female	806 (61%)	42 (21%)	170 (86%)
Median age (IQR)	66.2 (63.5-70.5)	70.2 (67.1-74.7)	63.0 (61.4-64.6)
Smoking status			
· Current	51 (4%)	3 (2%)	28 (14%)
· Former	541 (41%)	87 (44%)	88 (44%)
First degree relative	208 (16%)	74 (37%)	1 (1%)
Drink coffee	1187 (90%)	173 (87%)	194 (98%)
Drink alcohol	1143 (86%)	179 (90%)	177 (89%)
Hypertension	348 (26%)	59 (30%)	75 (38%)
NSAID use	83 (6%)	6 (3%)	19 (10%)
CCB use	155 (12%)	30 (15%)	25 (13%)
Beta blocker use	103 (8%)	30 (15%)	15 (8%)
Constipation	215 (16%)	73 (37%)	1 (1%)
Head injury	327 (25%)	86 (43%)	11 (6%)
Mood	159 (12%)	37 (19%)	9 (5%)
ED (males)	180 (35%)	132 (85%)	0 (0%)

Table 5.1: The prevalence of factors that contributed towards risk estimates shown for all participants and 15% of participants with highest and lowest risk estimates.

Legend: IQR = interquartile range; NSAID = non-steroidal anti-inflammatory drugs; CCB = calcium channel blockers; ED = erectile dysfunction.

Outcome	All subjects n=1,323	p-value*	Higher risk n=198	Lower risk n=198	p-value**
UPSIT					
n	888	-	138	130	-
Median (IQR)	32 (29 to 34)	<0.001	31 (28 to 34)	33 (31 to 35)	<0.001
n (%) ≤ 27	135 (15%)	-	30 (22%)	7 (5%)	<0.001
RBDSQ					
n	1323	-	198	198	-
Median (IQR)	2 (1 to 3)	<0.001	2.5 (1 to 4)	2 (0 to 3)	<0.001
n (%) ≥ 5	202 (15%)	-	46 (23%)	23 (12%)	0.003
KS					
n	1079	-	161	161	-
Mean (95% CI)	53.6 (53.0 to 54.2)	<0.001	50.9 (49.3 to 52.4)	54.6 (52.9 to 56.3)	0.002
n (%) ≤ 44	181 (17%)	-	34 (21%)	27 (17%)	0.394

Table 5.2: UPSIT, RBDSQ and KS in all subjects and comparing those with highest and lowest estimated risk of PD.

Legend: IQR = interquartile range, KS = kinsesia score for the worst hand, RBDSQ = REM sleep behaviour disorder screening questionnaire, UPSIT = University of Pennsylvania smell identification test.

* p-value derived from regression analyses; ** p-value from comparative analysis between higher and lower risk groups using Wilcoxon Rank Sum for UPSIT and RBDSQ, t-test for KS for continuous data, and Chi-squared tests for categorical data.

In the 15% of subjects with highest estimated risk, the median UPSIT score was 31 (IQR 28 to 34) and in the 15% of subjects with the lowest estimated risk it was 33 (IQR 31-35; $p < 0.001$). Using the lower 15th centile as a cut-off, corresponded to an UPSIT score of 27 or less.¹⁶⁰ Five percent (7/130) of the lower risk participants had an UPSIT score below or equal to 27 and 22% (30/138) of the higher risk participants ($p < 0.001$). The median RBDSQ score in the lowest and highest risk groups was 2 and 2.5 (IQR 0 to 3 and 1 to 4 respectively), and the sum of the ranks was larger in the higher risk group ($p < 0.001$). Using the RBDSQ cut-off score of ≥ 5 , more people had RBD in the higher compared to the lower-risk group (23% versus 12%, $p = 0.003$). For the BRAIN test, the mean KS scores in the worst hand for each subject in the higher and lower risk groups were 50.9 and 54.6 respectively ($p = 0.002$). There was no significant difference in the proportion of the higher and lower risk that fell beneath the 15th centile cut-off of ≤ 44 taps and there was no significant differences in the other three BRAIN test variables (data not shown).

UPSIT, RBDSQ and KS scores were plotted against estimated risk of PD in all participants (Figure 5.2). Estimated risk of PD as calculated by the algorithm doubled with a decrease of UPSIT scores by 0.67 points (95% CI 0.46 to 0.88; $p < 0.001$), an increase of RBDSQ scores by 9.5% (95% CI 6.2% to 12.7%; $p < 0.001$) and a decrease of KS scores in the worst hand by 1.0 points (95% CI 0.55 to 1.47; $p < 0.001$). Regression analyses for each outcome were repeated using risk estimates that excluded age and gender. The association between risk scores and intermediate markers remained statistically significant (UPSIT $p < 0.001$, RBDSQ $p < 0.001$, KS $p = 0.021$).

5.4 Discussion

Internet-based recruitment gave rise to a large sample size without high expenditure; the participants were recruited relatively easily and completion rates for individual stages of the study were high. The penetration of the internet has increased over the last decade, including use in the over 60's age group. This means that similar research can be undertaken via the internet as a means of accessing large populations, with frequent re-testing and relative convenience, whilst dramatically reducing the cost, when compared to traditional longitudinal studies. The methods relied on self-recruitment, which introduces potential for selection bias, and also on self-reporting without confirmation of results (except smell testing and tapping speed which were objectively measured). This method, even with future modification, is unlikely, on its own to be a reliable measure of pre-diagnostic PD. However, one outcome of this type of study would be to

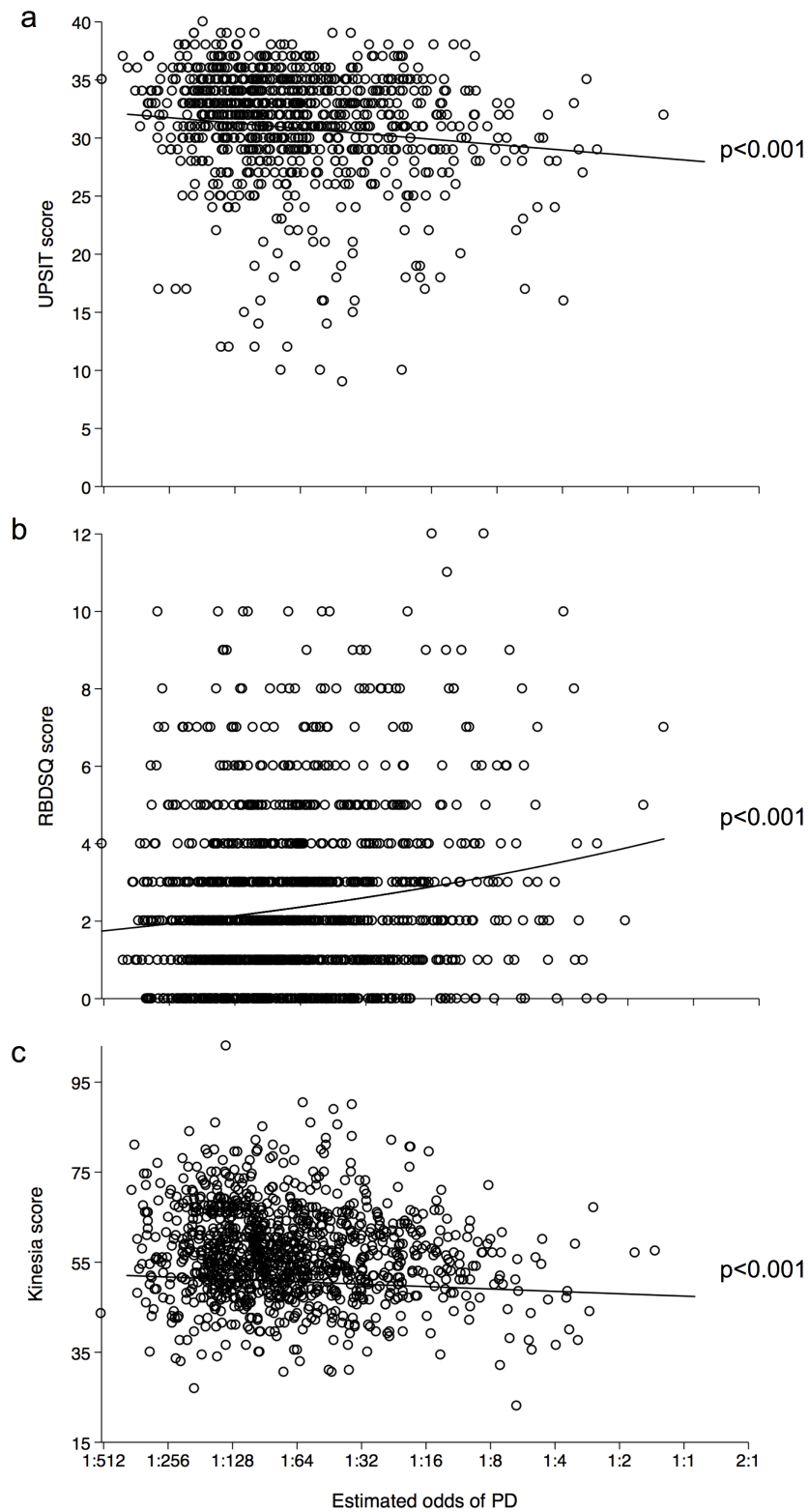


Fig. 5.2: (a) Smell test (UPSIT), (b) RBD questionnaire (RBDSQ) and (c) finger tapping scores plotted against estimated risk of PD with regression curves and p-values.

provide a mechanism by which a group at increased risk can be identified from the general community for inclusion in more detailed studies. These could be more demanding in terms of time, resources and effort by participants and researchers, including those using imaging and laboratory biomarkers, or potentially in the future, preventative trials (Figure 5.3).

A significant difference in the average UPSIT score between the higher and lower risk groups identified through the algorithm was observed, and a significant difference in proportion in the higher and lower risk group that had a score equal to or below the lower 15th centile cut-off of 27. Analogous results were reported from the PARS study where hyposmics were significantly more likely to report non-motor features, including anxiety and depression, constipation, and rapid eye movement sleep behaviour disorder symptoms, and to report changes in motor function.¹⁶⁰ RBD, as suggested by an RBDSQ score of ≥ 5 , was significantly more frequent in the high-risk than the low-risk group, and tapping speed was significantly lower in the high-risk group (in analyses using continuous data, with a trend observed in categorical data), indicating that at least some of the individuals in the higher risk group may be in the very earliest stages of motor impairment. Subtle changes in movement control have previously been reported in video footage of the footballer Ray Kennedy several years before onset of diagnosed PD and in patients with confirmed RBD.^{76,93} Of note is also that the scores for all three proxies for PD were significantly worse with increasing predicted risk of PD across all participants. These analyses remained statistically significant when age and gender were removed from the risk estimates. Given the role that age (and to a lesser extent gender) has on risk of PD and since each of the three outcomes could also be affected by age, it is reassuring to find that the remaining combined risk factors were still associated with outcomes.

Individually, none of these three outcomes is both specific and sensitive for early PD. However, evidence from pathological, epidemiological and imaging studies suggest that each of them is associated with an increased risk of PD, and all of them were found to be enriched in the higher risk group defined by the preliminary algorithm.^{9,161} Taken together, these results all indicate that this evidence-based algorithm has the potential to be a useful tool to identify groups at higher risk of future PD.

Limitations

Firstly, many of the participants volunteered following an advertisement by a Parkinson's charity. Therefore many had a family history of PD and may have been fearful about their own risk of the disease. This is a measurable bias in part and increases the likelihood of finding those at high-risk. Second, in using

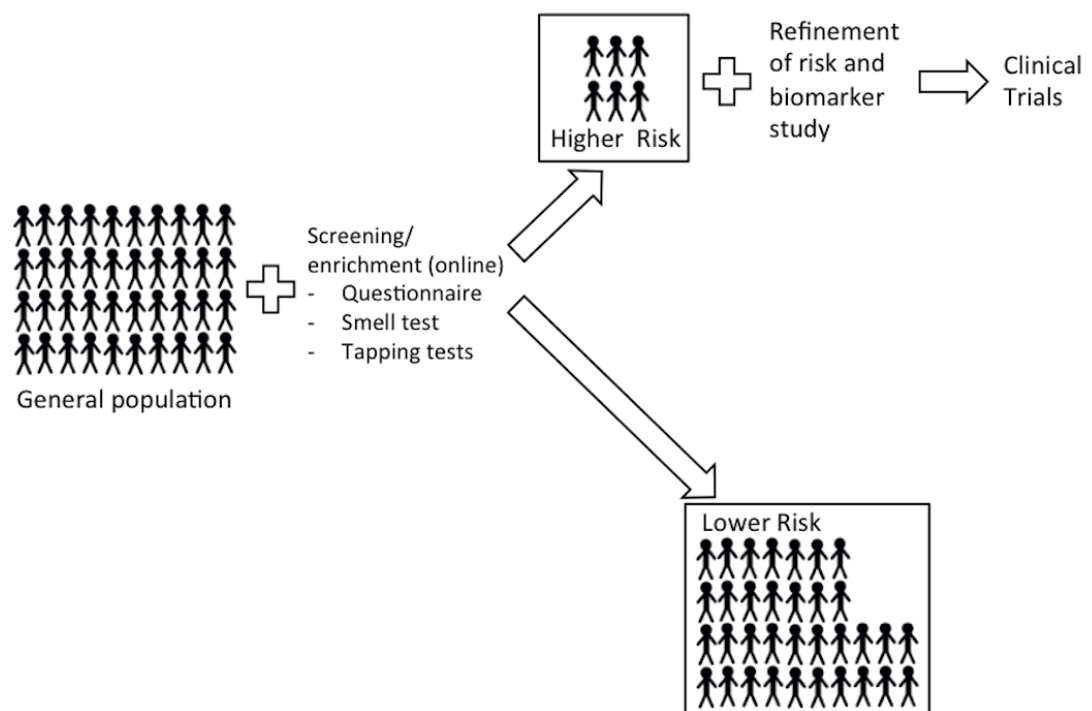


Fig. 5.3: Schematic to indicate how the PREDICT-PD approach might channel into detailed biomarker and risk-determination studies in groups identified as being higher risk than the background population. An eventual outcome could be preventative trials, were suitable drugs to be available.

proxies for future PD risk it is unknown currently what proportion of those that are at higher risk will go on to develop PD. The numbers are likely to be small given the number of participants in the study to-date, and the incidence of PD in the general population. Nonetheless, the conversion of individuals in the higher risk group and not the lower risk group to clinically established PD would offer strong evidence of the predictive nature of the algorithm. Third, for this analysis it was assumed the predictors were independent, whereas in fact there may be some association between individual factors. Although many of the effect size estimates from the systematic review were adjusted for confounders (see chapter 2), fully adjusted estimates were not available for a number of factors. The simple additive model used may therefore not fully replicate the early stages of the disease. Currently there are insufficient data in the literature to account for interactions between exposures, and one aim of the longitudinal study is to modify the results based on emerging prospective data. Fourth, subjective RBD and some other clinical features were not confirmed using PSG or other objective tests. This is likely to reduce the accuracy of these predictive factors; but the purpose of this methodology is to be easily available and non-invasive, which objective confirmatory tests often are not. It was notable that the proportion of subjects exceeding the cut-off for RBD was high (15% of the entire cohort). Fifthly, due to the design of the study, there is an additional bias in that participants that are English-speaking and computer literate have been identified. Finally, in order to test the initial validity of the approach a conservative method was adopted, excluding the most promising risk factors of smell loss, RBD and tapping speed from the algorithm, and these used as proxies to evaluate its performance. The results were statistically significant despite their exclusion and it is likely that subsequent inclusion of smell loss, RBD and tapping speed would improve the performance of the algorithm. However to do this will require estimation of the magnitude of risk conveyed by each since absolute data are currently not available in the literature. These hypotheses will be tested further when longitudinal results are available from this study, which will likely result in modifications to the preliminary algorithm.

Conclusions

This is one of largest cross-sectional studies to-date examining methods to identify a group of individuals with risk factors for developing PD. The methods are based on a comprehensive systematic review of the literature on early features and risk factors of PD that can be identified through history taking, and on calculating estimates of risk through combination of these factors. Support for the hypothesis that those deemed to be at higher risk of PD would have poorer smell sense,

increased rates of RBD and slower finger tapping speed, when compared to lower risk, was found. Ultimately, confirmation of the validity of this algorithm requires recording an increased rate of incident PD in the higher risk group, and this information will only become available during longitudinal follow-up.

Chapter 6

GBA and *LRRK2* variants in PREDICT-PD

6.1 Introduction

Variants in the glucocerebrosidase gene (*GBA*) are the most common genetic risk factor for PD, found in approximately 9% of unselected UK PD patients and 4% of healthy controls.^{32,162} Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are the most common Mendelian cause of PD (1% of sporadic UK cases) but rare in controls (<0.1%).²¹

In earlier chapters an evidence-based process of risk stratifying members of the population for future PD was described. In chapter 5, higher risk subjects from the PREDICT-PD study were found more likely to have objective smell impairment and slowed finger-tapping, as well as subjective RBD, compared with participants estimated to be at lower risk.

Recently, early non-motor features of PD were reported in carriers of *GBA* mutations including depression, subjective RBD and objective olfactory disturbance, when compared with healthy controls.³⁴ Objective motor impairment was observed in the same subjects and this was the first report from a longitudinal study in non-manifesting *GBA* mutation carriers. Away from asymptomatic *GBA* variant carriers, there have been a number of studies reporting on the phenotype of parkinsonian *GBA* carriers, who tend to have a worse cognitive profile than patients with idiopathic PD and may have an increase in neuropsychiatric symptoms.^{32,163,164}

There has been limited study in non-manifesting carriers of *LRRK2* mutations to-date, but some observations suggest higher rates of non-motor features of PD, such as constipation and impairment of colour vision, compared with non-carriers.¹⁶⁵ In parkinsonian *LRRK2* carriers, olfactory deficit has been variably

reported, with some saying it is as frequent and severe as that found in idiopathic PD, and others suggesting it is milder.^{26,27} Other observations include potentially lower frequency of gastrointestinal dysfunction and RBD compared with idiopathic PD.¹⁶⁶ Some of the heterogeneity of *LRRK2*-related parkinsonism may depend on the underlying pathology, with non-motor features such as cognitive impairment and anxiety occurring more commonly in those subsequently found to have Lewy bodies.¹⁶⁷

Acknowledging the frequency of these mutations (particularly *GBA* variants), it was hypothesised that if PREDICT-PD risk stratification were effective, an excess of *GBA* variants and perhaps *LRRK2* variants would be found in higher risk subjects compared to lower risk, and this would provide further evidence in favour of enrichment for PD risk.

6.2 Methods

Detailed information about recruitment and risk stratification are given in chapters 2 and 5. In brief, 1,323 eligible participants were recruited via the study website, following a limited advertising campaign which included an email to members of Parkinson's UK. Inclusion criteria were residency in the UK and age 60-80 years. Exclusion criteria were pre-existing PD, neurological disease or medication associated with iatrogenic parkinsonism. Participants completed an online consent form, followed by a survey comprising demographic questions and items related to early non-motor features and risk factors for PD identified through systematic review (see chapter 2), and a keyboard tapping task (the BRAIN-tap test; see chapter 4). Subjects were sent the US version of the University of Pennsylvania smell identification test (UPSIT), the answers to which were also completed online. The Queen Square Research Ethics Committee approved the study (reference 10/H0716/85).

A preliminary algorithm was developed to estimate PD risk based on questionnaire answers. Risk scores were ranked to enable comparative analyses between subjects with the highest 15% and lowest 15% risk scores. Invitations were extended to these subjects, and additional subjects sampled randomly from the middle risk group, to participate with in-person clinical (including motor and brief cognitive assessment) and genetic studies. A greater likelihood of identifying differences in outcomes was anticipated if extremes of risk were sampled. Therefore higher, middle and lower risk subjects were approached with a ratio of 2:1:2 (80/40/80 subjects respectively).

