Clinical and biochemical features of Parkinson's disease: A cross-sectional and longitudinal analysis of the Oxford Discovery cohort dataset



Doctoral thesis

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Abstract

Parkinson's disease (PD) is a heterogenous multisystem neurodegenerative disorder displaying a marked variation in phenotype and prognosis. Predicting prognosis has important implications for individual prognostication, empowering clinical trials and targeting novel treatments. This study examined which clinical and biochemical factors predicted prognosis in a large cohort of early PD subjects (Discovery cohort, n=945).

A systematic review revealed evidence that older age at onset, mild cognitive impairment, verbal fluency, male gender, REM sleep behaviour disorder, and greater axial impairment may predict more motor and cognitive progression. Comparisons between the Discovery cohort and similar published disease and control cohorts established Discovery as being highly representative of the target population. Older age and poor performance on the Purdue assembly, visuo-spatial and verbal fluency tasks at baseline predicted more rapid cognitive decline. Older age, postural instability, pain and anxiety predicted a more rapid functional decline and dependency. Combinations of each of these clinical assessments were specific but not sensitive. Uric acid, vitamin D, epidermal growth factor, apolipoprotein-A1 and C-reactive protein have been cited as the most promising biochemical biomarkers, although there was a lack of high quality studies. In the Discovery cohort, while a lower uric acid level at baseline was found in PD compared to controls, the difference was insufficient to be of diagnostic value. None of the proposed biochemical markers predicted future progression on careful longitudinal follow up. This important negative study should help redirect precious resources to more useful biomarker candidates.

In conclusion, a combination of clinic-ready clinical factors at baseline predict a more rapid cognitive and functional decline in PD with good specificity but not sensitivity, and could be used to help tailor prognosis on an individualised basis.

“It is a capital mistake to theorise before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.”

— Arthur Conan Doyle, Sherlock Holmes

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Contributions

This thesis represents primarily my own work including the design, analysis and interpretation of the included projects. However, it also includes contains the outcomes of data collection, biochemical analysis and data clustering techniques which are not my own work. Recruitment started to the Discovery cohort, on which this work was based, 3 years before I joined the project. After joining the team, I formed part of the clinical team with regular meetings to review strategy and implementation of data collection. The data collection itself was performed by the Oxford Parkinson's Disease Centre clinical team, the Thames Valley Dementias & Neurodegenerative Diseases Research Network team as well as myself. Greg Toulson performed the assays to analyse the serum for uric acid, epidermal growth factor, vitamin D, C-reactive protein and apolipoprotein-A1, under the supervision of Dr Reza Morovat. Michael Lawton performed k-means cluster analysis on the clinical dataset to determine the data driven subtypes referenced in the study.

Peer-reviewed publications to date directly related to the work in this thesis for which I am the lead author are:

1. Baig F, Lawton MA, Rolinski M, Ruffmann C, Klein JC, Nithi K, et al. Personality and addictive behaviours in early Parkinson's disease and REM sleep behaviour disorder. Parkinsonism & Related Disorders. 2017;37:72-8.
2. Baig F, Lawton M, Rolinski M, Ruffmann C, Nithi K, Evetts SG, et al. Delineating non-motor symptoms in early Parkinson's disease and first-degree relatives. Movement Disorders. 2015;30(13):1759-66.

ABBREVIATIONS

AD	Alzheimer's disease
ADL	Activities of daily living
ApoA1	Apolipoprotein-A1
AR	Akinetic-rigid phenotype
CRP	C-reactive protein
DLB	Dementia with Lewy bodies
EDS	Excessive daytime somnolence
EGF	Epidermal growth factor
GBA	Glucocerebrosidase
HRQoL	Health related quality of life
ICB	Impulse control behaviours
IQR	Interquartile range
LRRK2	Leucine-rich repeat kinase 2
LEDD	Levodopa equivalent daily dose
MCI	Mild cognitive impairment
MDS	Movement Disorders Society
NMS	Non-motor symptoms
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission topography
PIGD	Postural instability and gait disorder phenotype
PND	Predominantly non-dopaminergic domains
RBD	Rapid eye movement sleep behaviour disorder
RBD	REM sleep behaviour disorder
S&E	Schwab and England activities of daily living scale
SD	Standard deviation
TD	Tremor dominant phenotype
TUG	Timed get up and go test
UPDRS	Unified Parkinson's Disease Rating Scale
YOPD	Young onset Parkinson's disease