During clinical visits, further written consent was obtained and participants provided saliva samples via specialised collection tubes, which were kept at room

temperature during transit for 1-4 days, before being cooled to +4°C for longer term storage. DNA was extracted using standard methods. Sanger sequencing was used to screen exon 41 of *LRRK2* (which contains the commonest c.6055 G>A; p.G2019S mutation) and exons 8-11 of the *GBA* gene (contains almost 90% of recognised *GBA* pathogenic variants).³² The sequence reference and exon numbering were those of RefSeq accession number NM000157.3 (*GBA*) and XM058513 (*LRRK2*). The conventional nomenclature for *GBA* mutated alleles was used, referring to the processed protein, excluding the 39-residue signal peptide.ⁱ

Statistical methods

The proportion of the higher risk group carrying gene variants and the proportion of pooled lower and middle risk groups were compared using Fisher's exact test and an odds ratio (OR) and 95% confidence interval (CI) were calculated. Cases of PD identified at the time of examination were excluded from all analyses. Statistics were performed using Stata and GraphPad Prism for Mac.

6.3 Results

At the time of analysis, 192 saliva samples had been collected from participants. Sequencing was undertaken in 79/195 higher, 45/927 middle, and 68/198 lower-risk subjects (Table 6.1). After excluding 7 sequencing failures, *GBA* variants were found in 6/75 higher (1 N370S, 2 E326K, 3 T369M), 0/43 middle and 1/67 lower-risk subjects (T369M homozygous). The OR of having a *GBA* variant in the higher versus pooled middle and lower-risk group was 9.5 (95% CI 1.10 to 440; $p=0.018$). *LRRK2* G2019S mutations were not found in any participant.

Comparisons between *GBA* variant carriers from the higher risk group, with the remainder of the higher, middle and lower risk groups were undertaken (Table 6.2). Results in higher risk *GBA* variant carriers reflected some of the characteristics upon which risk was calculated (age, gender, family history), as well as a tendency for lower smell test scores overall. In subjects carrying *GBA* variants, mild motor impairment was found in three (mainly soft signs e.g. stooped posture, not sufficient to diagnose PD), mild cognitive impairment in three and hyposmia in two, with one subject scoring highly on the REM-sleep behaviour disorder (RBD) screening questionnaire (Table 6.3).

ⁱExperiments were performed by Alastair Noyce and Niccolo Mencacci.

GBA and LRRK2 variants in PREDICT-PD

	Higher risk participants	Middle risk participants	Lower risk participants	p-value
Subjects per group	195	927	198	
Number screened	79	45	68	
Sequencing failures	4	2	1	
Sequenced (% of group)	75 (38%)	43 (5%)	67 (34%)	
<i>GBA</i> variants	6	0	1	0.018*
<i>LRRK2</i> G2019S mutations	0	0	0	

Table 6.1: Participants, proportions tested and variants across groups.
Legend: *p-value from Fisher’s exact test.

	Higher risk <i>GBA</i> carriers	Higher risk non-carriers	Middle risk participants	Lower risk participants
Number	6	189	927	198
Median age in years (range)	70 (67-76)	70 (60-81)	66 (60-80)	62 (60-73)
Female (%)	1 (17%)	39 (21%)	597 (64%)	168 (85%)
First-degree relative (%)	4 (67%)	68 (36%)	134 (15%)	1 (1%)
Median UPSIT score (IQR)	30 (23-33)	31 (28-34)	32 (29-34)	33 (31-35)
KS (95% CI)	56 (48-64)	51 (50-53)	54 (53-55)	55 (53-57)

Table 6.2: Clinical details of higher risk *GBA* variant carriers and other groups.
Legend: UPSIT = University of Pennsylvania smell identification test; IQR = interquartile range; CI = confidence interval.

Rank (out of 1,323)	Variant	UPDRS	RBDSQ	MoCA	UPSIT
1	N370S	3	7	25	32
14	T369M	6	3	25	24
41	E326K	0	2	25	29
100	E326K	3	1	28	21
108	T369M	5	1	30	30
143	T369M	4	0	28	35
1185	T369M*	0	1	27	31

Table 6.3: Further details of individual *GBA* variant carriers (6 carriers from higher risk group above line and 1 carrier from the lower risk group below line).
Legend: UPDRS = unified Parkinson’s disease rating scale (motor section); RBDSQ = REM sleep behaviour disorder screening questionnaire; MoCA = Montreal cognitive assessment; UPSIT = University of Pennsylvania smell identification test. *Homozygous variant.

6.4 Discussion

PREDICT-PD uses a combination of environmental determinants and early phenotypic (non-motor) features to develop a prediction algorithm for PD that estimates risk. *GBA* variants were found in 8% of higher-risk subjects, a proportion similar to estimates of frequency in sporadic PD.³² Conversely, less than 1% of lower-risk subjects were found to have a *GBA* variant. An overall rate of 3.6% in the total sample was observed, which is similar to the 4% reported in healthy controls.¹⁶² Age is a powerful determinant of PD risk but has the potential to confound given its effect on many outcome measures used in predictive studies (e.g. sense of smell, objective motor function and functional imaging). By using *GBA* variants as an outcome for risk stratification, age-independent support for enrichment by the preliminary algorithm was observed.

Of the variants identified from exons 8-11 of the *GBA* gene, one subject had N370S, two subjects had E326K and four subjects had T369M variants. The N370S variant has a deleterious effect on glucocerebrosidase enzyme activity and is known to cause Gaucher's disease (GD) as well as being a clear risk factor for PD. Although not a cause of GD, the E326K variant is also a known risk factor for PD, and there is emerging evidence to support the T369M variant similarly.¹⁶⁸ The fact that no *LRRK2* mutations were found in subjects screened was perhaps unsurprising given the rarity of the G2019S mutation in healthy controls and its presence in only 1-2% of patients with PD.²¹ However, if any subjects had been identified as *LRRK2* mutation positive this would have been a potentially important observation.

GBA-associated PD can be indistinguishable from sporadic PD, with typical motor impairment that has a good response to standard treatment, and presence of non-motor features.³² *GBA* variants may convey younger age of onset and preponderance towards cognitive impairment. In kind with studies of non-manifesting *GBA* variant carriers, low-normal smell, mild motor signs and mild cognitive impairment were observed in PREDICT-PD *GBA* heterozygotes.³⁴ One of the *GBA* heterozygotes also scored highly on the RBDSQ, whereas others fell short of the 5 point threshold score for RBD.¹⁵² Here *GBA* variant carriers were identified using early phenotypic features for PD, but the motor and non-motor features listed in Table 6.3 were not included in risk score calculations. This suggests clustering of early phenotypic non-motor features as has been observed in other studies.^{118,160} Of great interest, although perhaps a chance finding, is that the subject with the highest estimated risk in the whole study at baseline was a carrier of the N370S mutation. Carriers of other variants were otherwise distributed evenly throughout the higher-risk group.

Limitations

There are some limitations to the current study. Firstly, the findings are based on small numbers, making it difficult to draw robust conclusions. Despite this, the difference in proportions is clinically meaningful and statistically significant. Furthermore the methods (in-person assessment of a proportion of the online cohort) favoured sampling from the extremes of risk, resulting in a smaller proportion being sampled from the middle risk group. This raises the possibility of underestimating the frequency *GBA* variants, but to assess an equivalent proportion of middle risk participants (35-40%) would have required an additional 350 clinical visits, which was not feasible. In light of these limitations, self-administered saliva collection will be used to obtain DNA from all participants to enable further testing for *GBA* and *LRRK2* in the cohort (see chapter 9 for further work). The purpose of this is to confirm the observations made in this smaller sample and also because *GBA* variants are risk factors in their own right and may eventually contribute to the risk scoring process.

Of the three variants discovered, there is some controversy over whether T369M constitutes a true disease-associated variant. Even if subjects carrying T369M variant were excluded from the analysis (3 from higher-risk and 1 from lower-risk), the results were still of borderline statistical significance ($p=0.06$). Nonetheless, recent reports suggest that although T369M is not sufficient to cause GD, there is change in glucocerebrosidase activity in heterozygous carriers of the T369M and E326K variants and it is feasible that these could confer an increase in risk of PD.¹⁶⁸

Conclusions

Taken with earlier observations in chapter 5, these data using *GBA* variants as an outcome for PD risk, support the notion that the preliminary PREDICT-PD algorithm can separate healthy controls into a higher risk group with a mutation frequency of 8% compared with 0.9% in lower risk subjects. Validation of the PREDICT-PD risk algorithm will ultimately be achieved through finding an excess of converters to clinically established PD in the higher risk group with prolonged follow-up. The higher risk group (and similar numbers of lower risk) will continue to be studied using a range of clinical and imaging studies, with further genetic evaluation undertaken across the cohort.

Chapter 7

Imaging studies in PREDICT-PD

7.1 Introduction to TCS and SPECT

Ultrasound may play a role in early diagnosis of PD and is attractive due to its availability, simplicity and cost.¹⁰⁵ The technique of transcranial sonography (TCS) has been most studied in mainland Europe and at the time of beginning this work there was only believed to be one other centre in the United Kingdom using TCS for the assessment of movement disorders. Hyperechogenicity describes an enlarged and contiguous area of increased echogenic signal and when found in the region of the substantia nigra (SN) is referred to as SN hyperechogenicity (Figure 7.1). This appearance has been observed in 90% of patients with established PD.¹⁶⁹ However, SN hyperechogenicity is not correlated with disease severity or duration, and does not change over time in patients with PD.^{169,170} It may also be observed as a static marker of PD risk, which occurs before the onset of motor symptoms and does not change with follow-up.^{171,172}

A study in individuals with mild parkinsonian features, found a sensitivity of 91%, specificity of 82% and positive predictive value (PPV) of 93% for SN hyperechogenicity and PD diagnosis after follow-up, despite blinding those performing sonography to clinical details at baseline.¹⁷³ The PRIPS study in Germany and Austria screened more than 1,800 participants over the age of 50 years with physical examination and TCS, and 304 had SN hyperechogenicity. At 3 years follow-up, 11 had developed PD and the relative risk of incident PD was 17.3 (95% CI 3.7 to 81.3) if there was SN hyperechogenicity at baseline.¹⁰⁶

The pathological abnormality that is being observed using TCS is uncertain, but post-mortem studies implicate activated microglial, iron accumulation and neuromelanin as contributing to the altered signal.¹⁷⁴ A further limitation of the technique is that 10% of people lack the necessary temporal bone window needed for evaluation.¹⁰⁵

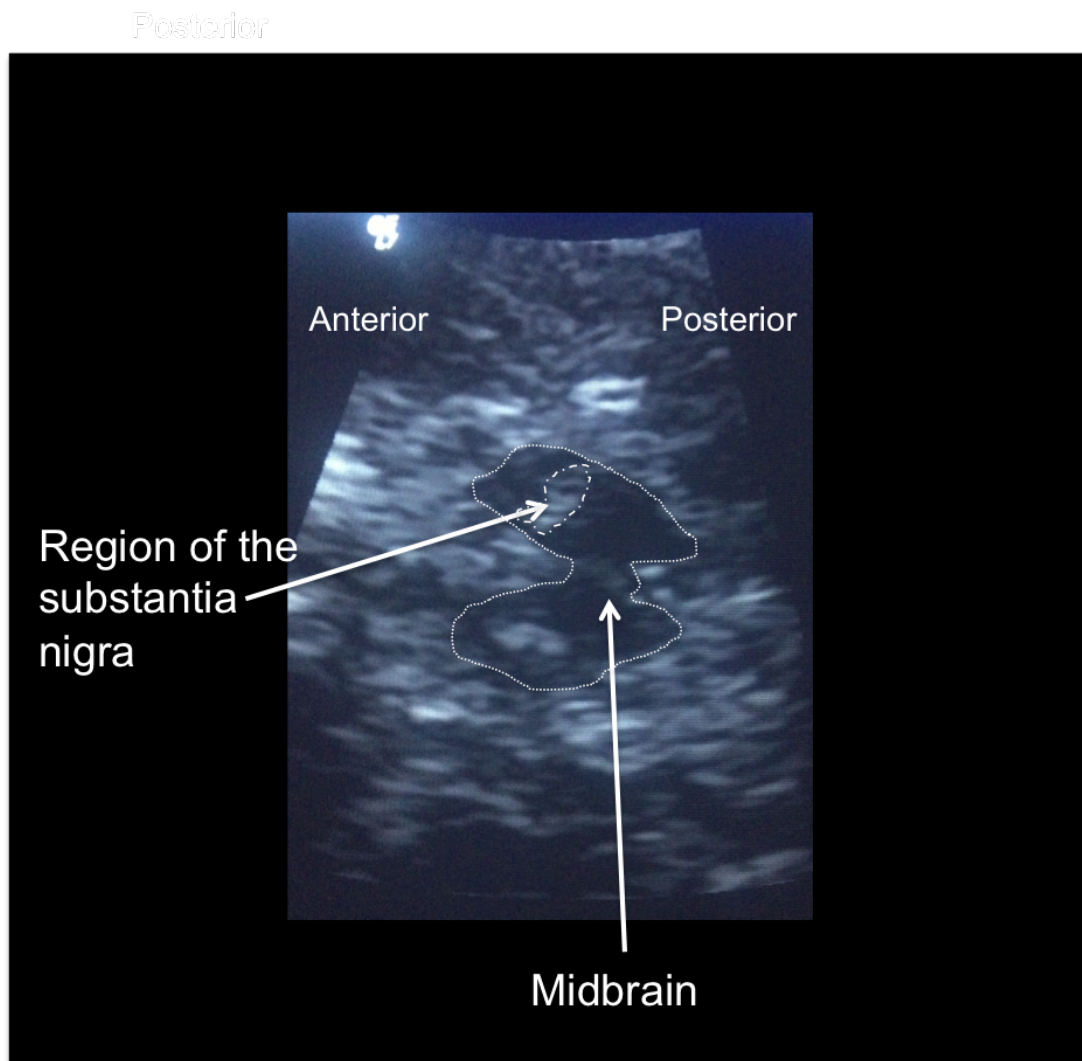


Fig. 7.1: An example of hyperechogenicity in the region of the substantia nigra in a patient with PD. Picture obtained by Alastair Noyce using GE Logiq 7 ultrasound machine in Innsbruck, Austria.

^{123}I -FP-CIT SPECT and ^{123}I - β -CIT SPECT are analogous to one another and are widely used in the work-up of parkinsonian disorders. SPECT may be helpful in cases where parkinsonism is indeterminate (e.g. isolated rest tremor) or a degenerative aetiology is uncertain (e.g. if vascular and drug-induced causes are included in the differential diagnosis).^{98,99} In a study that recruited patients with uncertain diagnosis at baseline, ^{123}I -FP-CIT SPECT alone had a low false positive rate compared with cases diagnosed on clinical grounds alone (3% versus 54%, i.e. high specificity) at acceptable sensitivity (78%).¹⁷⁵

The notion that ^{123}I -FP-CIT SPECT might be abnormal prior to PD diagnosis was prompted by studies showing bilateral changes in patients with clinically unilateral parkinsonism.¹⁷⁶ In keeping with estimates from neuropathological study, clinical diagnosis of PD tended to be made once there was 40-50% reduction in tracer binding.^{4,176} An average 11.2% decline in striatal binding has been observed each year after diagnosis.¹⁷⁷ ^{123}I - β -CIT SPECT has been used to assess those with risk factors for PD including those with RBD and idiopathic anosmia.^{69,70,73} It is being used in the PPMI study, which compares *de novo* PD with healthy controls, and in the PARS study which operates a two-step screening process for PD, with objective smell testing at the first stage and ^{123}I - β -CIT SPECT at the second. The PARS investigators recently reported differences in dopamine transporter (DaT) binding in hyposmic subjects compared with those that were normosmic.¹⁰⁴

TCS and ^{123}I -FP-CIT SPECT were used as outcome measures to determine whether subjects estimated to be at higher risk of PD using the PREDICT-PD preliminary algorithm, were more likely to display abnormal imaging features associated with PD, compared with those estimated to be at lower risk.

7.2 Training to perform TCS

Training was undertaken during a one-month period between February and March 2013 at the Department of Neurosonologie, Ländeskrankenhaus Innsbruck Austria, and the Department of Neurology, University of Innsbruck. Training in sonography was overseen by Dr Martin Sojer (Oberarzt) and Dr Heike Stockner (Post Doctoral Research Fellow), who have extensive experience in the use of TCS of the mesencephalon and vascular system.

7.2.1 Methods

Patients that were attending for routine vascular sonography of the extra- and intra-cranial arteries (undertaken by a trained sonographer) gave further verbal consent for sonography of midbrain structures so that familiarity with the technique

could be gained. Additional experience was gained with patients in the emergency room setting and the intensive care unit. A smaller number of patients with movement disorders were invited to the Department of Neurosonologie including those with PD, MSA, essential tremor, and dystonia, as well as some patients with RBD (with and without parkinsonism).

Scanning was undertaken using a range of ultrasound machines including the GE Logiq 7, GE Logiq 9 and Acuson Sequoia. Experience was also gained with the GE Vivid-E, which is a portable machine, and another portable Philips machine. Most patients were scanned in the semi-recumbent position with the operator seated on the patient's right hand side. The right side of the head was examined first with the patient's head turned slightly to the left, then the left side of the head was examined with the head turned to the right. The GE Logiq 7 was most frequently used and was the machine for which all analyses were undertaken. A standardised setup was used comprising a 2.5MHz probe, with preset grey-scale and image gain, and tissue harmonics switched off (i.e. non-harmonic mode). Focus and depth were set to the centre of the screen, where the midbrain was expected to be identified.

The mesencephalic scanning plane was identified, in which the midbrain has a typical butterfly appearance (Figure 7.1). The region of the SN was identified as a contiguous echogenic area. When best visualised, the image was frozen and the area of echogenicity was measured using both elliptical and free hand methods, to give an estimate of the area in centimetres squared (cm^2). The same steps were followed when scanning both sides and a measurement was recorded for each, along with the side that had the largest area (SN-Max) and the average of both sides (SN-Mean). The width of the third ventricle was recorded in cm. During training, further examination was made to identify the anterior horn of the lateral ventricle, the caudate nucleus, the putamen, the thalamus, the raphe nucleus, the cerebral aqueduct, the rubral nucleus, the amygdala, the hippocampus and the pineal gland, which to-date are structures that are incompletely defined using TCS. If subjects had a temporal bone window that prevented visualisation of the mesencephalon, note was made that it was inadequate.

Statistical methods

Subjects were included in the analyses if they were scanned using the GE Logiq 7 machine and had at least one adequate temporal bone window. Descriptive statistics were calculated for controls and patients with parkinsonism. Normally distributed data were reported as group means with 95% confidence intervals (CI) and compared using t-tests. Non-normally distributed data were reported using medians and interquartile ranges (IQR), and groups compared with the

Mann Whitney U test. Receiver operated characteristic (ROC) curves were drawn to determine cut-offs that separated cases with parkinsonism from controls with corresponding sensitivity and specificity. Fisher's exact test was used for categorical data to determine differences in the proportion of cases and controls with hyperechogenicity. Spearman's correlation was undertaken in controls to determine the role of age on non-normally distributed continuous measures.

7.2.2 Results

During the four-week time period, 108 patients were scanned (Table 7.1). Gender and diagnosis was recorded for all subjects (68 males, 40 females) and age was recorded in 59 of these. Most subjects were attending for routine examination of the intra- and extra-cranial arteries, and are hereby referred to as vascular controls (n=70). Other diagnoses included PD (n=8), MSA (n=1), essential tremor (n=1), dystonia (n=2), ptosis (n=2), intra-cranial haemorrhage (ICH, n=7), RBD (n=5), acute stroke (n=3), ataxia (n=1), head injury (n=1), abnormal gait (n=1), dementia (n=1), seizure (n=1), dizziness/syncope (n=2), amaurosis fugax (n=1) and temporal arteritis (n=1).

Eighty-nine subjects were scanned using the GE Logiq 7 machine. In thirteen subjects, both bone windows were inadequate for visualisation of the midbrain and four had vascular ultrasound only without additional parenchymal sonography. Therefore 72 subjects had at least one bone window that was sufficient for examination of the midbrain. Analysis was conducted on measurements taken only after sufficient technical experience had been gained and the area of interest could be identified confidently and independently of the trainers. Measurement of at least one area of the SN was made for 51 subjects. Of these, six patients with parkinsonism (4 PD and 2 RBD plus parkinsonism) were compared to 42 non-degenerative, non-movement disorder control subjects (39 vascular controls, 2 ICH and 1 syncope).ⁱ

The median SN-Max in control subjects was 0.15cm² (IQR 0.09 to 0.20cm²) and was 0.37cm² (IQR 0.24 to 0.55cm²) in PD patients (p<0.001; Figure 7.2a). ROC curves were constructed for SN-MAX and SN-Mean. The ROC curve for SN-Max had an area under curve (AUC) of 0.94, with a sensitivity of 100% and specificity of 81% for identifying parkinsonism if a cut-off of 0.23cm² was used (Figure 7.2b). The ROC curve for SN-Mean had an AUC of 0.95, with a sensitivity of 83% and specificity of 93% in identifying PD if a cut-off of 0.23cm² was used (not shown). Using an SN-Max cut-off of 0.23cm² meant that 8/42 (19%) of

ⁱThe remaining three non-parkinsonian cases were excluded (1 with muscular dystrophy, 1 with essential tremor and 1 with isolated RBD).

controls had SN hyperechogenicity compared with 6/6 (100%) of parkinsonian patients ($p < 0.001$).

Information regarding year of birth was available for 32/42 controls. There was no correlation between year of birth and SN-Max ($r = -0.097$, $p = 0.60$) or SN-Mean ($r = -0.155$, $p = 0.40$), and no gender difference in either parameter ($p = 0.29$ and 0.69 respectively).

Subjects (n=108)	
Gender (male/female)	68/40
Median age (IQR)*	66.0 (57.0 to 72.0)
Diagnosis:	
· Vascular controls	70
· Parkinsonism**	11
· Other neurological	27
Machine used:	
· GE Logiq 7	89
· GE Logiq 9	9
· Sequoia	6
· Vivid-e	3
· Portable Philips	1

Table 7.1: Subjects scanned during TCS training.

Legend: IQR = interquartile range.

* age available on 59 subjects; ** group comprised 8 PD, 2 RBD with parkinsonism, 1 MSA.

7.2.3 Discussion

This period of training and investigation provided support for the use of TCS in the evaluation of parkinsonian subjects and the results suggested that training was sufficient to practice independently in the UK.ⁱⁱ The results were comparable to previous studies comparing subjects with PD to healthy controls.¹⁰⁵ TCS has operator- and machine-dependent ranges for the echogenicity of basal ganglia structures, which must be established for the specific setting in which the method is to be applied.¹⁰⁵ In order that TCS could be used to assess participants from the PREDICT-PD study, validation of the technique was required in patients attending the Movement Disorder clinic at the National Hospital for Neurology and Neurosurgery (NHNN) and a suitable control group.

ⁱⁱFour weeks training in TCS for use in parkinsonian subjects is considered an unusually long time with other courses typically lasting 2-7 days.

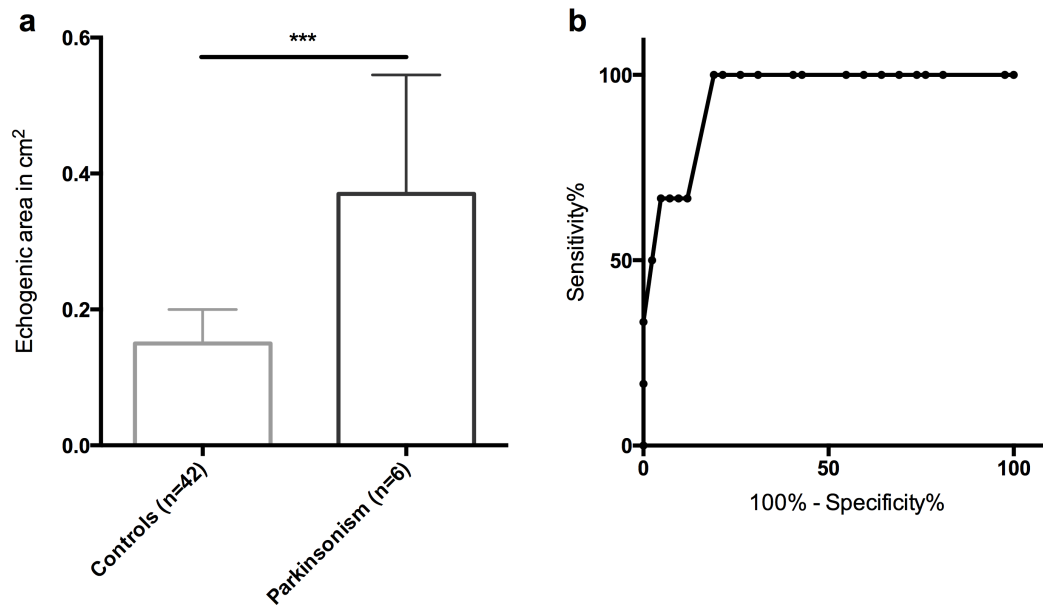


Fig. 7.2: Comparison of SN-Max in parkinsonian subjects versus controls: a) bar graph showing median and 75th centile, b) ROC curve of sensitivity against 1-specificity displayed as percentages. *** $p < 0.001$.

7.3 Validation of TCS in UK subjects

7.3.1 Methods

A validation study was undertaken in the Lysholm Department of Neuroradiology at the NHNN. Patients with PD according to UK Queen Square Brain Bank criteria were identified from the Movement Disorders clinic and invited to participate, along with a group of healthy control subjects (partners of patients, spouses, students and staff).³ Ethical approval for the study was gained from the Queen Square Research Ethics Committee (reference 13/LO/1457). The Department of Neuroradiology houses a Philips iU22 (Figure 7.3). Like the GE Logiq 7, the Philips iU22 is a high-end sonography machine suitable for transcranial studies. A 2.5MHz probe was used and the machine was operated in the ‘non-harmonic’ setting, consistent with guidance from trainers in Innsbruck. Greyscale and gain were preset to identify midbrain structures, and depth and focus were adjusted to centre the midbrain on the screen.

Participants were scanned in the semi-recumbent position, except for one parkinsonian subject who was unable to transfer from a wheelchair to the trolley. A standardised procedure was followed in which the right side of the head was scanned first and then the left. The probe was applied to the temporal bone window in the mesencephalic scanning plane and the midbrain identified. The probe was angled rostrally to identify the third ventricle before returning to the



Fig. 7.3: Philips iU22 ultrasound machine with 2.5MHz probe in the Lysholm Department of Neuroradiology at the National Hospital for Neurology and Neurosurgery.

mesencephalic scanning plane for detailed examination of the midbrain and the region of the SN. A contiguous area of echogenicity in the region of the SN was measured free-hand (rather than elliptically) for each subject with an adequate bone window. Measurements were taken from both sides (where possible) and SN-Max and SN-Mean were calculated. Additional imaging and measurement of other structures was not undertaken (examples listed in section 7.2.1).

Statistical analysis was undertaken using the same methods described above (section 7.2.1) to determine normal and disease-related operator- and machine-dependent ranges and cut-off values in the UK. In addition, linear regression (instead of correlation) was used to explore relationships between continuous outcomes (SN-Max and SN-Mean) and exposures (age and disease duration).

7.3.2 Results

Fifty patients with parkinsonism were scanned during a 15-month period, as well as 35 healthy controls (Table 7.2). Patients were older than controls and the proportion of male patients was higher than male controls. Two patients were excluded from the analysis because they had indeterminate parkinsonism rather than PD according to diagnostic criteria.³ Three control subjects were also excluded from the analysis (one had generalised myoclonus and two carried recessive genes associated with PD - *PARK2* and *PINK1*). At least one adequate bone window was present in 44/48 PD subjects (91.7%) and 32/32 controls (100%). The median disease duration in PD subjects was 7.0 years (IQR 4.0 to 10.0 years).

	PD (n=48)	Controls (n=32)	p-value
Mean age in years (SD)	63.2 (10.7)	48.8 (18.1)	
Gender (M/F)	40/8	10/22	
Adequate bone window	44/48	32/32	
Median SN-Max (IQR)	0.24 (0.20 to 0.34)	0.12 (0.08 to 0.14)	<0.001
Median SN-Mean (IQR)	0.22 (0.17 to 0.26)	0.09 (0.07 to 0.12)	<0.001
SN-Max cut-off >0.16cm ²	Sensitivity 93.2%	Specificity 90.6%	
SN-Mean cut-off >0.13cm ²	Sensitivity 90.9%	Specificity 84.4%	
SN +ve	41/44	3/32	<0.001
PPV	93.2%		

Table 7.2: Subjects scanned during TCS validation in the UK.
Legend: SD = standard deviation; IQR = interquartile range;
CI = confidence interval; PPV = positive predictive value.

The median SN-Max in patients was 0.24cm^2 (IQR 0.20 to 0.34cm^2) compared with 0.12cm^2 (IQR 0.08 to 0.14cm^2) in controls (Figure 7.4a; $p < 0.001$). Median SN-Mean in patients was 0.22cm^2 (IQR 0.17 to 0.26cm^2) compared with 0.09cm^2 (IQR 0.07 to 0.12cm^2) in controls ($p < 0.001$). A ROC curve (Figure 7.4b) for SN-Max gave a specificity of 90.6% for a sensitivity of 93.2% using a cut-off of 0.16cm^2 to diagnose PD. The corresponding ROC curve for SN-Mean gave a specificity of 84.4% for a sensitivity of 90.9% using a cut-off of 0.13cm^2 (not shown). Examples of observations from a case and a control are shown in Figure 7.5. Age was not associated with SN-Max in either patients ($\beta = 0.0013\text{cm}^2$ per year, $p = 0.339$) or controls ($\beta = 0.0005\text{cm}^2$ per year, $p = 0.307$). There were no gender differences in SN-Max for patients (0.25cm^2 in males versus 0.24cm^2 in females, $p = 0.720$) or controls (0.12cm^2 in males versus 0.11cm^2 in females, $p = 0.528$). In patients, disease duration was not associated with area of echogenicity (Spearman's $\rho = 0.198$ (95% CI -0.114 to 0.475 , $p = 0.197$)).

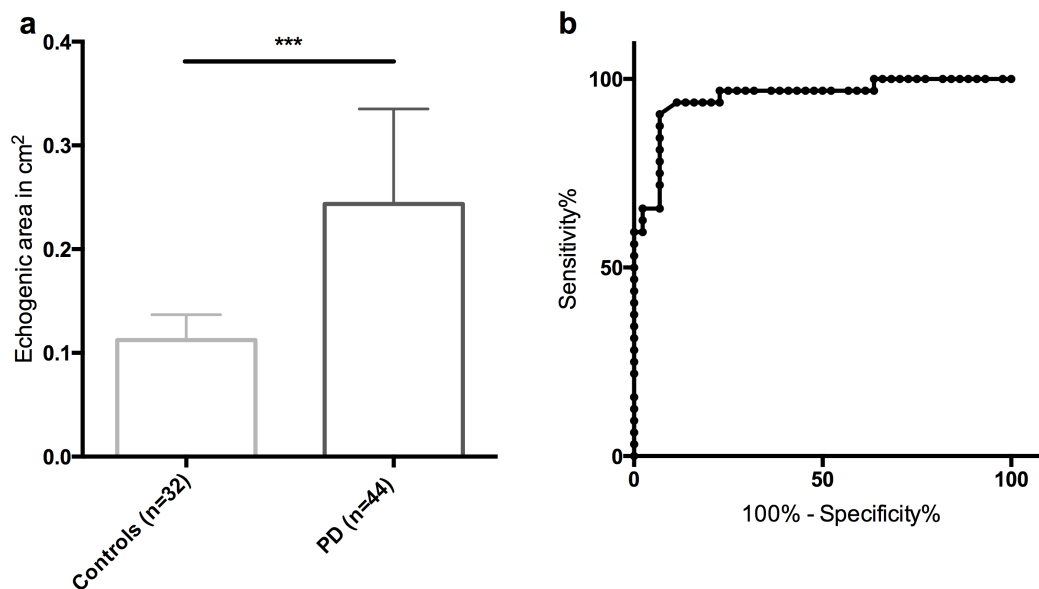


Fig. 7.4: Comparison of SN-Max in PD subjects versus controls: a) bar graph showing median and 75th centile, b) ROC curve of sensitivity against 1-specificity displayed as percentages.*** $p < 0.001$.

7.3.3 Discussion

A difference in echogenicity was demonstrated between patients and controls in line with differences identified during the training period and in the published literature.¹⁰⁵ The difference was both statistically significant and clinically meaningful, enabling machine- and operator-dependent cut-off values to be established for the next phase of the study. SN-Max offered marginally better screening

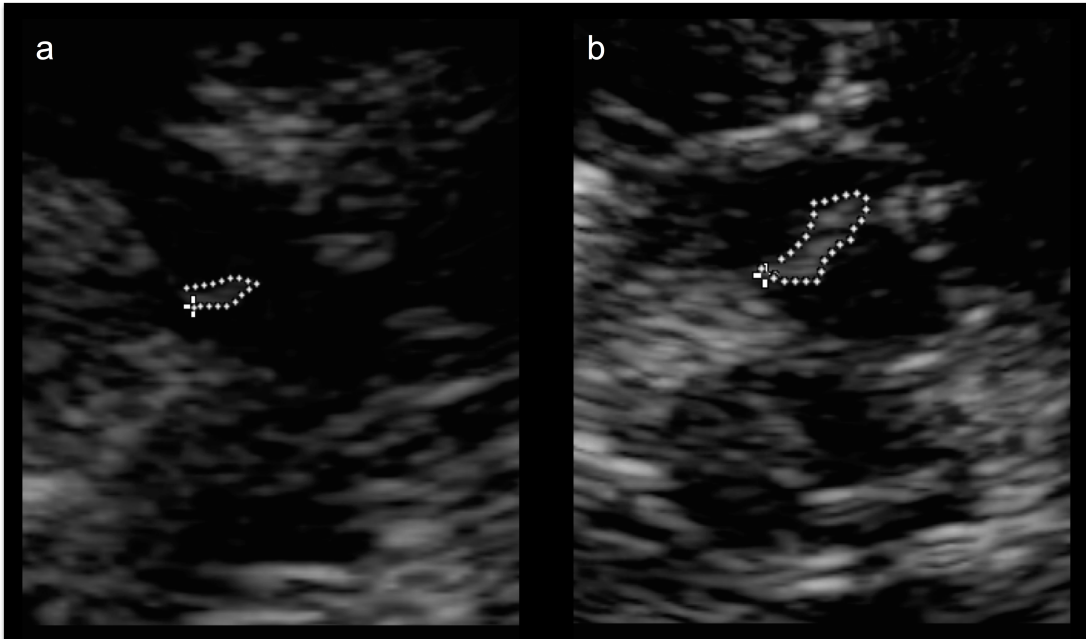


Fig. 7.5: Comparison of SN area of echogenicity in a control subject (a) and a patient with PD (b), who has SN hyperechogenicity.

performance than SN-Mean and was selected as the parameter by which to assess PREDICT-PD participants. Age and gender did not appear to significantly affect area of echogenicity when patients and controls were analysed separately, consistent with observations from the training period. Age and gender have been inconsistently associated with SN hyperechogenicity previously.^{106,169,178} Ideally comparisons between cases and controls would have been stratified by age and gender to assess the role of these as confounding factors, particularly given that cases were older and more likely to be male than controls. However, the low number of controls with SN hyperechogenicity prevented stratified analysis using either simple Mantel-Haenszel methods or logistic regression (empty strata due to sparse data). Further work would aim to clarify better whether SN hyperechogenicity is independent of age and gender.

Previous studies of TCS and PD have defined hyperechogenicity as being above the 90th centile of echogenic area in control subjects assessed using that specific experimental setup (ultrasound machine, transducer and operator).^{105,106} This is analogous to creating a cut-off value based on a 10% false-positive rate. From this validation study, an SN-Max of $>0.16\text{cm}^2$ was observed in approximately 10% of controls and this was selected as the cut-off for hyperechogenicity for this experimental setup and the cut-off for assessment of PREDICT-PD participants.

Previous studies have tended to observe larger areas of echogenicity in the region of the nigra than were observed in this study, in both PD subjects and controls.¹⁰⁵ This further underlines the need to validate and calculate reference

ranges for each centre using this method, rather than relying on cut-offs determined in other centres. Nonetheless data on areas of echogenicity similar to that reported here have been previously published.¹⁷⁹ Similarly, 10% is frequently quoted as the proportion of subjects with an inadequate bone window, which prevents assessment with TCS.¹⁰⁵ Using the current experimental setup, a smaller proportion of 5% was observed.

7.4 TCS & SPECT in PREDICT-PD subjects

TCS and ¹²³I-FP-CIT SPECT were then used as outcome measures to test the hypothesis that subjects estimated to be at higher risk of PD using the preliminary PREDICT-PD algorithm, would be more likely to display abnormal imaging features, such as SN hyperechogenicity and DaT deficit, compared with those estimated to be at lower risk.

7.4.1 Methods

Sample size calculations

A sample size calculation was performed for TCS based on the published literature, given that results from the validation study were not available at that time. In other centres, marked hyperechogenicity was represented by SN area of $>0.25\text{cm}^2$, with normal areas $<0.20\text{cm}^2$. It was unknown how much higher risk subjects might differ from lower risk subjects in terms of TCS appearances, so a conservative calculation was performed. Detection of an average 0.02cm^2 difference between SN-Max for higher versus lower risk controls (90% power, $\alpha=0.05$) required 25 individuals in each group. An SN-Max $>0.16\text{cm}^2$ was used to determine SN positive or negative status (see section 7.3).

For ¹²³I-FP-CIT SPECT, a sample size estimate was calculated after referring to unpublished data from the Parkinson's Progression Markers Initiative (PPMI) study.ⁱⁱⁱ Striatal binding ratios (SBRs) were reported in PPMI data and are derived from tracer uptake in the striatum compared with uptake in the occiput. In PPMI, PD patients with mean disease duration of 6.6 months had a mean SBR of 1.41 and the mean SBR of controls was 2.56. The difference between higher and lower risk subjects in PREDICT-PD was anticipated to be substantially less than the difference between cases and controls in PPMI. Performing scans in 29 of the higher-risk group and 22 of the lower risk was expected to demonstrate a difference in ratios of 0.57 (half of the difference observed in cases and controls from PPMI; 80% power, $\alpha=0.05$).

ⁱⁱⁱData available via formal application to PPMI investigators via www.ppmi-info.org.

Participant selection

Subjects who had been participating in the PREDICT-PD study since baseline were invited to undergo imaging investigations between March 2014 and April 2015. Participants were sampled from the upper extreme of risk, along with a smaller number of participants that had lower risk estimates (Figure 7.6). Individual risk status was not revealed. Participants were sent information and returned signed acceptance via post. Invitations were restricted to those under the age of 80 years because of the potential for long-distance travel in order to participate in imaging studies. Invitations were sent regardless of participant's location in the UK (there was no geographical bias to recruitment). If willing to participate, subjects were sent iodine tablets, to block uptake of radioactive iodine by the thyroid gland. These were taken before and after SPECT scanning. Subjects were apprised of anticipated risks associated with ^{123}I -FP-CIT SPECT including radiation exposure and the potential of allergic reaction to iodine contained within the radiotracer. Information relating to potential exclusion criteria (below) and current medication (some antidepressants and sympathomimetics alter dopamine transporter binding¹⁸⁰) was gathered over the telephone prior to participant travel. There were no anticipated risks associated with TCS.

Exclusion criteria for ^{123}I -FP-CIT SPECT were:

- Criteria from the main PREDICT-PD study (diagnosed PD, movement disorder, dementia, motor neurone disease, stroke).
- Previous hypersensitivity to a radioisotope or related product/component.
- Liver disease.
- Epilepsy.
- Pregnant or breast feeding women.