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1 INTRODUCTION

Parkinson's disease (PD) is a heterogeneous multisystem neurodegenerative disorder displaying a marked variation in phenotype and prognosis. Predicting the future outcome in an individual with newly diagnosed PD is therefore very challenging. There is a critical need for biomarkers to predict both motor and non-motor outcomes. In addition to the visible motor symptoms of Parkinson's (tremor, stiffness and slowness of movement), there is increasing evidence that it is the non-motor symptoms which patients develop that play a major impact in causing disability and undermining their quality of life. Non-motor symptoms commonly include memory problems or dementia, mood disorders (such as depression and anxiety) and sleep disorders. For example, a person with Parkinson's is 4-6 times more likely to develop dementia than someone of a similar age without Parkinson's (Aarsland et al. 2010), which has a major impact on the patient (as well as to their family and carers) frequently resulting in a loss of independence, institutionalization, care-giver burden and early death.

Biomarkers are urgently needed to guide research into disease mechanisms, empower clinical trials and delineate subtypes. The ultimate aim would be to use this information to identify patients at risk of early deterioration for treatment with drugs aimed at slowing or halting the disease process itself. In clinical practice, this would have significant implications for the individual (personalised treatment and future planning), as well as directing health and social service resource use in an increasing prevalent disease. The discovery of clinical features which predict prognosis would help address these issues and aid biomarker discovery.

This study looks to examine which clinical and biochemical factors in early PD can be used to predict prognosis.

1.1 WHAT IS PARKINSON'S DISEASE?

Parkinson's disease is a chronic multisystem disorder of the nervous system characterised by its motor syndrome of bradykinesia with at least one of rigidity, tremor or postural instability. It is the second most common neurodegenerative disorder, after Alzheimer's disease, with an estimated crude incidence of 13.0 (10.9–15.1)/100,000 person-years (Evans et al. 2016). Parkinson's currently affects up to 127,000 people in the UK with this number expected to rise exponentially with the ageing population (Bach et al. 2011). Treatment remains symptomatic with no disease modifying treatments available.

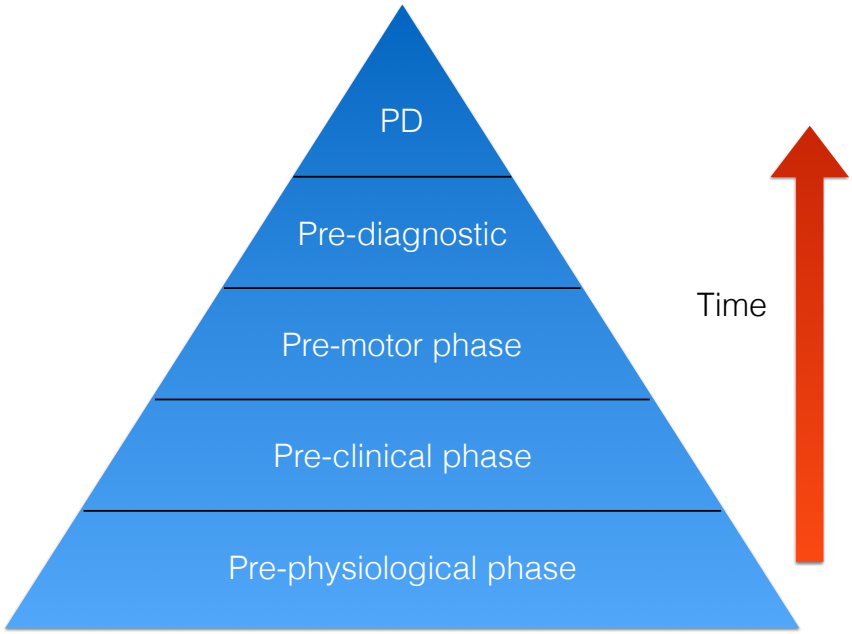
Ante-mortem, in the absence of a cheap and robustly accurate diagnostic biomarker, clinical phenotyping has remained the primary diagnostic method in both clinical practice and for research studies. This typically includes a motor syndrome of parkinsonism, levodopa responsiveness and a lack of features suggesting an alternative diagnosis. Dopamine transporter neuroimaging, in combination with clinical phenotyping, may improve diagnostic accuracy in vivo to some extent by excluding a few alternative disorders, but lacks specificity. The most commonly used clinical diagnostic criteria in research studies has been the UK Brain Bank criteria (Hughes et al. 1992), which is primarily based on the parkinsonian motor syndrome.

However, the gold standard criteria for a diagnosis of Parkinson's is pathological confirmation following this typical clinical picture. Deposition of α-synuclein and dopamine neuronal loss in the substantia nigra pars compacta, in the context of a typical clinical picture, is considered 'definite' PD. Clinico-pathological studies have shown that there remains a significant false positive and false negative rate of diagnosis on just clinical grounds, although this does improve up to 85% with longitudinal follow-up (Adler et al. 2014). Thus, ascertaining the diagnosis in early PD remains difficult, even

by neurologists with an interest in movement disorders specialists using established diagnostic criteria.

There are controversies around this approach though (Berg et al. 2014). These include the requirement of dopaminergic loss in the substantia nigra, as this is likely a later finding given the premotor non-motor syndrome has a pathological correlate (Halliday et al. 2011) and that α-synuclein deposition may not be the first sign of degeneration (Milber et al. 2012) (see Figure 1.1). In addition, α-synuclein is found 'incidentally' in 10% to 30% of asymptomatic post-mortem cases, which is higher than the expected prevalence of clinical synucleinopathies (Dickson et al. 2008, Parkkinen et al. 2008).

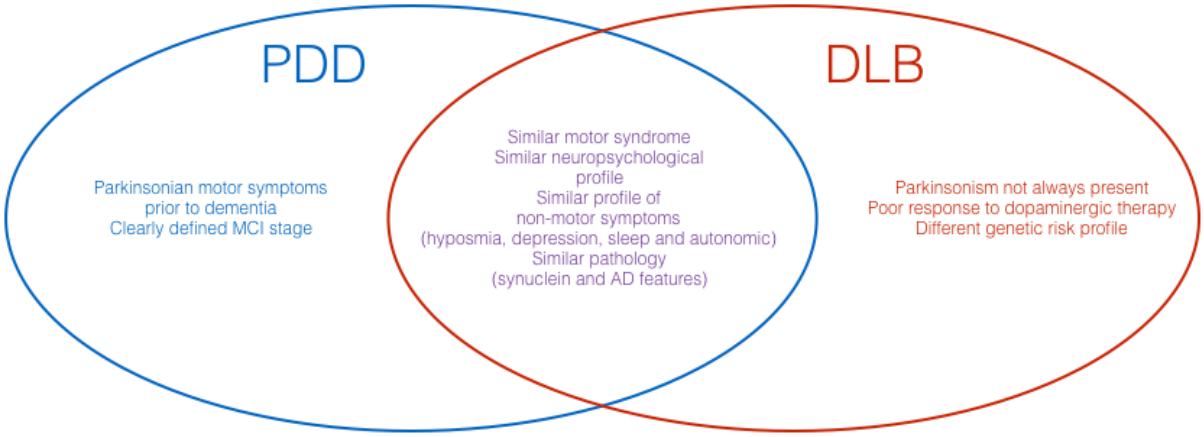
Figure 1.1 - This figure illustrates the concept of the evolution of PD. Initially, patients are in the **pre-physiological phase** where they may have genetic susceptibility and be exposed to environmental factors. They then enter the **pre-clinical phase**, during which neuronal damage occurs in the absence of clinically significant symptoms. This is followed by a **pre-motor phase** where they can develop a non-motor syndrome including symptoms such as hyposmia, constipation, depression and RBD. In the **pre-diagnostic phase** they have started to develop the motor syndrome of PD but do not yet fulfil the criteria for a diagnosis. This process varies in length, but thought to occur over decades. Figure adapted from (Siderowf et al. 2012).