Upon arrival, participants gave written consent to participate in the imaging studies. TCS was performed at the Lysholm Department of Neuroradiology at the NHNN and followed the standardised procedures described in sections 7.2 and 7.3. ^{123}I -FP-CIT SPECT was performed at the Institute of Nuclear Medicine, University College London Hospital using a GE Discovery 670 SPECT/CT machine. A standardised SPECT protocol was followed with images obtained approximately 3 hours after injection with 185 MBq of ^{123}I -FP-CIT (GE Healthcare). The imaging parameters used have been described in detail elsewhere.¹⁸¹

For semiquantitative analysis of tracer binding, BRASS software was used (HERMES Medical Solutions). BRASS software is a 3-dimensional semiautomatic

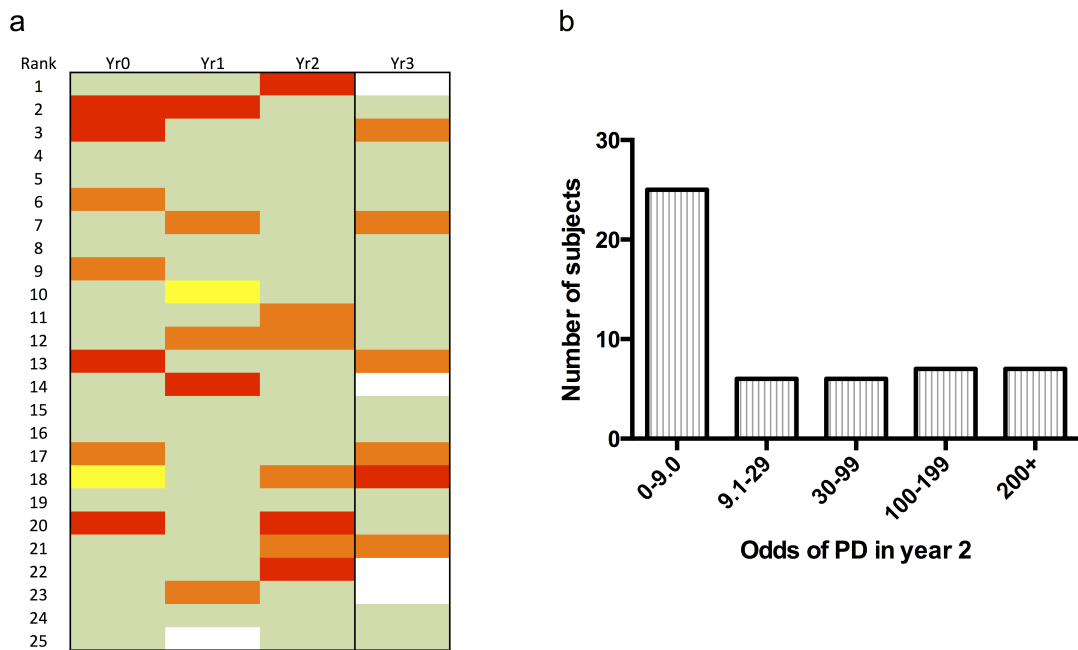


Fig. 7.6: Subjects selected for imaging studies within PREDICT-PD: a) Highest risk 25 subjects in each year (yr). Subjects were either: invited and scanned (green), declined to have scan or lost to follow-up (red), over 80 years old therefore not invited (orange), had a significant change in risk away from highest risk after one year (yellow), or were not contacted for scanning (white). Selection was based on year 2 or earlier risk estimates because year 3 scores were not finalised at time of scanning. b) Frequency distribution of subjects that were scanned using risk estimates in year 2. Note the preference for highest risk and sampling of lower risk subjects across a range of scores.

brain analysis package in which the subject's brain was first registered to a standard anatomic atlas, then tracer binding in the whole striatum, caudate nucleus, and putamen was assessed. Volumes of interest (VOIs) were automatically defined over the caudate nucleus and putamen to assess specific tracer binding and over a reference region, the occipital cortex (OCC), to assess non-specific binding. The count concentrations in these regions were used to calculate striatum specific binding ratios (SBRs) as $[VOI_{\text{striatum}} - OCC]/OCC$, where VOI_{striatum} and OCC are the count concentrations in the striatum and occipital cortex, respectively. Binding ratios were also calculated for the caudate and the putamen compared to the occiput. Separately, a reference group of over 100 healthy European controls was used to determine mean age-expected striatal uptake (not expressed as a ratio), along with age-related cut-offs at 80% of the mean and 65% of the mean.¹⁸¹ These approaches were consistent with the methods used in the PARS study.¹⁰⁴ An experienced image processor performed all semiquantitative analysis.^{iv}

Statistical methods

Risk scores were calculated using the same algorithm described in chapter 5, which calculated a combined odds of PD derived from meta-analysis (chapter 2), and these risk scores were used as the exposure variable. Outcomes were SN-Max for TCS and binding ratios from the side with least uptake of tracer for ¹²³I-FP-CIT SPECT. Linear regression was used to study relationships between continuous variables, including estimated risk (transformed to log odds) with SN-Max and with binding ratios. T-tests and Wilcoxon Rank Sum tests were used to compare continuous outcomes (SN-Max and binding ratios) in categorical exposure groups (higher versus lower risk) depending on whether data were normally or non-normally distributed. Categorical outcomes were compared in higher and lower risk groups using Fisher's exact test where relevant. Linear or median regression was used to examine the effect of age and intermediate markers from year 3 (UPSIT, RBDSQ, KS) on continuous outcomes (SN-Max and binding ratios). Logistic regression using a binary outcome (SN hyperechogenicity positive or negative) was used to assess the effects of higher risk status, age (as a linear term) and gender.

7.4.2 Results

Fifty two subjects participated in PREDICT-PD imaging studies. Of these 49 underwent both TCS and ¹²³I-FP-CIT SPECT, two underwent TCS only and one underwent ¹²³I-FP-CIT SPECT only. The reason for only undergoing TCS

^{iv}Dr John Dickson performed all semi-quantitative analysis described in this paragraph.

was recent cancer in one subject^v and the other subject had suffered a significant allergic reaction to iodine in the past. The reason that one subject underwent ¹²³I-FP-CIT SPECT only, was because the ultrasound machine was being used indefinitely at the time of their appointment. Another subject attended for imaging but had clinical features of PD (which was diagnosed soon after the appointment). His results were not included in the analysis.

Eleven subjects were sent information about imaging studies but declined to participate, despite nine continuing to participate in the wider project. A further 6 subjects who were in the top 25 highest risk subjects were over the age of 80 years and were not invited for imaging studies (Figure 7.6). Two subjects were taking antidepressants known to have the potential to interfere with DaT binding and these were withheld after discussion with their health practitioner.

Risk estimates in years 2 and 3 of follow-up (the most recent years of surveying for scanned participants) were available for 49 of 51 subjects and ranged from odds of 1 in 2.5 to 1 in 310 (median 1 in 8.9). Higher risk subjects were defined as those with a risk estimate less than 9 (essentially an odds 1 in 9 or greater) in either one or both of the last two years of follow-up (years 2 and 3). Lower risk subjects had scores greater than 9 (an odds of PD less than 1 in 9). Higher risk subjects were older and were all male.

TCS

Of the 50 subjects that underwent TCS, 49 had at least one sufficient bone window through which SN-Max could be ascertained and measured. Of these 29 exceeded the cut-off for hyperechogenicity (SN-Max >0.16cm²). Using linear regression, SN-Max was associated with risk estimates each year, from baseline to year three of follow-up (Table 7.3).

Risk score year	Slope of line	95% CI	p-value
· 3	0.0644	0.0348 to 0.0940	<0.001
· 2	0.0520	0.0234 to 0.0806	0.001
· 1	0.0522	0.0217 to 0.0827	0.001
· 0	0.0544	0.0279 to 0.0807	<0.001

Table 7.3: Associations between annual risk estimates and side of maximum echogenicity (SN-Max) determined through linear regression modelling log odds of risk.

Legend: CI = confidence interval.

SN-Max was larger in higher risk subjects and smaller in lower risk subjects (0.22cm² versus 0.14cm²; p<0.001; Table 7.4). A greater proportion of higher risk

^vNot a specific exclusion criterion, but reluctance to expose them to unnecessary radiation.

7.4 TCS & SPECT in PREDICT-PD subjects

subjects had SN hyperechogenicity compared with those at lower risk (78% versus 36%; $p=0.004$). A more stringent definition of higher risk was then used, in which subjects had to have highest risk estimates in all four years of follow-up (odds of PD 1 in 9 or more in every year). Fourteen subjects were in the higher risk group every year and 85.7% of these had SN hyperechogenicity, compared with 48.6% of the remaining 35 subjects ($p=0.024$).

	Higher risk	Lower risk	p-value
n	27	22	
Median age in year 3 (IQR)	73.8 (69.6 to 78.2)	68.3 (66.7 to 71.2)	
Gender (male/female)	27/0	14/8	
Mean SN-Max cm^2 (95% CI)	0.22 (0.19 to 0.26)	0.14 (0.12 to 0.17)	<0.001*
SN hyperechogenicity	21 (78%)	8 (36%)	0.004**

Table 7.4: Mean area of maximum echogenicity (SN-Max) and proportion with SN hyperechogenicity in higher versus lower risk subjects.

Legend: CI = confidence interval; IQR = interquartile range.

*t-test; **Fisher's exact test.

Intermediate markers measured in year 3 (UPSIT, RBDSQ and worst hand KS) were used to predict SN-Max using linear regression for UPSIT and KS, and median regression for RBDSQ. There was no association between SN-Max and any of the intermediate markers. SN-Max was not associated with age ($\beta=0.0035\text{cm}^2$ per year, $p=0.150$), in keeping with the earlier case-control validation phase.

Logistic regression was used to model odds of having SN hyperechogenicity if higher risk compared with lower risk (Table 7.5). Age and gender were also modelled in univariate analyses but neither were significantly associated with SN hyperechogenicity, and therefore multivariate analysis was not undertaken.

	OR	95% CI
Univariate analysis		
· Higher risk in year 2 or 3*	6.13	1.74 to 21.51
· Age in year 3** (per additional year)	1.07	0.95 to 1.20
· Male gender	2.89	0.60 to 13.83

Table 7.5: Logistic regression giving odds of SN hyperechogenicity in higher risk subjects compared to lower risk. No effect of age or gender on hyperechogenicity.

Legend: OR = odds ratio; CI = confidence interval. *ORs varied between 3.8 and 6.9 if higher risk any single year (baseline to year 3); **Age modelled as a linear term.

¹²³I-FP-CIT SPECT

Forty-nine subjects underwent ¹²³I-FP-CIT SPECT. SBRs were not significantly associated with risk estimates in any year (Table 7.6), but the slope of the line favoured a reduction in binding with increasing risk. Putaminal binding ratios were also not associated with risk estimates in any year, and caudate binding ratios showed an association of borderline significance in year 3 and a trend towards an association at year 1 (Table 7.6). However, scatter plots of log odds of risk against binding ratios suggested that any borderline associations were dependent on 2-3 outliers (not shown). A sensitivity analysis showed that the p-value increased if any one of the outliers was removed from the analysis (not shown).

Risk score year	Slope of line	95% CI	p-value
Striatal BR			
· 3	-0.1609	-0.3331 to 0.0114	0.067
· 2	-0.1053	-0.2866 to 0.0760	0.249
· 1	-0.1389	-0.3310 to 0.0531	0.152
· 0	-0.1042	-0.2787 to 0.0702	0.235
Putaminal BR			
· 3	-0.1295	-0.2988 to 0.0397	0.130
· 2	-0.0892	-0.2726 to 0.0943	0.333
· 1	-0.1057	-0.3012 to 0.0897	0.282
· 0	-0.0785	-0.2555 to 0.0985	0.377
Caudate BR			
· 3	-0.1975	-0.3853 to -0.0097	0.040
· 2	-0.1456	-0.3353 to 0.0441	0.129
· 1	-0.1962	-0.3952 to 0.0028	0.053
· 0	-0.1504	-0.3324 to 0.0136	0.103

Table 7.6: Linear regression to assess associations between annual risk estimates (modelled as log odds) and binding ratios in the whole striatum, and separately for the putamen and caudate, on the side with least binding.

Legend: CI = confidence interval; BR = binding ratio.

Higher and lower risk groups were defined in the same way that they were for TCS analysis above (cut-off using median odds 1 in 9). Comparisons between higher and lower risk subjects found no significant difference in striatal, putaminal or caudate binding ratios, although there was perhaps a slight trend towards lower binding ratios in higher risk subjects (Table 7.7).

Cut-off values for striatal uptake were calculated using 80% and 65% of age-expected mean from a database of over 100 European controls.¹⁸¹ There was no significant difference between proportion of higher and lower risk subjects that fell beneath these cut-offs (Table 7.7 and Figure 7.7). However lower risk subjects were more likely to have striatal uptake counts greater than the age-expected mean

7.4 TCS & SPECT in PREDICT-PD subjects

compared with lower risk (36.4% versus 7.4%, $p=0.029$; Figure 7.7). Examples of scans from those with the lowest striatal uptake are shown in Figure 7.8.

Intermediate markers measured in year 3 (UPSIT, RBDSQ and worst hand KS) were used to predict SBRs using linear regression for UPSIT and KS, and median regression for RBDSQ. Significant associations were observed between binding ratios and UPSIT and KS in year 3, but not RBDSQ (Table 7.8). Similar observations were noted if putamen and caudate binding ratios were used (data not shown). Age and gender were also significantly associated with striatal binding ratio ($p=0.036$ and 0.003 respectively). However, if gender analysis was restricted to subjects under 70 years the effect of gender lessened significantly ($p=0.066$). Logistic regression analysis was not possible due to insufficient numbers meeting categorical outcomes (i.e. normal versus abnormal ^{123}I -FP-CIT SPECT scan). Finally no association was observed between striatal binding ratio and SN-Max analysed using linear regression ($p=0.823$).

	Higher risk	Lower risk	p-value
n	27	22	
Median age in year 3 (IQR)	73.8 (69.6 to 78.2)	68.3 (66.7 to 71.2)	
Gender (male/female)	27/0	14/8	
Median Striatal BR (IQR)	2.790 2.453 to 2.897	2.888 2.474 to 3.197	0.213*
Median Putaminal BR (IQR)	2.675 2.361 to 2.878	2.826 2.339 to 3.065	0.278*
Median Caudate BR (IQR)	2.799 2.458 to 3.076	2.973 2.686 to 3.295	0.148*
DaT binding <80% of age-expected mean	9 (33.3%)	7 (31.8%)	1.00**
DaT binding <65% of age-expected mean	2 (7.4%)	2 (9.1%)	1.00**

Table 7.7: Median binding ratios in the whole striatum, and separately for the putamen and caudate for subjects categorised as higher and lower risk. Legend: IQR = interquartile range; DaT = dopamine transporter; BR = binding ratio. *Wilcoxon Rank Sum test; **Fisher's exact test.

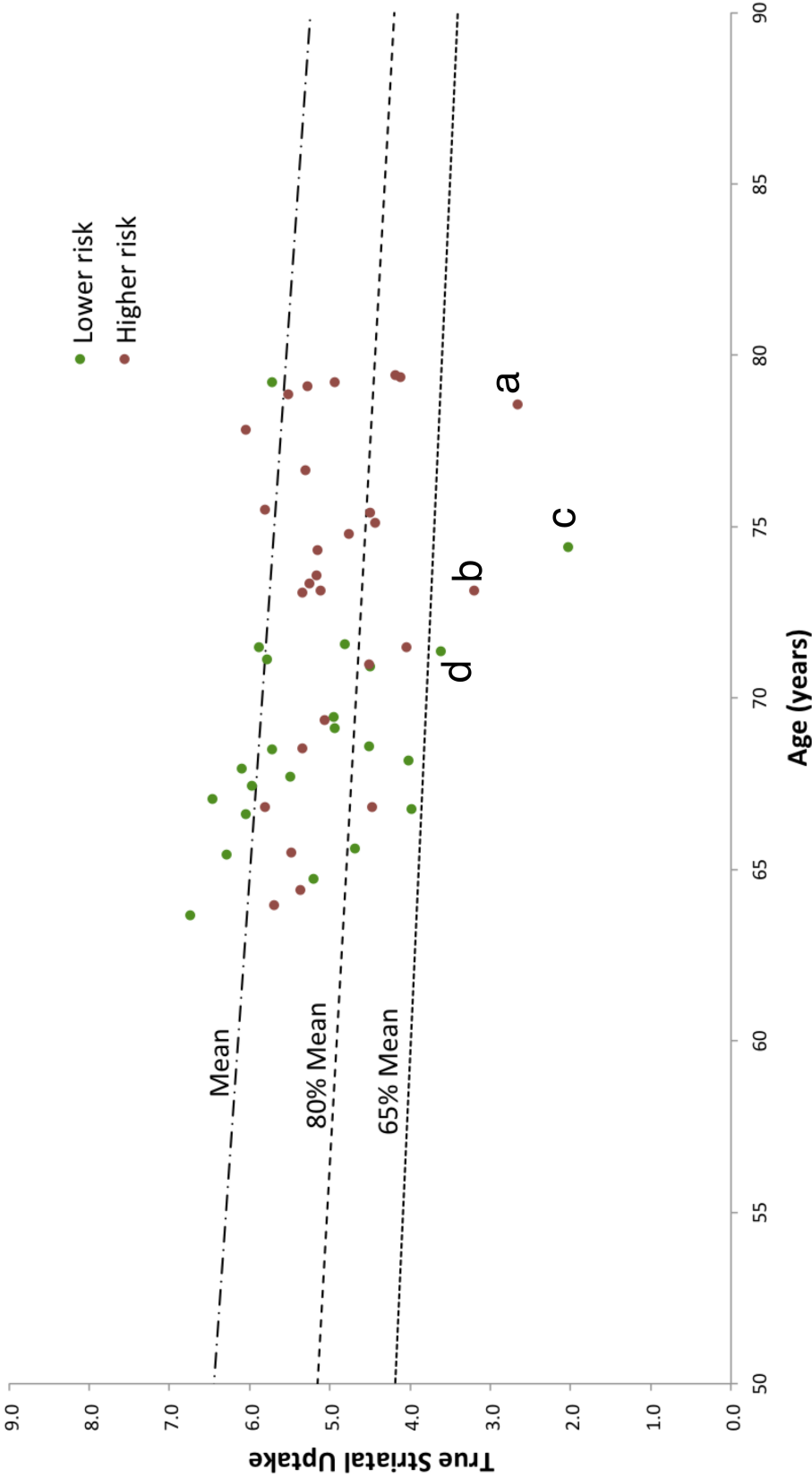


Fig. 7.7: Higher (red) and lower risk (green) subjects plotted on a graph of age-expected striatal uptake derived from a European control group of over 100 subjects. Four subjects (a-d) had uptake counts below 65% of age-expected mean and their scans are shown opposite.

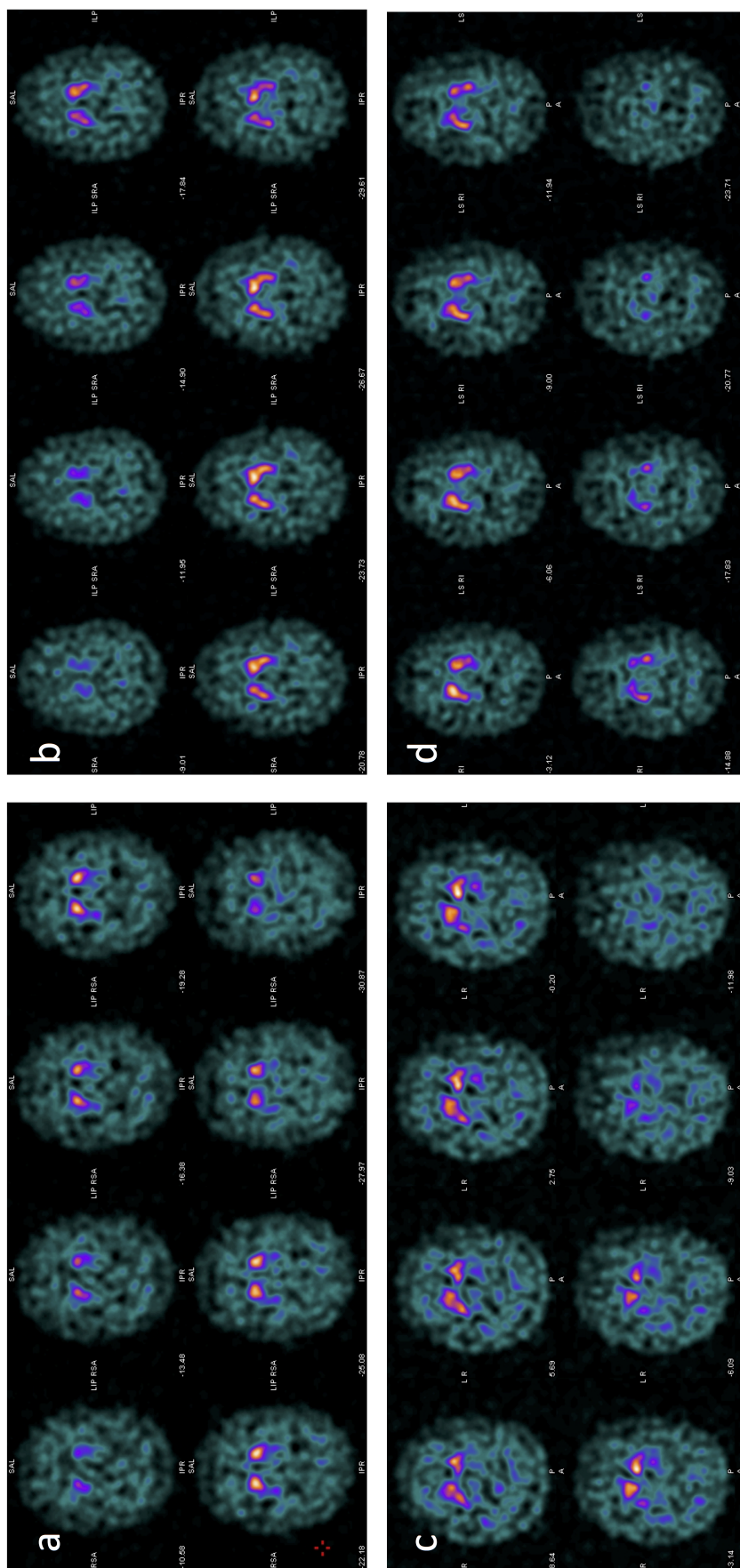


Fig. 7.8: SPECT scans in which striatal uptake counts were below the 65% of the mean from a European control group of over 100 subjects. Two images (a & b) come from higher risk and two (c & d) come from lower risk subjects.

Year 3 intermediate markers	Slope of line	95% CI	p-value
UPSIT	0.0327	0.0128 to 0.0527	0.002
RBDSQ	-0.0447	-0.1031 to 0.0137	0.130
KS worst hand	0.0153	0.0034 to 0.0272	0.013

Table 7.8: Regression analysis of associations between intermediate markers measured in year 3 of follow-up and striatal binding ratios, on the side with least binding.

Legend: CI = confidence interval.

7.4.3 Discussion

TCS and ^{123}I -FP-CIT SPECT are currently used as part of the diagnostic work-up of parkinsonism. For each modality, a variety of studies have demonstrated differences between subjects with idiopathic PD compared with controls and subjects with other causes of parkinsonism. Here TCS and ^{123}I -FP-CIT SPECT were used as outcomes to assess risk scoring in the PREDICT-PD study.

TCS

Area of echogenicity was strongly associated with risk estimates overall and SN hyperechogenicity occurred in a significantly greater proportion of higher risk subjects compared with lower risk. SN hyperechogenicity is recognised as being a risk marker for PD and finding it in higher risk subjects reinforces the notion that this group has been enriched for PD by the algorithm.⁹⁵ However, there were no statistically significant associations observed between SN hyperechogenicity and age, gender, or intermediate markers measured in year 3.

The PRIPS study was the largest study using TCS to determine PD risk in subjects over the age of 50 years. In the German arm of the study, healthy subjects with SN hyperechogenicity (n=193) were more likely to have abnormal smell test scores (using Sniffin sticks rather than UPSIT) and also to have mild parkinsonian signs, measured with the UPDRS.¹⁸² When the same analysis was undertaken in the full PRIPS cohort (approximately 1,600 subjects), similar results were observed.¹⁸³ Intermediate markers (smell and finger tapping) in PREDICT-PD subjects were not associated with SN hyperechogenicity, but this may be due to the small proportion of participants scanned from the project overall or, for motor testing, because quantitative finger tapping does not pick up the wide range of mild parkinsonian signs that the UPDRS does.

The PRIPS study reported a non-significant trend in favour of male gender in those with SN hyperechogenicity but no association with age.^{182,183} The results for

the lack of effect of age on SN hyperechogenicity are consistent between PRIPS and PREDICT-PD, and the trend in favour of male subjects was also observed in PREDICT-PD. However, male gender is a risk factor in the algorithm which may have biased results, since higher risk subjects were recruited for imaging preferentially in this study. No effect of age or gender was observed at the validation stage, but again female participants were under-represented.

In another study, TCS was performed in small numbers of subjects with idiopathic anosmia and SN hyperechogenicity was reported in 11 out of 26 (42%).⁷⁰ The combination of SN hyperechogenicity and hyposmia (with and without motor features) has been used for distinguishing PD from other types of parkinsonism or movement disorder.^{184,185} Data from Innsbruck (Bruneck cohort) have suggested that the combination of SN hyperechogenicity and hyposmia increases the odds of having mild parkinsonian signs on clinical examination.¹⁸⁶

Subjects diagnosed with RBD using overnight sleep study and polysomnography (PSG) have been observed to have SN hyperechogenicity more frequently than control subjects.^{187,188} That a similar association was not observed between SN hyperechogenicity and scoring above the threshold of the RBDSQ in PREDICT-PD, may be due to the small number of subjects included in the imaging studies. However, poor performance of the RBDSQ in diagnosing RBD in certain settings has emerged recently, particularly if used without prior sleep history and PSG.¹⁸⁹ It is noteworthy that approximately 15% of PREDICT-PD participants have RBDSQ scores which exceed the cut-off (see chapter 5), suggesting a significant rate of over-diagnosis. Similar over-diagnosis of RBD with the RBDSQ has been observed elsewhere, with 15% of healthy controls exceeding the cut-off on the RBDSQ compared with 2% meeting PSG criteria in the DeNoPa study.¹⁷⁹

The rate of SN hyperechogenicity in PREDICT-PD subjects exceeds that observed in published controls and in control subjects enrolled in the validation stages (59% versus 10%). Even in the lower risk group, the proportion with SN hyperechogenicity was 36%. This may be in part due to enrichment of PD risk factors and early features in the PREDICT-PD cohort compared to community controls (i.e. selection bias), but is perhaps more likely to result from observer bias due to unblinded ultrasonographic assessment. Blinded assessment is preferable when performing TCS due to inherent subjectivity of measurement, but was not feasible in this study. In addition the cut-offs identified from the validation stage tended to be lower than previously published cut-offs using other equipment.¹⁰⁵ This may in turn have resulted in an excess of PREDICT-PD subjects exceeding the cut-off for SN hyperechogenicity. Although the proportion of subjects with SN hyperechogenicity was relatively high, the median scores for higher and lower

risk were similar in magnitude to the differences observed between cases of PD and healthy controls at the validation stage.

As far as is known, this is the first time SN hyperechogenicity has been used as an outcome of risk stratification. Rather like olfaction, RBD and other early non-motor features, it is more usual that it be used as the exposure variable that defines risk status. However, similar to finding that there are differences in the smell, sleep and finger tapping in higher risk subjects from PREDICT-PD, the excess of subjects with SN hyperechogenicity is reassuring. In line with the other intermediate markers, SN hyperechogenicity is thought to occur some time before diagnosis (perhaps five or more years before), although unlike smell and motor features, it appears to be static and not to vary over time.^{170,171,172}

Further work could examine the expanded use of TCS in PREDICT-PD, not simply as an outcome, but along with other intermediate markers to determine those higher risk subjects that are most likely to develop incident PD. TCS is insufficient as a risk marker in isolation because its PPV alone is very low.¹⁰⁶ However, in combination with objective smell and motor tests, it may predict those most likely to convert to PD, particularly if used in a group that had already undergone preliminary screening.

¹²³I-FP-CIT SPECT

In contrast with TCS, binding ratios measured using ¹²³I-FP-CIT SPECT tended not to be associated with estimated risk scores in the PREDICT-PD participants that underwent scanning. Nor were higher risk subjects more likely to have striatal uptake below cut-offs defined *a priori* (below 65% and 80% of age-expected mean DaT binding in a large group of control subjects). As far as is known this is the first time ¹²³I-FP-CIT SPECT has been used to assess a composite risk score for PD. Examples wherein functional imaging has been used, include the assessment of subjects with individual risk factors for PD such as idiopathic anosmia, RBD and single gene carriers.^{25,69,103,104}

Binding ratios were negatively associated with age, showing a decline over time, which is consistent with previous studies.¹⁹⁰ Female gender was also associated with significantly higher binding ratios than those seen in males, again broadly consistent with what has been observed previously.¹⁹⁰ However, as with TCS, gender analyses should be viewed with caution given the high proportion of males compared to females. The females that were included tended to be younger than the males, and when analysis was restricted to participants under 70 years of age, the association between gender and DaT binding attenuated significantly.

SBRs were associated with UPSIT and KS measured in year 3 of follow-up. There was no significant association between binding ratios and RBDSQ scores, but

this may be due to poor specificity of the RBDSQ (discussed above). Nonetheless, these observations are encouraging because in the longer term, intermediate markers could be incorporated into the preliminary risk algorithm to augment its performance in identifying those at higher risk of PD. Currently there exist insufficient observational study data to model effect size estimates for hyposmia and possible RBD (were it possible to measure RBD accurately without PSG) and update the algorithm accordingly. The observed association between binding ratios and UPSIT scores is consistent with recently reported results from the PARS study, showing greater deficits in DaT binding in subjects with hyposmia compared to those without.¹⁰⁴ Here PARS methods were replicated using the same cut-offs, but using risk score as the exposure rather than hyposmia, however similar results were not achieved.

It has been previously observed that SN hyperechogenicity and DaT binding tend not to correlate with one another, suggesting that they identify different pathophysiological processes (e.g. microglial activation versus pre-synaptic denervation).^{191,192} Similar to what has been reported previously, no association between SN hyperechogenicity and DaT binding deficit was observed in PREDICT-PD subjects.

Although trends were observed in the expected direction, that risk scores generated by the preliminary algorithm were not significantly associated with binding ratios is perhaps unsurprising. The number of participants scanned was relatively few and whereas observed TCS differences are marked between higher and lower risk subjects, differences were far smaller for ¹²³I-FP-CIT SPECT. The modest sample size may have meant the study was underpowered to detect a difference. A greater effect may have been observed if lower risk subjects had been sampled from the extreme low risk, rather than across moderate and low risk estimates (Figure 7.6).

Although not statistically significant, the directionality of effect in analyses considering continuous data suggested that a larger sample size may have had a greater chance of demonstrating significant results. One important consideration is that the degree of binding deficit is likely to change over time whereas in comparison, SN hyperechogenicity is unlikely to change.^{171,177,193} It may be that if there are differences to be observed between higher and lower risk subjects using ¹²³I-FP-CIT SPECT, the curves are not yet far enough apart for a difference to be detected (Figure 7.9). Moreover, if Braak staging of PD is accepted as broadly accurate, then binding deficit may occur relatively close to the point at which a diagnosis is typically made.⁹ Differences between higher and lower risk subjects may be enhanced by repeating ¹²³I-FP-CIT SPECT in subjects that have already been scanned at two further years of follow-up to assess differences in rate of

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change between higher versus lower risk subjects. However, this assumes that the repeated measurement of binding ratios in subjects is accurate which it may not be, and as far as is known there are no published data on this topic. An alternative option would be re-draft sample size calculations based on the observed differences here, and scan greater numbers of higher and lower risk subjects, with preferential sampling from the extreme low risk.

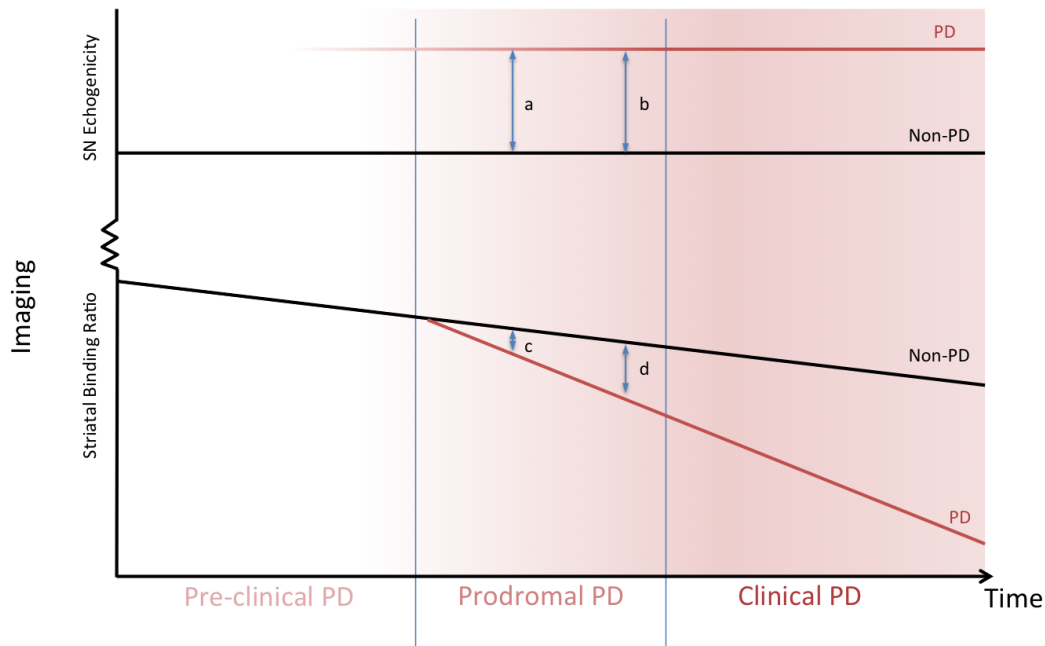


Fig. 7.9: A schematic showing how appearances of TCS and ^{123}I -FP-CIT SPECT may change during the pre-diagnostic stage of PD. TCS is thought to be a static marker for the PD prodrome meaning that differences ought to be observed throughout the period (comparisons at point (a) and point (b)). The time point within the prodromal phase that SN hyperechogenicity appears is not known and is depicted by fading of the line. In comparison to TCS, ^{123}I -FP-CIT SPECT tends to become progressively abnormal during the clinical phase of the disease and likely before diagnosis as well. Sufficient differences in DaT binding may not be demonstrable until late in the prodromal phase at point (d), meaning that there may be no clear difference if observed at point (c).

Another possibility is that the preliminary algorithm in its current format does not adequately identify those at risk of PD. There are currently multiple examples that support the notion of enrichment, including the results of TCS above. Furthermore, it was observed that lower risk subjects were significantly more likely to have striatal uptake above age-expected mean, which suggests that risk stratification is playing at least a minor role. The two lower risk subjects that

had striatal uptake below 65% age-expected mean had odds of 1 in 128 and 1 in 90 at the time when they were last measured. Both would therefore be ranked in the middle risk group from the wider study, rather than the extreme low risk. In contrast, the two higher risk subjects that had low striatal uptake, have been ranked in the top 15 highest risk subjects since the study began. Reviewing these images (Figure 7.8) one can see either marked abnormalities or clear asymmetry in all four subjects. Further work will also include blinded clinical rating of the existing scans from higher and lower risk subjects to determine if there are differences exist that were not picked up by quantitative measures.

Limitations

The major limitation of use of TCS in PREDICT-PD subjects was the unblinded nature of assessment, which could have resulted in observer bias. The options to avoid this were limited due to the fact that TCS assessments were performed by the investigator, who had prior knowledge of the risk status of individuals. To avoid observer bias, a second investigator would have needed to have been trained to perform blinded TCS assessments. Another possibility was to have the static TCS images independently rated, but there was no way to standardise measurement after images had been exported from the ultrasound machine.

The ^{123}I -FP-CIT SPECT arm of the study was underpowered and could not demonstrate statistically significant differences between binding ratios in higher and lower risk subjects. The expected difference in binding ratios between higher and lower risk subjects that was used in power calculations was an overestimate, and the observed difference was substantially less. However, there were some reassuring findings, including observing expected associations with age and smell test scores, and reassuring associations with finger tapping speed. Analyses using categorical data were also likely to be underpowered given sampling across a range of lower risk subjects (rather than just the extreme low risk) and due to sampling fewer lower risk than higher risk. This method left the analysis vulnerable to type 2 error in which a single abnormal result in the lower risk group would lead to acceptance of the null hypothesis. An abnormal result in the low risk group was quite possible given that, whilst the current algorithm includes a variety of risk factors for PD, it excludes some of the strongest ones (e.g. genetic risk factors, smell loss and RBD).

Conclusions

The current algorithm is preliminary and will benefit from refinement of effect size estimates and inclusion of additional predictors. The validity of the algorithm

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will be tested ultimately if sufficient cases of incident PD are diagnosed during follow-up and the majority of these had been stratified into the higher risk group at an early stage. Over time statistical power may be improved by combining incident PD diagnosis and DaT deficit in those as yet undiagnosed as a composite outcome. This composite outcome could be used in turn to refine the selection of predictive factors used in the algorithm.

Chapter 8

Risk of PD in the community: follow-up from PREDICT-PD

8.1 Introduction

The PREDICT-PD study has been running for the three years of this thesis and gathering data from participants annually. Here follow-up data are presented to determine whether the preliminary algorithm continued to identify a higher risk group enriched for intermediate markers of PD beyond baseline, and crucially, whether the higher risk group had an elevated incidence of PD. Further objectives included determining whether participants who were re-scored each year, moved between risk groups over time and whether, if baseline groups were preserved, intermediate markers remained different during follow-up.

8.2 Methods

Data collection

Full methods relating to the baseline year of data collection are detailed in chapter 5. Each year participants were prompted via email to return to the website to complete an annual submission of the risk factor questionnaire and the BRAIN-tap test. The assessment included a question on whether subjects have been given any new diagnoses and listed ‘Parkinson’s disease’ and/or ‘Movement Disorder’ specifically. Positive responses to either of these were followed-up in person or by telephone interview. Smell testing was repeated in year 3 (having been previously tested at baseline) using the same US-version of the University of Pennsylvania smell identification test (UPSIT). Participants that were ‘lost to follow-up’ were defined as subjects that did not complete a submission for any year after baseline. No additional participants were recruited after the baseline year.

Construction of risk groups

The same preliminary algorithm was used each year to provide PD risk estimates (expressed as odds of PD) for each participant. Exposures were ascertained in a binary fashion, except for age which was included as a continuous variable. Where data existed on multiple levels (i.e. bowel movement frequency, erectile function, anxiety and depression), cut-offs were used in the same way that they were at baseline. Laxative use and antidepressants were used as surrogate markers to suggest constipation and depression respectively. Information on use of drugs such as sildenafil (Viagra) was only collected in year three, but was considered sufficient to suggest erectile dysfunction (ED).

Steps to eliminate inconsistencies in the reporting of risk factors and early non-motor features were employed, such as checking that those reported ED were male. A standardised method for handling inconsistencies was used, in which newly reported factors such as head injury or family history were accepted as plausible, as well as being a smoker one year and ex-smoker the following year. However, where a factor had been reported previously and in a subsequent year not reported, data from all years were reviewed to determine whether the factor should be included or excluded. For example, in a subject that reported a past head injury at baseline and at year 1 and 3, but not at year 2, the year 2 data were re-coded so that the subject would be recorded as having a past head injury for all years of follow-up. Where the presence of a factor had been reported in 50% or more years of follow-up, it was re-coded as being present for all years. If reported in less than 50% it was re-coded as having been absent. These methods were applied to head injury, and smoking, coffee and alcohol consumption, and family history, where further information was not available.