As knowledge advances, further controversies exist around factors historically considered to exclude PD. For example, a first-degree relative with PD has historically been considered an exclusion criteria (Hughes et al. 1992). However, as our understanding of the genetic basis of inherited forms of PD improves, this is no longer widely used in favour of testing for common genotypes.

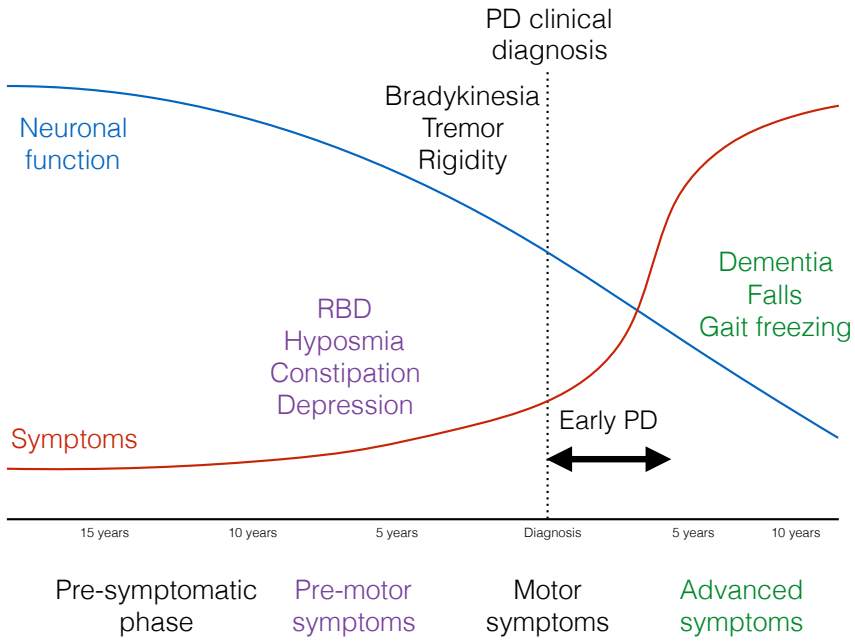
Another example is the highly controversial area of dementia with Lewy bodies (DLB). This is defined, relatively arbitrarily, as the onset of dementia prior to or within a year of developing the parkinsonian motor syndrome. Although differences do exist (see Figure 1.2), PDD and DLB share many similarities in dementia presentation, neuropsychological findings, non-motor profile (olfactory loss, depression, sleep disorders and autonomic dysfunction in both), imaging, genetics and pathology. Thus there are arguments as to whether this represents a subtype or an alternative disease (Berg et al. 2014, Boeve et al. 2016).

Figure 1.2 – The overlapping syndromes of PDD and DLB



Accurate early diagnosis would enrich clinical trials and improve clinical care, particularly as disease modifying trials in PD have increasing focus on early untreated disease to reduce the potential masking effect of symptomatic dopaminergic therapy. The concept of PD has evolved over the past 20 years with increasing recognition and understanding of the prevalence and importance of non-motor symptoms (NMS). These symptoms can pre-date, sometimes even by several years or decades, the onset of the motor symptoms. Thus the pathophysiological processes which underpin this disease start earlier than the symptoms (see Figure 1.3). By diagnosis, there is a 50 to 80% loss of dopamine transporter function in the nigra-striatum. Therefore there is a debate as to when PD 'begins' (Berg et al. 2014), in essence when the diagnosis can be made with acceptable accuracy. Novel diagnostic criteria, published in 2015 and gaining popularity (Postuma et al. 2015), include non-motor symptoms as supportive criteria to help refine this process.