Hyposmia (measured with the UPSIT), RBD (measured with the RBDSQ) and finger tapping speed (measured with KS, the most discriminate BRAIN-test parameter) were not included and were used as 'intermediate' markers of PD risk. KS scores were reported as worst hand KS (the result for the hand with the lower KS). Annual risk estimates in all subjects were ranked from highest to lowest. Centile-based cut-offs for higher and lower-risk were used so that the groups reduced in proportion to the reduction in size of the overall cohort during follow-up. Data using 15th and 85th centiles to denote higher and lower risk are reported here. As was the case at baseline, it was hypothesised that the higher risk group would have significantly lower UPSIT scores, higher rates of RBD and slower finger tapping speeds than the lower risk group, and that intermediate markers would correlate with estimated PD risk in the whole cohort. Further hypotheses were that the higher risk group defined at baseline was expected to maintain

differences in intermediate markers during follow-up, and that individuals defined as higher risk would be more likely to be diagnosed with PD.

Statistical methods

The longitudinal performance of the algorithm was assessed in two ways. First, risk scores were calculated for each individual separately, based on their survey answers in that year. Subjects in the higher and lower risk groups were compared in terms of intermediate markers collected that year (except smell which was tested at baseline and year 3 only) and associations between risk scores in the whole group and intermediate markers for that year were also examined. Secondly, the higher and lower-risk groups defined at baseline were compared longitudinally according to their intermediate marker results. Further analyses considered the movement between higher, lower and middle risk groups each year, and rates of PD diagnosis at one, two and three years follow-up in each group were also recorded.

In group-wise analyses, KS in higher and lower risk subjects was compared using t-tests and described using means and 95% confidence intervals (CI). UPSIT and RBDSQ scores did not follow a Gaussian distribution and therefore medians, interquartile range (IQR) and Wilcoxon Rank Sum tests were used. Comparisons for categorical data (defined by cut-off values for each intermediate marker) and for new diagnoses of PD were made using Fisher's exact test. Cut-off values for smell loss, RBD and tapping speed corresponding to the 15th centile for each intermediate marker using UPSIT, RBDSQ and KS were identified (≤ 27 , ≥ 5 and ≤ 44 respectively). These were comparable to those in the published literature.^{85,152,160} Relationships between estimated risk of PD in the whole dataset (independent variable) with UPSIT, RBDSQ and KS (dependent variables) were examined using median, linear and Poisson regression, respectively. Regression analyses excluding the factors age and gender from the algorithm were also undertaken as these may be independently associated with the intermediate markers of PD of smell loss, RBD and tapping speed. Subjects with newly diagnosed PD were excluded from analyses of data following diagnosis but not those preceding diagnosis. All analyses were performed using Stata.

8.3 Results

At baseline, 1,323 eligible participants were recruited (see chapter 5). After contacting participants for follow-up testing, 1,040 responded in year 1, 939 in year 2 (90% of year 1 and 71% of baseline respondents), and 846 in year 3 (90%

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of year 2 and 64% of baseline). Using 15th centile risk cut-offs, there were 155 subjects each in the higher and lower-risk groups at year 1, 140 subject in each in year 2, and 125 and 126 in higher and lower risk groups in year 3 respectively. Figure 8.1 shows the flow of participants in the study and Table 8.1 shows the demographic and risk factor information for subjects in each group each year. There were no marked differences between those that continued to participate in the study and those that were lost to follow-up (Table 8.2).

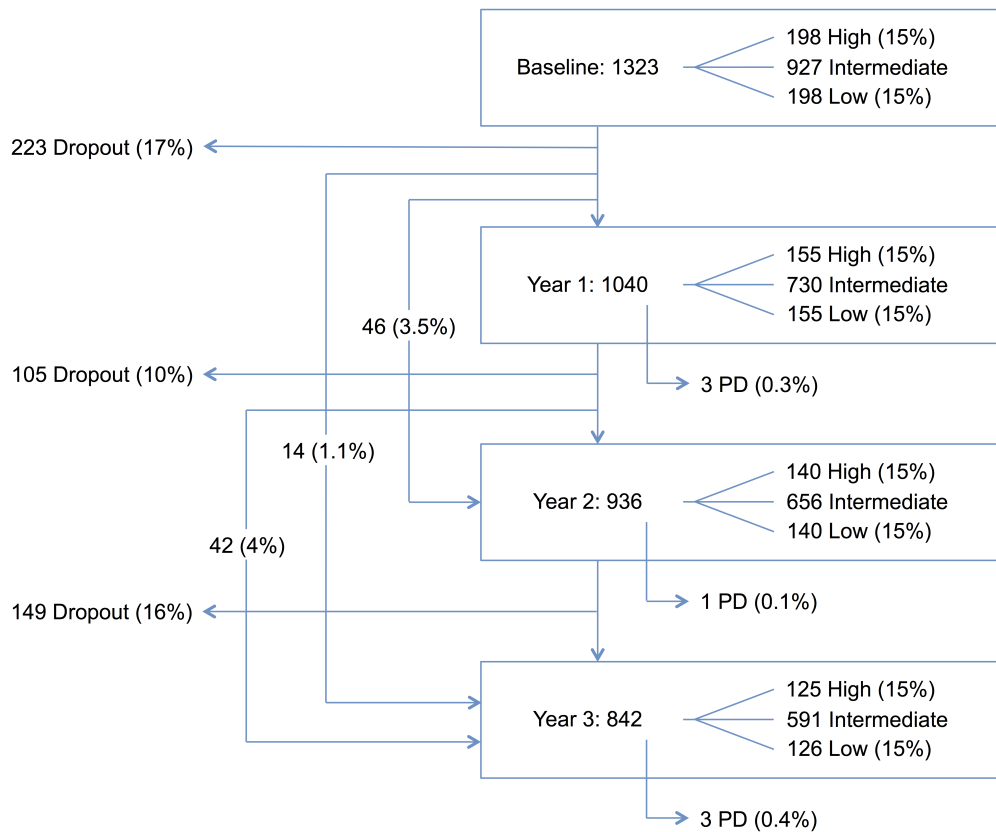


Fig. 8.1: Flowchart of participants during each year of follow-up, drop-out rates, risk group sizes and PD diagnoses.

	Year 1			Year 2			Year 3		
	All	Higher	Lower	All	Higher	Lower	All	Higher	Lower
Female	642 (61.9%)	34 (21.9%)	134 (86.5%)	582 (62.2%)	27 (19.3%)	128 (91.4%)	512 (60.8%)	24 (19%)	113 (90.4%)
Age (IQR)	67.4 (64.9-71.3)	71.2 (68.5-74.9)	64.5 (62.7-65.8)	68.1 (65.7-72.0)	71.6 (68.4-76.3)	65.5 (63.8-67.5)	69.1 (66.7-72.9)	72.5 (68.9-76.1)	66.7 (64.9-68.5)
Current smoker	29 (2.8%)	1 (0.6%)	15 (9.7%)	22 (2.4%)	1 (0.7%)	12 (8.6%)	20 (2.4%)	2 (1.6%)	12 (9.6%)
Past smoker	416 (40.1%)	71 (45.8%)	62 (40%)	371 (39.7%)	65 (46.4%)	51 (36.4%)	334 (39.7%)	55 (43.7%)	46 (36.8%)
First degree relative	179 (17.3%)	63 (40.6%)	0 (0%)	173 (18.5%)	65 (46.4%)	0 (0%)	155 (18.4%)	59 (46.8%)	0 (0%)
Drink coffee	945 (91.1%)	142 (91.6%)	151 (97.4%)	869 (92.9%)	131 (93.6%)	140 (100%)	791 (93.9%)	118 (93.7%)	125 (100%)
Drink alcohol	915 (88.2%)	139 (89.7%)	143 (92.3%)	836 (89.4%)	126 (90%)	132 (94.3%)	756 (89.8%)	110 (87.3%)	120 (96%)
Hypertension	278 (26.8%)	43 (27.7%)	60 (38.7%)	266 (28.4%)	45 (32.1%)	57 (40.7%)	244 (29%)	36 (28.6%)	49 (39.2%)
NSAID use	56 (5.4%)	8 (5.2%)	8 (5.2%)	49 (5.2%)	7 (5%)	9 (6.4%)	42 (5%)	6 (4.8%)	7 (5.6%)
CCB use	121 (11.7%)	25 (16.1%)	26 (16.8%)	119 (12.7%)	21 (15%)	29 (20.7%)	135 (16%)	25 (19.8%)	27 (21.6%)
Beta blocker use	92 (8.9%)	24 (15.5%)	10 (6.5%)	79 (8.4%)	26 (18.6%)	6 (4.3%)	77 (9.1%)	25 (19.8%)	5 (4%)
Constipation	185 (17.9%)	55 (35.5%)	1 (0.6%)	155 (16.6%)	52 (37.1%)	0 (0%)	137 (16.3%)	38 (30.2%)	1 (0.8%)
Head injury	320 (30.9%)	77 (49.7%)	10 (6.5%)	335 (35.8%)	71 (50.7%)	13 (9.3%)	317 (37.6%)	71 (56.3%)	10 (8%)
Anxiety or depression	131 (12.6%)	30 (19.4%)	5 (3.2%)	122 (13%)	31 (22.1%)	4 (2.9%)	108 (12.8%)	32 (25.4%)	4 (3.2%)
ED (males)	145 (36.7%)	107 (88.4%)	0 (0%)	143 (40.5%)	96 (85%)	0 (0%)	154 (46.7%)	93 (90.3%)	0 (0%)

Table 8.1: Prevalence of factors that contributed towards risk scores in follow-up years 1, 2 and 3.

Legend: IQR = interquartile range; NSAID = non-steroidal anti-inflammatory drugs; CCB = calcium channel blockers; ED = erectile dysfunction.

	Followed-up	Lost to follow-up
n	1,100	223
Age (IQR)	66.1 (63.5 to 69.9)	67.5 (63.5 to 72.3)
Female	672 (61.1%)	134 (60.1%)
First degree relative	178 (16.2%)	30 (13.5%)
Current smoker	38 (3.5%)	13 (5.8%)
Past smoker	440 (40.0%)	101 (45.3%)
Drink coffee	988 (89.8%)	199 (89.2%)
Alcohol	957 (87.0%)	186 (83.4%)
Hypertension	285 (25.9%)	63 (28.3%)
NSAID use	67 (6.1%)	16 (7.2%)
CCB use	128 (11.6%)	27 (12.1%)
Beta blockers	84 (7.6%)	19 (8.5%)
Constipation	181 (16.5%)	34 (15.3%)
Anxiety/Depression	133 (12.1%)	223 (11.7%)
Head injury	257 (23.4%)	70 (31.4%)
ED (males)	145 (32.7%)	37 (41.6%)
Median UPSIT (IQR)	32 (30 to 34)	32 (28 to 35)
Median RBDSQ (IQR)	2 (1 to 3)	2 (1 to 4)
Mean KS (95% CI)	53.9 (53.2 to 54.5)	52.2 (50.5 to 53.9)

Table 8.2: Prevalence of factors in subjects that were lost to follow-up compared to those that remained under follow-up.

Legend: IQR = interquartile range; CI = confidence interval; NSAID = non-steroidal anti-inflammatory drugs; CCB = calcium channel blockers; ED = erectile dysfunction.

Comparison of ‘intermediate’ markers of PD between higher and lower risk groups

The differences in median UPSIT, RBDSQ and mean KS were significant between the higher and lower risk groups in all years of follow-up (Table 8.3). In addition, a greater proportion of individuals with smell loss, RBD and slowed finger tapping, according to predefined cut-offs were found in the higher risk compared to lower risk group each year. Results for categorical outcomes were statistically significant in all years, except the association between risk group and abnormal smell in year 2 ($p=0.094$).

The majority of individuals remained in the same risk group (higher, middle, lower) each year. However, annual changes of risk led to movement of some subjects between higher, middle and lower risk groups (Figure 8.2). Between baseline and year 1, approximately 20% of both the higher and lower risk group moved to the middle risk group, in year 2, 26% and 15% moved groups, and similar changes were observed in year 3.

Outcome	Year 1			Year 2			Year 3			
	All	Higher	Lower	All	Higher	Lower	All	Higher	Lower	p-value*
UPSIT										
n	766	113	105	699	106	100	783	117	118	-
Median	32	30	33	32	31	33	32	30	33	<0.001
(IQR)	(30 - 34)	(27 - 33)	(31 - 35)	(30 - 35)	(28 - 33)	(31 - 35)	(29 - 34)	(26 - 33)	(30 - 35)	<0.001
n (%) ≤27	114 (15)	30 (27)	11 (10)	96 (14)	23 (22)	12 (12)	128 (16)	39 (33)	14 (12)	0.094
RBDSQ										
n	1034	154	154	935	140	140	839	125	126	-
Median	2	2	1	2	2	1	2	3	1	<0.001
(IQR)	(1 - 3)	(1 - 4)	(0 - 3)	(1 - 3)	(1 - 5)	(1 - 3)	(1 - 3)	(1 - 5)	(0 - 3)	<0.001
n (%) ≥5	125 (12)	30 (19)	13 (8)	128 (14)	35 (25)	7 (5)	103 (12)	34 (27)	5 (4)	<0.001
KS										
n	878	131	128	792	123	114	791	123	119	-
Mean	54.1	52.2	57.6	54.2	51.8	56.2	53.8	50.9	55.5	0.001
(95% CI)	(53 - 55)	(50 - 54)	(56 - 60)	(53 - 55)	(50 - 54)	(54 - 58)	(53 - 55)	(49 - 53)	(53 - 58)	0.001
n (%) ≤44	149 (17)	37 (28)	15 (12)	132 (17)	29 (24)	14 (12)	160 (20)	36 (29)	19 (16)	0.028

Table 8.3: UPSIT, RBDSQ and KS in participants with risk estimates calculated each year, and comparing those with the higher and lower estimated risks of PD.

Legend: IQR = interquartile range; CI = confidence interval; KS = kinesis score for the worst hand; RBDSQ = REM sleep behaviour disorder screening questionnaire; UPSIT = University of Pennsylvania smell identification test.

* p-value from comparative analysis between higher and lower risk groups using Wilcoxon Rank Sum test for UPSIT and RBDSQ, t-test for KS for continuous data, and Fisher's exact test for categorical data.

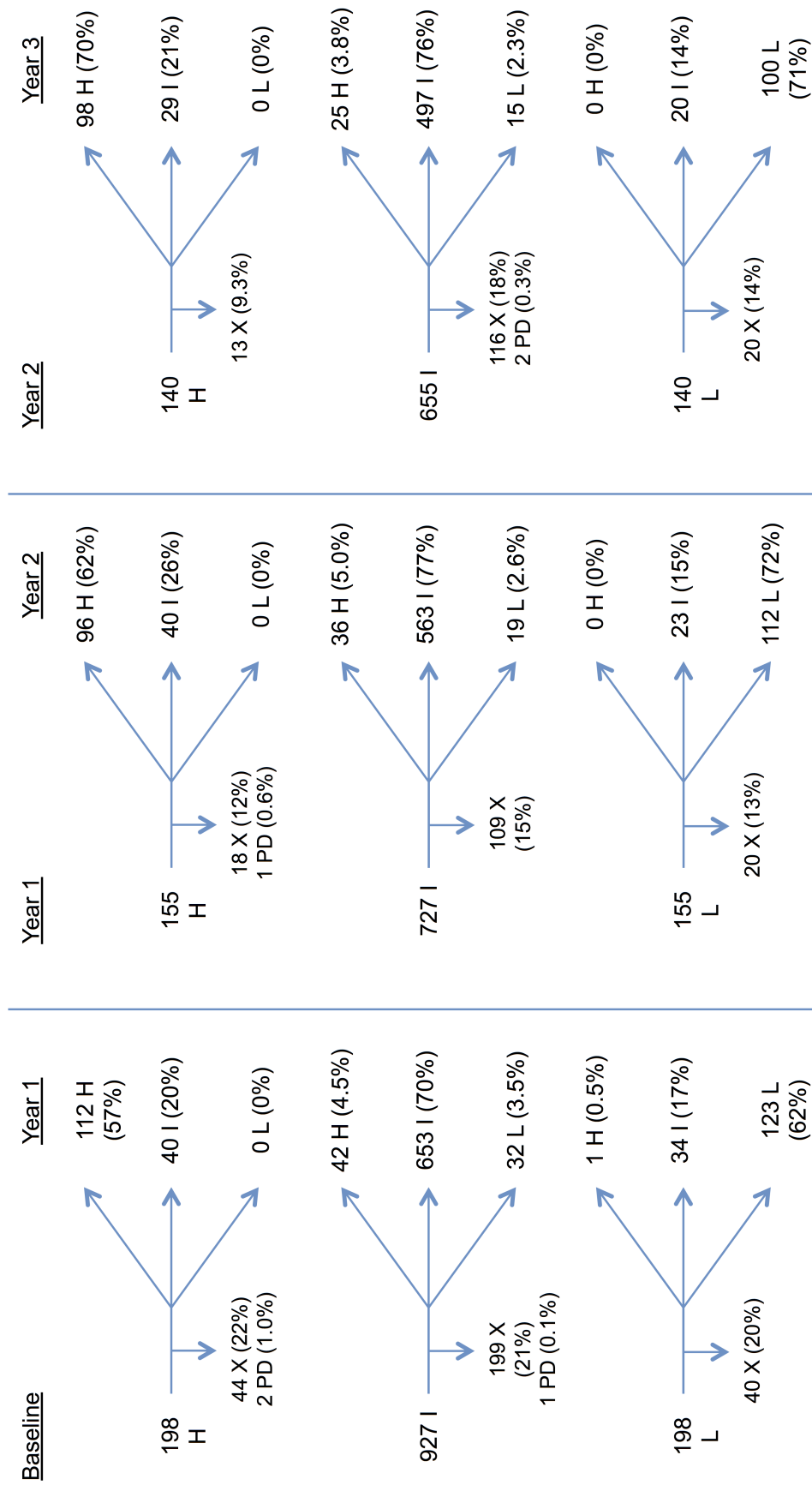


Fig. 8.2: Flowchart of participant movement between risk groups when scored each year of follow-up.

When analysis was restricted to the higher and lower risk groups defined at baseline and followed prospectively for three years, the two groups had significantly different median UPSIT and RBDSQ, and mean KS in all years (Table 8.4). In addition, a greater proportion of individuals with smell loss, RBD and slowed finger-tapping according to predefined cut-offs were found in the higher risk group in every year of follow-up. Results for categorical outcomes were statistically significant in all years, except for the association between risk group and abnormal finger tapping in year 2 ($p=0.080$).

Associations of estimated risk of PD with ‘intermediate’ markers in the whole cohort

Risk scores were strongly associated with each of the intermediate markers across the whole group (all p -values <0.001). After exclusion of age and gender from the algorithm, results comparing smell and RBD scores to risk at each year of follow-up were similar (all p -values <0.001). Significant associations between risk excluding age and gender and KS were observed at year 2 and year 3 ($p=0.035$ and $p=0.004$ respectively), with a trend observed in year 1 ($p=0.079$). Figure 8.3 shows regression analyses for association between intermediate markers and risk estimates excluding age and gender in year 3.

Difference in diagnosis of PD between risk groups during follow-up

At year one of follow-up, three patients had been newly diagnosed with PD, a further subject was diagnosed by the year two survey, and three more by year three (seven in total). Of the participants with newly diagnosed with PD at year 1, all 3 were in the higher risk group that year, and 2 were in the higher risk group at baseline. The subject diagnosed at year 2 was in the higher risk group from baseline. Of the three subjects diagnosed by year 3, none were in the higher risk group at baseline and two subjects had missing data for either year 1 or year 2 of follow-up. However, one of the three was flagged in the higher risk group in a year prior to diagnosis, and for the other two there were marked changes in their risk estimates prior to diagnosis. Annual rankings from risk scoring, year of reported diagnosis and intermediate markers for the seven subjects with incident PD are shown in Table 8.5.

At baseline, the relative risk of being diagnosed with PD over 3 years in the higher-risk group (3 subjects out of 198) compared to middle and lower-risk groups (4 subject out of 1125) was 4.3 (95% CI 0.96 to 19.5) and there was weak evidence to suggest a difference in proportions ($p=0.072$). However, if risk scores were used from one or two years before diagnosis as opposed to at baseline only, then 4 of

Risk of PD in the community: follow-up from PREDICT-PD

265 (1.5%) subjects that had ever been in the higher risk group received diagnosis of PD, compared with 3 of 1058 subjects that had never been higher risk (0.2%). The OR for the association was 5.39 (95% CI 1.20 to 24.24; $p=0.033$). Incidence of PD in the higher risk group at 3 years was 1.6% per year (6 subjects out of 126 higher risk) and 0.2% across the whole cohort.