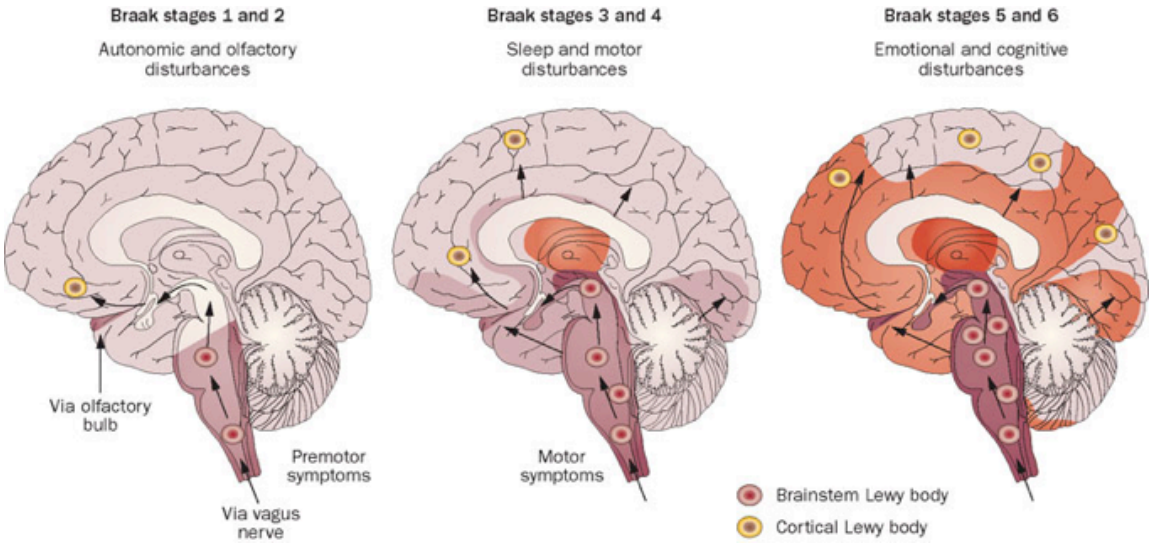
Figure 1.3 – This graph illustrates the temporal relationship between neuronal function and the progression of Parkinson's symptoms, adapted from (Lebouvier et al. 2010)



1.2 NEUROBIOLOGY OF PD PROGRESSION

The aetiology of PD has been linked to both genetic and environmental factors but their contribution to pathology, clinical features, treatment response, and progression is unclear. The hallmark of PD pathology is the degeneration of nigrostriatal dopamine neurons and the pathological deposition of the misfolded protein alpha-synuclein. These protein aggregations are found as in intra-neuronal Lewy bodies within vulnerable populations of neurons. Lewy bodies and neurites (degenerating neurites with α-synuclein) have been considered the defining features of disease. Braak and colleagues have proposed a staging schema by which the distribution of Lewy-pathology spreads through the brainstem in a prion-like fashion into the midbrain and subsequently throughout the cortex (Braak et al. 2003, Braak et al. 2005) (see Figure 1.4). This distribution maps closely to the progression of clinical symptoms in some studies (Kempster et al. 2010, Doty 2012, Kalia et al. 2015). While degeneration of the substantia nigra (and thus disruption of the dopaminergic pathway) accounts for the classical motor symptoms of PD, other neurotransmitter systems (such as cholinergic, serotoninergic and noradrenergic) are also affected. Disruption of these other pathways account for non-motor symptoms such as cognitive impairment, depression and apathy. These pathological changes occur gradually and progressively over many years. Therefore, a significant period of clinically silent cell dysfunction and death commonly precedes prodromal symptoms (such as hyposmia, autonomic, sleep, or mood symptoms) before the onset of the motor syndrome.