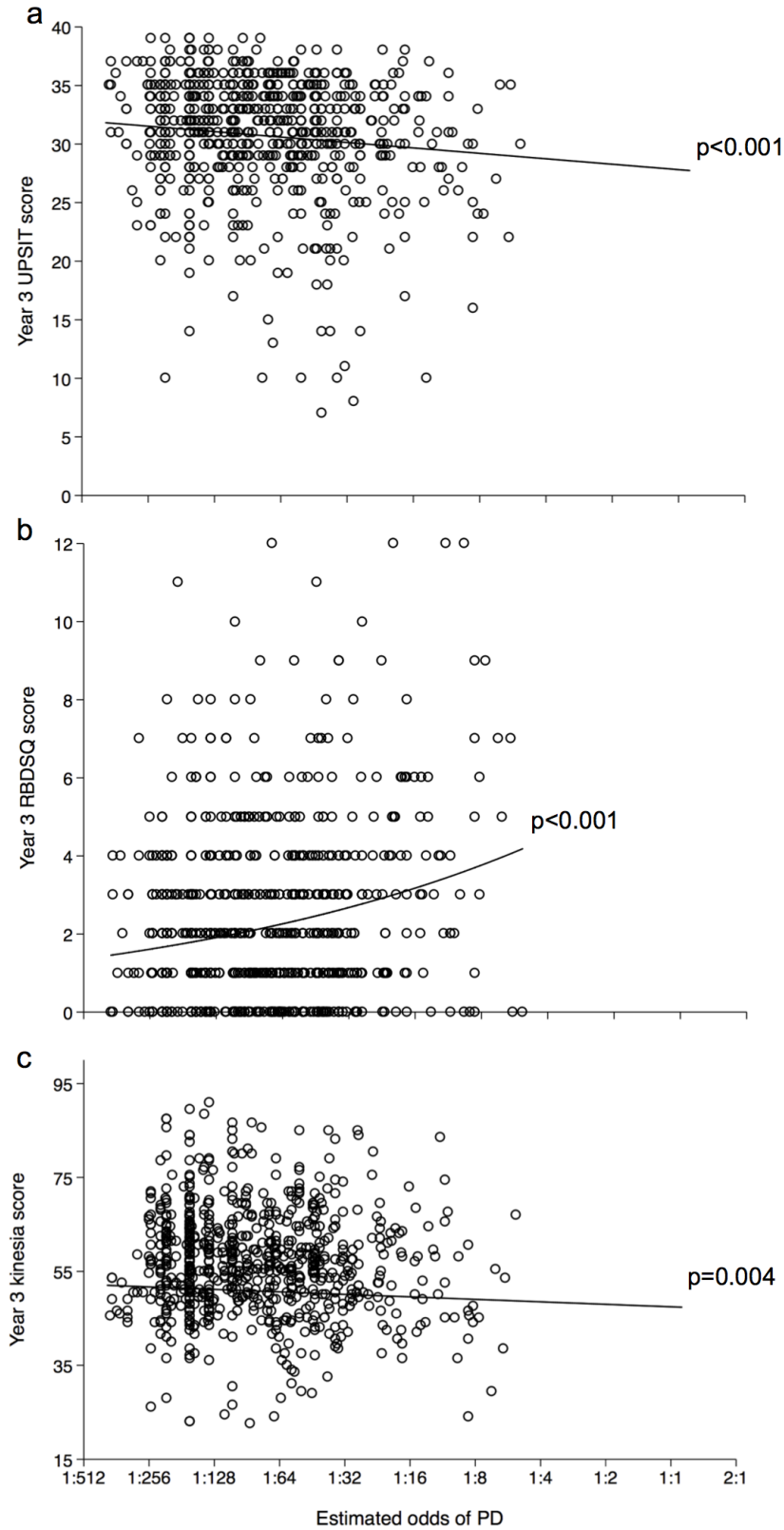


Fig. 8.3: Associations between risk estimates in year 3 excluding age and gender and (a) smell test (UPSIT) scores, (b) RBD questionnaire (RBDSQ) scores and (c) finger tapping scores for the worst hand. Note the leftward shift of risk estimates on the x-axis due to the exclusion of age and gender from the calculations.

Subject	Rank	yr0	yr1	yr2	yr3	UPSIT	yr0	yr1	yr2	yr3	RBDSQ	yr0	yr1	yr2	yr3	Worst-KS	yr0	yr1	yr2	yr3
A		86	68*	67	242		NA	20				0	1	3	1		24	33	39	22
B		106	97*	115	42		35	35				12	13	12	12		56	60	55	NA
C		886	64*	28	56		29	34				2	2	6	6		57	54	NA	72
D		19	13	2*	2		24	22				7	9	4	7		41	38	40	37
E		1086	NA	233	209*		NA	14				2	NA	4	3		43	NA	42	29
F		321	52	173	22*		NA	25				0	0	0	0		41	43	26	23
G		477	156	NA	53*		NA	31				1	3	NA	5		NA	86	NA	38

Table 8.5: Annual rankings and intermediate marker scores in seven subjects diagnosed with PD during follow-up.

Legend: yr = year; NA = not available; UPSIT = University of Pennsylvania smell identification test; RBDSQ = REM sleep behaviour disorder screening questionnaire; KS = kinesia score.

* denotes the year that PD diagnosis was reported in the survey.

8.4 Discussion

Further support for PREDICT-PD is provided here by reproducible results at years 1, 2 and 3 of follow-up, which are consistent with the differences in intermediate markers observed between the higher and lower risk groups at baseline. In all years, data using continuous measures of olfaction, RBD and finger tapping showed strong statistically significant differences, with similar trends noted in almost all years using categorical outcomes defined by cut-offs. For analysis in which risk estimates were calculated each year, a limited degree of change was observed between higher, middle and lower risk groups. The maximum percentage change in group was seen in higher risk subjects between years 1 and 2 (26% moving to the middle risk group) but in general changes were limited to no greater than 20%. Some change in groups was to be expected given the subjective, unobserved nature of online surveying and the potential for symptoms to vary over time (e.g. mood and bowel habit). This method of screening should not be thought of as a diagnostic test and risk group membership alone is not strongly ‘predictive’ of subsequent PD diagnosis in individual participants, but it is designed as a method of population enrichment for risk of PD. It suggests that screening may perhaps need to be repeated on more than one occasion. Moreover these results demonstrate that recruitment and screening longitudinally using a web-portal is a cost-effective and efficient means of study, with high annual retention rates (approximately 90% each year after baseline).

Significant differences in intermediate markers of PD were observed between higher and lower risk groups, regardless of whether subjects had risk estimates re-calculated each year or whether groups identified at baseline were compared longitudinally during follow-up. Given that these results have been replicated over three additional time points, the likelihood that the observations made at baseline (see chapter 5) were a chance finding, is significantly reduced. The potential role of bias and confounding is discussed in the concluding chapter of this thesis.

The effectiveness of the algorithm in identifying a group of individuals with increased risk of PD is underlined by the fact that all participants with newly diagnosed PD during follow-up were either in the higher risk group at baseline (three patients), appeared in the higher risk group before diagnosis (two patients), or were observed to have substantial changes in risk estimates prior to diagnosis (two patients). None were ever in the lower risk group. Although numbers are currently small, new diagnoses in the overall cohort are being made at a rate comparable to that predicted in the 60-80 years age group from the general population (1-3 per 1000 per year)¹⁴³ and the higher risk group appears to be enriched approximately 5-fold.

The differences in the presence of intermediate markers in these seven incident cases further underlines the heterogeneity in prodromes of PD, as only one subject had all three (RBD, smell loss, abnormal tapping speed). The other six subjects were only positive for one or two markers. The clustering of early non-motor features has been studied in the PARS and TREND studies, but the co-occurrence of features is still in need of further clarification.^{118,160} However it is clear that individual prodromal markers are not present in all subjects that are later diagnosed with PD. Strategies that measure multiple prodromal features and generate composite exposure information will be more likely to yield the greatest sensitivity and specificity for future diagnosis. PREDICT-PD may provide such a composite measure and future challenges and opportunities relating to this possibility are explored in the next chapter.

Limitations

Specific limitations relating to the intermediate marker analysis are discussed here, with limitations of the study overall considered in chapter 9, including the role of selection and information bias, and confounding, and the steps taken to try to minimise these. Age and gender are likely confounders in the association between risk scores and intermediate markers. However, once removed from the algorithm, significant relationships remained between risk scores and intermediate markers almost every year. Stratification using the preliminary risk algorithm which includes age and gender, yields a higher risk group which contains more elderly males. The overall differences in intermediate markers are relatively small, meaning that a greater sample size would be required to fully test associations stratified for age and gender.

Intermediate markers were selected on the basis that changes in one or more of them would be expected prior to a clinical diagnosis of PD. However, there are some limitations to their use. None of these intermediate markers are 100% specific and sensitive for PD, with RBD having high specificity and low sensitivity, and anosmia having comparatively high sensitivity and low specificity. The tool we used for RBDSQ also has high sensitivity for identifying RBD when compared against the gold standard, polysomnography (PSG) but low specificity. This may account for the high proportion of individuals that were observed to exceed the RBDSQ cut-off for RBD. Smell test data were only collected at baseline and in year 3 but not in years 1 or 2. Hence comparisons with new risk scores and groups in the subsequent 2 years were made using these baseline smell results.

Conclusions

These data support the reproducibility and validity of the PREDICT-PD approach in identifying individuals with increased risk. However, increased risk must be distinguished from future diagnosis of PD and a proportion of individuals moved from a higher to the middle risk group during follow-up. Future work is required to refine the algorithm to reduce flux between groups, in particular to identify those with a consistently high risk profile who eventually convert to clinical PD. Longer follow-up, with in-depth study (including eventual post-mortem examination), will identify a greater number of participants who have developed PD and allow further characterisation of the higher and lower risk groups.

Chapter 9

Overall Discussion

9.1 Main results and approach

This thesis describes an approach to community-level screening for PD risk. A systematic exploration of risk factors and early disease features formed the foundation for a longitudinal pilot study investigating intermediate phenotypes, prodromal markers and incident cases. The PREDICT-PD study is now in its fourth year and continues to gather data from participants annually. Participants in the upper 15th centile of risk were found to have more objective prodromal features of PD (hyposmia and slowed finger-tapping), subjective prodromal symptoms (RBD), gene variants known to increase PD risk, and some imaging findings associated with PD, compared with participants at lower risk. Incident PD cases are being recorded in the 15th centile of risk at a greater rate than incident cases in subjects at lower risk (the remaining 85%), providing the strongest evidence that the preliminary algorithm can enrich for future PD.

Previous studies have attempted to identify subjects at risk of PD by studying individuals with a family history of PD or with idiopathic anosmia or RBD, or imaging abnormalities associated with increased risk.^{69,70,71,72,74,75,106} A number of other ongoing approaches (apart from the TREND study) have focussed on subjects with RBD, idiopathic anosmia, and non-manifesting carriers of *LRRK2* or *GBA* mutations.^{34,77,104,118} Such studies are vital to the understanding of pre-diagnostic PD because they examine relatively homogenous populations and will provide estimates of ‘time to conversion’, and provide an excellent opportunity to investigate potential pre-diagnostic biomarkers and targeted disease-modifying therapies. However they may fail to take account of the heterogeneity of PD and be unrepresentative of the overall clinical spectrum. They may capture or alternatively fail to identify those for which the prodromal marker is transient (for example transient anosmia following sinus disease) but many of these strategies

do not include repeat testing due to cost and practicality. Such limitations may have further importance because in preparing proof-of-concept clinical trials, the markers that change prior to conversion, and their temporal relationship to conversion, are likely to be key to the study design and perhaps dictate power calculations.

Studies like PREDICT-PD may provide new knowledge relating to the temporality and natural history of the prodromes of PD, and provide tools for the further in-depth study of individuals with elevated risk of PD. Whilst the pilot study described here provides support for this approach, it appears unlikely that a sample size of approximately 1,000 subjects would achieve the intended goals of identifying sufficient numbers of higher risk subjects (with sufficient enrichment) for biomarker studies, and potentially for recruitment to disease-modifying trials. In order to meet these long-term aims, the sample size would need to be increased substantially to enable further prospective exploration adequately powered for outcomes that may not rely on incident diagnosis.

Longitudinal studies provide information and protection from some of the bias that occurs in cross-sectional studies or retrospective case-control studies. They can demonstrate the temporality of the effect that factors have on an outcome such as PD and provide risk estimates based on incidence within a cohort rather than odds ratios arising from comparison of cases and controls. Longitudinal studies are also essential to understanding PD and its prodromes, given that the pathology is likely to be present and undiagnosed for many years before it manifests with the classic features. Ideally longitudinal studies should be large enough that they can exclude incident cases diagnosed within the first 2-4 years of follow-up and avoid the bias created through confounding by prevalent disease.

Longitudinal studies of the PD prodrome including TREND and PARS, are also in progress.^{104,118} These studies have great potential to be able to delineate the features and course of prodromal PD, but widespread screening appears less achievable since they depend on expensive tests or heavy manpower. PD, compared to other age-related diseases such as cardiovascular disease and cancer, is relatively uncommon; the incidence in individuals over the age of 60 years is 0.1-0.3% per year.¹⁴³ This means that large numbers of subjects need to be screened in order to detect small numbers of potential cases. Early-identification strategies require platforms that can be made widely available and at low-cost. The approach described in this thesis is scalable and cost-effective, and the web-platform could test 100 times as many subjects for a negligible increase in price. It is also now clear, even from the incident PD cases in this study, that hyposmia, RBD and other individual non-motor features are not always present in the pre-

diagnostic or immediately post-diagnostic phase, which is another reason why the PREDICT-PD approach may prove superior.

9.2 Limitations

Algorithm

The preliminary, evidence-based algorithm was developed to estimate the risk of being diagnosed with PD expressed as an odds. Importantly, some of the strongest risk factors and early features of PD have been left out of the preliminary algorithm, first to act as intermediate markers of risk and provide reassurance that stratification was effective, but secondly because true risk estimates for the effect of hyposmia and RBD are not available (except for one study for hyposmia using the B-SIT which provided an estimate with very wide confidence intervals). Using odds as a measure of disease risk has potential limitations. The equation that underlies odds is *number of events in the exposed / number of events in the unexposed*, which is different to that for risk (*number of events in the exposed / number at risk at the beginning of the study period*). However, one can see that if the event is uncommon (the rare disease assumption) then odds and risk approximate one another, as do their ratios. In a community cohort such as this, incident PD is uncommon meaning that estimates of odds and risk will not be very different.

Calculation of combined odds were derived from multiplying the background odds (e.g. age-related odds) by the presence of an additional measured factor. However this approach does not make adjustments in the absence of a measured factor, which is simply treated as if it were unmeasured. For example, if a 65 year old male had his sense of smell objectively tested and it was found to be abnormal then he would have a higher risk of PD. If his smell is found to be normal (particularly at the upper extreme of possible scores) then one would expect him to have a lower risk of PD than a 65 year old male who had not had their sense of smell tested at all. To offer an extreme example, a 70 year old female, lifelong smoker, coffee and alcohol consumer, without any history of constipation, anxiety or depression would be expected to be at lower risk than a 70 year old female in whom none of these factors had been ascertained, but with the current algorithm, their risk scores would be the same. Ranking subjects according to risk estimates is likely to offer better accuracy than using the risk estimates themselves, and was the approach used here for analyses. This ranking of odds enabled comparative analysis to be undertaken between the extremes of risk in the cohort and cut-offs for higher and lower risk were created at the 15th centile and 85th centile.

The next phase of the algorithm will incorporate likelihood ratios for individual risk and protective factors which are due to be released soon by a Movement Disorders Society (MDS) Task Force.ⁱ Likelihood ratios enable the adjustment of the combined risk estimate upwards or downwards depending on the results of tests for measured factors. Furthermore the risk estimates for a number of early non-motor features have been refined since the original systematic review and meta-analysis (chapter 2) was undertaken, and any changes to these will be reflected in the upcoming likelihood ratio estimates.

Most of the *a priori* hypotheses of this study have been met. Higher risk subjects had an increased presence of intermediate markers for PD during every year of study, they had an excess of gene variants associated with PD and some imaging findings associated with PD. Most importantly higher risk subjects appear more likely to be diagnosed with PD during follow-up. The statistical tests which underpin the observed associations suggested that these were unlikely to be chance observations, given the small p-values in general. Repeat testing for intermediate markers and magnitude of effect for SN hyperechogenicity also make chance an unlikely explanation for the the observed differences between higher and lower risk. However, before these associations can be accepted as true, one must consider the roles that bias and confounding could have played in the study overall.

Bias

Selection bias occurs when the sample has different characteristics to the population from which it was drawn. The participants in the PREDICT-PD pilot study were recruited through a variety of methods, including some coverage on local radio and print media with an older readership. However most participants were recruited following an email to members of Parkinson's UK, calling on participation by individuals that did not have PD. This meant that relatives and spouses of patients with PD were more likely to take part than individuals with no connection with PD, an observation that is reflected in the relatively high prevalence of family history and spouse PD in the cohort overall. This in turn may have elevated risk overall in the cohort, not only via genetic risk factors, but also shared environment (and environmental risk factors) and cause the incidence of PD to be greater in this cohort than in a truly unselected population sample. However, this has not been reflected in the incidence to-date, which appears consistent with prior estimates.¹⁴³

Living with or caring for someone with PD may increase the chance of experiencing early-non motor features (for example, co-existent depression or anxiety)

ⁱA consensus statement from the MDS Task Force is in the final stages of preparation.

and in doing so has the potential to falsely elevate risk estimates in these participants. Separately, having a strong connection with PD may have also improved recruitment and retention rates, which may have influenced the longitudinal validity of the study. Overall selection bias can affect the validity and generalisability of the results and mean that similar outcomes would not be observed if the approach was applied more widely. Selection bias could have specifically affected imaging and genetic sub-studies given that participants were preferentially selected from the upper extreme of risk with less sampling across the remainder of the risk spectrum. This sampling method may have been vulnerable to false positives in the control group or conversely underestimate the frequency of an outcome in that group, leading to type 2 (incorrectly accepting the null hypothesis) or type 1 (incorrectly rejecting the null hypothesis) error respectively.

Information bias or measurement error can arise in a number of ways. Observer bias was unlikely for most methods employed in this study. Regarding exposures, the algorithm contained all the factors identified in chapter 2 that could be measured in an appropriate way. No additional factors were added or removed and therefore the results reflect *a priori* hypotheses about the preliminary algorithm, which was not altered by the investigator. For the outcomes, only SN hyperechogenicity may have been affected by observer bias. SPECT quantification was undertaken by a researcher that was blinded to participant risk status and was not unblinded until after the analysis stage, meaning that observer bias could not have arisen.ⁱⁱ The remaining outcomes (intermediate markers, genotyping) were measured without observer influence because genotyping results were binary (the presence or absence of a mutation) and were repeated, and intermediate markers were measured unobserved in the participant's own home.

Measurement bias could have arisen through unobserved collection of intermediate markers or indeed exposures recorded through the survey. Outcomes may have been affected if participants sought help from others when completing the smell test or used two hands to complete the BRAIN-tap test. Both of these possibilities were anticipated and steps were taken to minimise them (i.e. clear instructions, cross-checking smell test booklets with web results, and exclusion of improbable scores). Identifying inaccuracies in exposure information presented a greater challenge and the possibility of information bias here cannot be discounted. For example, with free text medication lists and past medical history, there exists the possibility that information may have been omitted intentionally or unintentionally. Furthermore individuals may tend to answer questions with a preference for immediate recall therefore discounting past symptoms and reporting them as normal, or declaring the presence of the symptom at that given time, which

ⁱⁱJohn Dickson undertook SPECT quantification.

Overall Discussion

subsequently was only transient. Methods of checking data were used to improve consistency and are described in chapters 5 and 8.