Figure 1.4 - This figure illustrates the Braak staging schema showing initiation sites via the olfactory bulb or vagus nerve and subsequent spread of Lewy pathology through the brain with corresponding clinical symptoms (Doty 2012).



However, neuronal loss can be present in the absence of Lewy pathology leading to the investigation of smaller α-synuclein aggregates (Dijkstra et al. 2014, Kalia et al. 2016). Uncertainty about the role of synuclein pathology is caused by the presence of Lewy bodies in the absence of parkinsonian symptoms, termed incidental Lewy body disease. There also appears to be a synergistic relationship between the α-synuclein, amyloid peptide and tau proteins (Irwin et al. 2013). The additional presence of AD pathology is associated with a shorter time to dementia and a higher burden of Lewy pathology in the cortex.

1.2.1 NEUROBIOLOGY OF COGNITIVE IMPAIRMENT IN PARKINSON'S

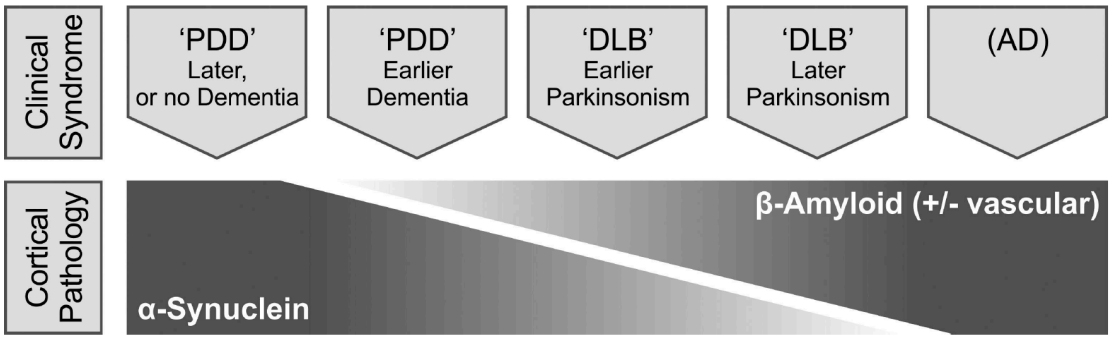
In the elderly, pathologies often occur on a background of age-related pathologies, which are extremely common in the over 75 age group. The pathologic substrate for PD dementia (PDD) and mild cognitive impairment (MCI) appears to be heterogeneous and includes Lewy bodies, Alzheimer's disease (AD) pathology, cerebrovascular disease, and other findings including cerebral amyloid angiopathy (Halliday et al. 2014).

The most compelling evidence to date suggests that Lewy-related pathology is the most important factor in the development of cognitive impairment in PD (see Figure 1.5). Ageing is commonly reported by cohort studies as being important driving factor in onset of dementia in PD patients, a factor also related to the prevalence of AD pathology in patients with dementia (Reid et al. 2011).

Neuropathological studies have also linked tau deposition to the Lewy body diseases. In both patients with DLB and those with PDD, the presence of pathological tau changes in combination with Aβ and α-synuclein has been shown to potentiate dementia (Howlett et al. 2015). Furthermore, as in patients with AD, tau aggregates in those with PD have been found to correlate with the severity of cognitive impairment, and tau aggregates measured at autopsy late in the course of disease are commonly observed in patients with PDD (Horvath et al. 2013).

In summary, it is very likely that there a synergistic effect between α-synuclein pathology and age related pathologies (including amyloid and tau aggregates) that drives early cognitive decline in PD.

Figure 1.5 - Dementia in DLB/PD is associated with two major pathologies: synucleinopathy (α-Synuclein i.e., Parkinson pathology) and neuritic amyloidopathy (i.e., Alzheimer pathology). In PD patients who develop dementia very late in their illness, or not at all (far left), neuritic amyloid deposition is minimal (or absent), and cortical pathology is mainly that of a-Synuclein deposition. At the other extreme, DLB patients with predominant neuritic amyloid deposition and very minimal a-Synuclein deposition would usually be diagnosed as AD during life, developing clinical DLB hallmarks late (if at all). Between these two extremes of the spectrum lie the most patients with PD and DLB (Berg et al. 2014).