Ascertainment bias occurs when cases (or controls) are not identified reliably. Those that reported having PD at baseline were excluded from analysis but others may have had PD at baseline and either not yet have been diagnosed or chose not to disclose this information. Attempts to verify information were made, including checking the reason for any dopaminergic medication in medication lists and, for cases of incident PD, checking the date of first symptoms and details about the neurologist diagnosis. Incident PD was defined as diagnosis with PD by a specialist, not onset of symptoms, and therefore some patients may have had symptoms predating diagnosis. However this is a flaw in most observational studies that rely on periodic review in-person or medical records, rather than date of first symptoms. Although the annual retention rate remains high, a further concern about ascertainment is for those that may have dropped out because they were diagnosed with PD, and were upset or did not want to continue in a study aiming to ‘predict’ PD. A table of baseline characteristics of those that completed at least one follow-up year and those that dropped out after baseline, showed no major differences but it is conceivable that some of those lost to follow-up may have included cases of PD.

Bias can only be acknowledged and cannot be accounted for at the analysis stage of a study (i.e. the results cannot be adjusted in light of perceived bias). Recognising bias allows one to consider how much the observed results can be relied upon and how generalisable they might be. Here, reduction in bias could be achieved at the data collection stage by increasing the number of objective measures obtained or by triangulating subjectively reported symptoms (e.g. multiple mood and sleep questionnaires or cross-referencing with primary care records). Case ascertainment could be strengthened by seeing all participants in-person, and selection bias reduced by advertising in a random and widespread fashion. However a degree of selection bias is always likely to be present, because people that participate in research are more likely to have certain socioeconomic characteristics. Likewise individuals that have a friend, relative or spouse with a given disease are more inclined to take part in research pertaining to this. Increasing the number of tests and in-person assessments for all participants contradicts the successful approach that has been described here. The best qualities of this project are that it shows a methodology for large scale, cost effective, low intensity research that results in high retention rates. Whilst elimination of bias is desirable wherever possible, it is often the case that practicality and cost are the key influences on the way that research is conducted.

Confounding

Away from bias, confounding is another reason that type 1 error can arise (incorrect rejection of the null hypothesis, a false positive). For most diseases, age is an important confounding factor and incidence tends to increase in older persons. Gender is also an important confounding factor with different prevalence occurring in males and females. Confounders identified *a priori* can be tested and adjusted for at the analysis stage of a study, to determine whether they have influenced the results. For PD, age and gender are important confounding factors, but they are also two of the most important risk factors for PD and were therefore included in the algorithm. Adjusting for age and gender at the analysis stage would increase error substantially due to the potential of multicollinearity (two modelled factors that are highly correlated i.e. the risk score and age, because age is part of the risk score). Removing both from the algorithm was another option but could have resulted in a study that was underpowered to detect differences between higher and lower risk in comparative analyses. Furthermore, simply removing age and gender from the algorithm does not account for the effect that they have on the outcome or indeed on other exposures contained within the algorithm. Limited analyses did show that when age and gender were dropped from the algorithm, associations between risk scores and intermediate markers were still observed, albeit less strongly than when they were included. A major problem with dropping age is that it is the only continuous variable in the risk score and its removal meant that lots of subjects ended up with identical scores, given that all other variables were collected in a binary fashion. The matter of a confounding effect of age and gender may be better explored in a larger study powered to detect an effect of the other risk factors in combination. However, in the long-term it is difficult to see how a combined risk score for PD would not include age and gender, and the important issue is that they do not explain all of the variance in risk, and that other factors do also contribute.

9.3 Further work

A few concurrent and possible future approaches are listed below.

Concurrent work

1. From the current group additional data have already been collected that have not yet been analysed and not included in this thesis. Over 200 of the current participants have been seen in-person as part of clinical visits to higher risk subjects and similar numbers of lower risk. These clinical

visits included a neurological examination, brief cognitive assessment, and a sample of handwriting. As part of the neurological examination, the motor section of the MDS-UPDRS was performed and recorded on video. The purpose of the video is for assessment by two movement disorders specialists (AJL and AS) who are blind to the risk status of the subjects and who will look for subtle motor dysfunction. This work may help clarify the nature of mild parkinsonian signs in subjects estimated to be at higher risk of future PD and characterise specifically what that motor dysfunction might look like. In turn this will guide what future remote and objective tests might be liable to capture this. Other studies have looked at mild parkinsonian signs in the elderly but have not captured them on video for blinded expert review.^{186,194}

2. Genotyping of *GBA* and *LRRK2* will be undertaken in the full cohort to confirm or refute the findings reported in chapter 6. DNA was obtained from approximately 800 of the participants following the year 3 follow-up survey in conjunction with repeat smell testing. Many of the samples have been genotyped (by LR and NM) but final results are not ready. Regardless of whether *GBA* variants are more prevalent in the higher risk group, they do impart increased risk of PD independently and so *GBA* variant status can be used to update risk estimates within the cohort and longitudinally. *LRRK2* mutations may be found in 1 or 2 participants in the wider cohort, but will be valuable because these individuals would be expected to be at very high risk of PD.
3. Genotyping of variants identified from GWAS studies is being undertaken using samples from PREDICT-PD participants, to ascertain the role that a combined clinical-genetic multifactorial risk score may have in predicting incident PD.
4. Recruitment of positive control groups has begun to assess 50 subjects with established PD, 30 subjects with idiopathic RBD confirmed using PSG, and 30 subjects with idiopathic anosmia confirmed with objective smell testing, endoscopy and imaging. Positive control subjects will participate in the same tests as regular PREDICT-PD subjects and will define reference ranges against which higher (and lower) risk subjects can be compared.

Future work

1. ¹²³I-FP-CIT SPECT will be carried in those subjects that were already scanned, 2 years after the first scan. This will examine whether DaT deficit

is progressing at a faster rate in higher risk participants, since cross-sectional analysis did not demonstrate statistically significant differences between higher and lower risk (see chapter 7). To improve statistical power, it is hoped to also increase the number of higher and lower risk subjects that are scanned. Power calculations based on the observed differences suggest that 3-4 times as many subjects would need to be scanned (150-200 participants, power 80%, $\alpha=0.05$). A final decision regarding further imaging will be made after blind clinical rating of the existing scans has been undertaken.

2. Plans to approach and consent current participants for brain donation or participation in parallel with wet and/or tissue biomarker initiatives are underway. The potential to strengthen the algorithm through pathological confirmation of PD diagnosis is an important consideration, whilst balancing the interests of the participants within the cohort.
3. In 1-2 years time, it is expected that there may be sufficient ‘converters’ or DaT positive subjects, that modelling of risk factors for conversion may be undertaken using data from within the cohort. Logistic regression (predictive approach rather than aetiological) will enable selection of factors that explain most of the variation in case load in order that the preliminary algorithm may be refined, along with inclusion of likelihood ratios reported from the MDS Task Force. As has been previously noted, the use of longitudinal data to inform understanding of risk of PD is vital because there is under-diagnosis in the community and the disease runs an insidious course. The temporality and lead time of risk factors is vital to the continued study of pre-diagnostic PD.

Expanding the study

In addition to ongoing work within the current pilot cohort, there exists great potential benefit in considering the expansion of this programme of work to recruit a much larger sample of participants. Anticipated benefits include:

1. Greater numbers of clinical converters, sufficient to power clinical trials with disease-modifying intentions. Expanding the current cohort by 5 or 10-fold would see 50-150 clinical converters over a five year period, which are sufficient numbers to randomise to RCTs of drugs with an estimate effect size of 0.5.
2. A generalisable sample reflecting the wider UK population. Furthermore there is no reason why recruitment should be limited to the UK using this

platform. Translation of web-based generic components could be used to ‘roll-out’ a study such as this to other countries.

3. Due to the current sample size, it is not possible to rank males and females separately, due to the risk of under-powering the study. Increasing numbers of participants would mean that gender could be stratified separately to ascertain risk for males and risk for females.
4. The implementation of a parallel biomarkers programme (invasive and non-invasive). To-date objective markers have been limited to clinical (smell and finger-tapping) and imaging. An expanded programme ought to include a parallel wet and/or tissue biomarkers programme to develop disease-specific measures for the prodromal period that can be applied as outcomes for clinical trials.
5. A conduit to clinical trials in the long-term, or as a recruitment portal and a place to register interest in trial participation in the short-term.
6. Linking the cohort to outcome data such as cause of death ascertained through vital registration.
7. Provision of opportunities to study a wider range of diseases and outcomes from longitudinal data such as dementia.

Improving the web portal

There are also opportunities to improve and evolve the current web-platform in terms of the way in which it gathers data.

1. The free-text options for medication and co-morbidity are not ideal, because they are laborious for participants at the point of data entry and for the investigator due to a need to clean the data prior to analysis. Options for this include:
 - Incorporating a drug search engine that works by identifying medications on the first few letters and then presenting list of options.
 - Requesting a photograph or copy of the participant’s repeat prescription.
 - Linking data to primary care records.
 - Presenting reported symptoms, medication and co-morbidities back to participants each year and asking them to update if there are differences from the year before.

2. To incorporate brief but valid, computerised tests of cognitive function. It is known the mild cognitive impairment occurs early in elderly patients diagnosed with PD, perhaps pre-diagnosis and objective cognitive data are vital to the long term success of the project.^{195,196} It would also help ascertain reliability of other gathered self-report data and support the expansion of the cohort for the assessment of other disease states.

9.4 Ethical issues

The aim of the PREDICT-PD study is to identify a group of individuals at higher risk of PD and demonstrate this through a variety of outcomes, including incident PD. Participants have so far been given no information about their test results or their estimated risk scores. As such many of the possible ethical issues have been avoided but as positive results relating to the above aims have started to emerge, unresolved ethical issues come to the fore.

In the long-term, the purpose of enriching a group for PD risk is to offer early disease-modifying treatment to slow or prevent PD. The necessary precursor to this is commencing proof-of-concept clinical trials. It is believed that the best chance of demonstrating disease-modifying potential will be in the earliest stages of disease (pre-diagnosis), which makes identification of those at higher risk of diagnosis a priority.⁶ The identification of reliable pre-diagnostic imaging or laboratory markers of PD, so that the outcome of clinical trials is not reliant on clinical diagnosis, is also an important focus of research.

For a re-purposed drug with previous data on safety and tolerance, the implications are perhaps less than for novel drugs with unknown safety profiles. Both types of drug are under investigation already, driven by increasing understanding about the basic biology of PD. It is unlikely that higher risk subjects identified through the basic PREDICT-PD approach are sufficiently enriched to be included directly in clinical trials, but if they were to be or whether they were to require a further layer of risk stratification (using smell or TCS or genetics), disclosure of their risk status will be a pre-requisite. Having a biomarker for PD would be helpful in many ways because it would provide reassurance about the accuracy of information relating to risk status, but ultimately participants would be likely to want to know what their risk is, in order to make an informed decision about drug trial participation.

9.5 Concluding comments

This thesis describes a pilot study aiming to enrich a sample of the general population for risk of PD, which could one day underpin efforts to recruit such subjects to clinical trials of disease-modifying therapy. The higher risk subjects have been found to have higher rates of intermediate markers, gene mutations and imaging appearances associated with PD, which adds credence to the notion that the higher risk group had been enriched for future PD. Follow-up data over three years have been presented and show the consistency of results over time, and some evidence that higher risk subjects are more likely to be diagnosed with incident PD. Modification of the algorithm using incident cases and abnormal ^{123}I -FP-CIT SPECT to determine the most predictive factors in the pilot cohort will further validate this approach. The next step will focus on how the approach might be expanded and utilised in further defining risk in the population, support biomarker initiatives and ultimately lead in to clinical trials with disease-modifying intentions.

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Appendix A

Details of studies from systematic review and meta-analysis presented in chapter 2

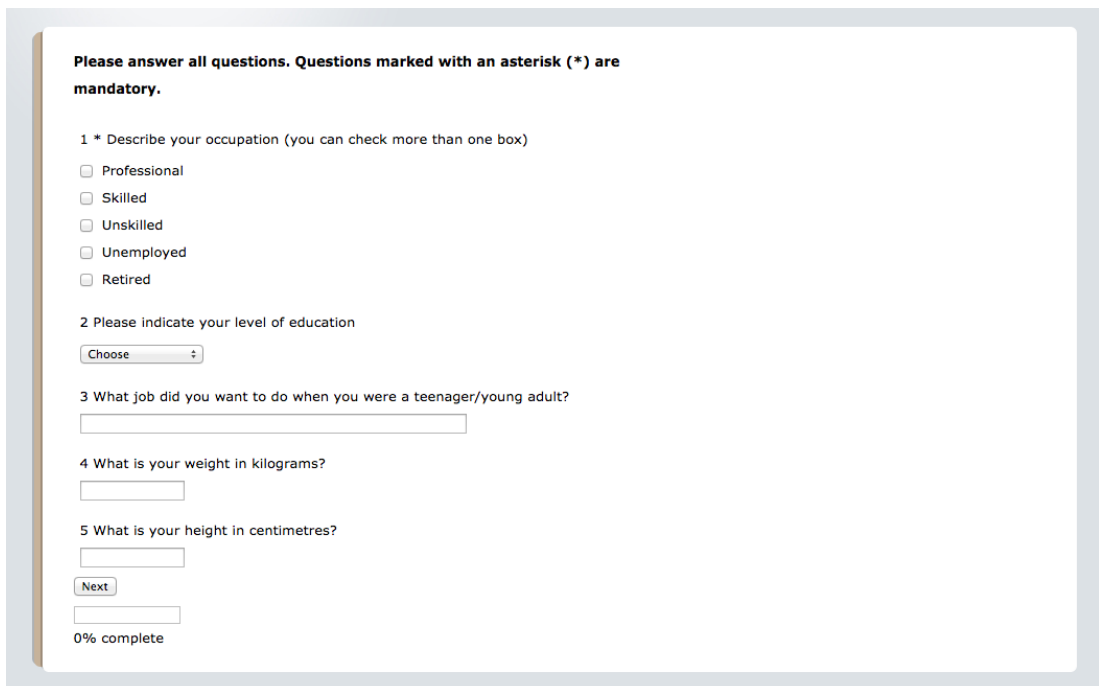
Tables providing details of studies included in the systematic review and meta-analysis are too numerous to be printed here but can be reviewed on the CD/DVD attached to the inside back cover of this thesis.

The following files are reproduced on the CD/DVD:

- Table 1 - Details of Studies included in the meta-analysis.
- Table 2 - Odds ratios and relative risks of studies included in the meta-analysis.
- Table 3 - Studies included for systematic review but not suitable for use in the meta-analysis.
- Extended reference list of studies included in the systematic review and meta-analysis.

Appendix B

Screenshots from the questionnaire of early non-motor features and risk factors embedded in the web portal



Please answer all questions. Questions marked with an asterisk (*) are mandatory.

1 * Describe your occupation (you can check more than one box)

- Professional
- Skilled
- Unskilled
- Unemployed
- Retired

2 Please indicate your level of education

Choose ▾

3 What job did you want to do when you were a teenager/young adult?

4 What is your weight in kilograms?

5 What is your height in centimetres?

Next

0% complete

Fig. B.1: Screenshot - Demographic information

Screenshots from the questionnaire of early non-motor features and risk factors embedded in the web portal

Please answer all questions. Questions marked with an asterisk (*) are mandatory.

6 * Does anyone in your family have Parkinson's (a blood relative)?
 Yes No

7 * Are you one of a pair of twins?
 Yes No

8 Which family member(s) has/had Parkinson's?
 Mother
 Father
 Sister
 Brother
 Grandmother (Mother's side)
 Grandfather (Mother's side)
 Grandmother (Father's side)
 Grandfather (Father's side)
 Other (i.e. uncle or cousin). Please enter in text

9 * Do you smoke?
 Yes No I used to smoke

10 For how long have you/did you smoke (in years)?

11 How many cigarettes do you/did you smoke each day on average?

12 * Do you drink coffee?
 Yes No

13 If yes, please enter approximate number of cups per week

14 * Do you drink alcohol?
 Yes No

15 If yes, please enter approximate number of alcohol-containing drinks per week

16 * Have you ever hit your head so strongly that you nearly fainted or lost consciousness, or sustained significant trauma to the face or nose?
 Yes No

17 * Do you think you have received significant exposure to pesticides in your lifetime?
 Yes
 No
 I don't know

18 If you answered 'yes' to question 17, please indicate estimate amount or frequency

16% complete

Fig. B.2: Screenshot - Risk and Protective Factors

Please answer all questions. Questions marked with an asterisk (*) are mandatory.

19 * Do you have any of the following medical conditions?

- Parkinsons disease
- Movement disorder
- Stroke
- Motor neurone disease
- Dementia
- None of these

20 * Do you have any other medical conditions?

- Heart disease
- High-blood pressure
- High-cholesterol
- Diabetes
- Cancer
- Gout
- None of these
- Other

21 * Please list all medication that you have taken regularly in the last 10 years (if none please state 'None')

33% complete

Fig. B.3: Screenshot - Co-morbidity and Medication

Screenshots from the questionnaire of early non-motor features and risk factors embedded in the web portal

Please answer all questions. Questions marked with an asterisk (*) are mandatory.

22 * How many bowel movements do you usually have in one week?

1 2 3-4 5-8 9-12 13-16 >17

23 * Do you ever use laxatives?

Yes No

24 * Does opening your bowels require a lot of effort?

Yes No Sometimes

25 * Do you suffer from hard stools?

Yes No Sometimes

26 * Rate your ability in the previous 3 months, without treatment, to have and maintain an erection adequate for intercourse

Poor Fair Good I'd rather not answer I am a female participant

27 Do you ever use Viagra or similar to improve ability to achieve an erection?

Yes No I am a female participant

28 * Do your hands, arms or legs ever shake?

Yes No

29 * Do you shuffle your feet and take tiny steps when you walk?

Yes No

50% complete

Fig. B.4: Screenshot - Non-motor and motor symptoms

Please answer all questions. Questions marked with an asterisk (*) are mandatory.

30 * I feel tense or wound-up

31 * I still enjoy the things I used to enjoy

32 * I get a sort of frightened feeling as if something awful is about to happen

33 * I can laugh and see the funny side of things

34 * Worrying thoughts go through my mind

35 * I feel very cheerful

36 * I can sit at ease and feel relaxed

37 * I feel as if I am slowed down

38 * I get a sort of frightened feeling like "butterflies" in the stomach

39 * I have lost interest in my appearance

40 * I feel restless as if I have to be on the move

41 * I look forward with enjoyment to things

42 * I get sudden feelings of panic

43 * I can enjoy a good book or radio or TV programme

Fig. B.5: Screenshot - Hospital Anxiety Depression Scale

Screenshots from the questionnaire of early non-motor features and risk factors embedded in the web portal

Please answer all questions. Questions marked with an asterisk (*) are mandatory.

44 * Have you ever been told, or suspected yourself, that you seem to act out your dreams while asleep (for example punching, flailing your arms in the air, making running movements, etc)?

Yes No

45 * I sometimes have very vivid dreams

Yes No

46 * My dreams frequently have an aggressive or action-packed content

Yes No

47 * The dream contents mostly match my nocturnal behaviour (for example, if you run in the dream do your feet move in bed?)

Yes No

48 * I know that my arms or legs move when I sleep

Yes No

49 * It thereby happened that I (almost) hurt my bed partner or myself

Yes No

50 * I have or had the following phenomena during my dreams: Speaking, shouting, swearing, laughing loudly

Yes No

51 * I have or had the following phenomena during my dreams: Sudden limb movements, fights

Yes No

52 * I have or had the following phenomena during my dreams: Gestures or complex movements, that are useless during sleep, e.g. to wave, salute, frighten mosquitoes, falls off the bed

Yes No

53 * I have or had the following phenomena during my dreams: Things that fell down around the bed, e.g. Bedside lamp, book, glasses

Yes No

54 * It happens that my movements awake me

Yes No

55 * After awakening I mostly remember the content of my dreams well

Yes No

56 * My sleep is frequently disturbed

Yes No

57 * I have / had a disease of the nervous system (e.g. Stroke, head trauma, parkinsonism, RLS, narcolepsy, depression, epilepsy, inflammatory disease of the brain)

Yes No

83% complete

Fig. B.6: Screenshot - RBD Screening Questionnaire