1.3 HOW DO YOU MEASURE SYMPTOM SEVERITY AND PROGRESSION IN PARKINSON'S?

An understanding of the natural history of PD is required to inform clinical practice and empower clinical trials. Until relatively recently, there have been few high quality studies which reflect the evolution of signs and symptoms of a real-world treated PD population over time. Available data includes information from the placebo arms of drug trials, which frequently have stringent inclusion criteria (Shoulson et al. 1993, Anonymous 1996, Ravina et al. 2006, Olanow et al. 2009). Retrospective longitudinal studies (Louis et al. 1999, Jankovic et al. 2001) and prospective cohort studies (Hely et al. 1999, Schrag et al. 2007, Marinus et al. 2014) had provided some insights but have tended to be small, have a short follow up, or include patients at baseline with a long disease duration. The publication of natural history studies including the ParkWest (Bjornestad et al. 2017), CamPaIGN (Evans et al. 2011), CARPA (Velseboer et al. 2013), PPMI (Fereshtehnejad et al. 2017) and DeNoPa (Mollenhauer et al. 2016) cohorts have significantly improved knowledge in this area and are discussed in more detail in this chapter.

There is obvious individual variation of clinical features (both motor and non-motor) at diagnosis and subsequent progression rates, even in pathologically defined cohorts. To date there are no widely accepted biomarkers by which disease progression (i.e. neuronal degeneration) could be measured. This is despite numerous attempts to use neuroimaging and laboratory techniques (McGhee et al. 2013), although new attempts are promising (Burciu et al. 2017). Clinical trials investigating neuroprotective agents in PD have universally failed to demonstrate benefit, potentially due to the agent or lack of a suitable biomarker or clinical assessment to demonstrate clinically meaningful benefit, or both. In the absence of a biomarker, clinical features are used as a marker for the underlying neuronal cell death / dysfunction.

There is currently no standard measure of disease progression in PD. A useful marker of progression would be a feature that changes in frequency or severity over time in parallel with the continuing disease process. Ideally it would be valid, reliable and sensitive to change over a short period (over and above measurement error) for use in clinical trials. Due to the variety of symptoms caused by PD, measuring the change in signs and symptoms is complex. Measures of progression could include motor deterioration, cognitive deterioration, disability, quality of life and other symptoms (such as autonomic dysfunction). Thus the concept of clinical progression is multi-factorial.

Some studies use the onset of clinical milestones (e.g. onset of dementia, need for dopamine therapy or dyskinesia) as an alternative, but these lack the sensitivity to monitor change over shorter time periods. In addition, they are often a later manifestation thus less useful to monitor early changes. However, they have the advantage of allowing clinically relevant end-points to be analysed, in many cases including the ability to include withdrawn participants if their disease status can be ascertained.

This difficulty is made more challenging by individual symptoms evolving at different rates, many of which in a non-linear fashion (Jankovic et al. 2001). Combined with a lack of consistency in measures of progression between cohorts, this complex problem remains a significant barrier to our understanding of the evolution of PD. Clinical features unresponsive to dopamine therapies are increasingly recognised as being more useful markers of progression as they are less responsive to conventional treatment changes (Antonini et al. 2012, van der Heeden et al. 2014). They may also follow a more linear progression than current measures of motor severity that decline more rapidly earlier in the disease course (Maetzler et al. 2009).

Longitudinal cohorts and clinical trials have used different measures of progression, although until recently there has been a major emphasis on motor features. These have

included changes in rating scales (e.g the Unified Parkinson's Disease Rating Scale (UPDRS) part III motor score), the onset of specific milestones (e.g. dyskinesias) and imaging markers (e.g. positron emission tomography (PET) measuring dopamine transporter function). However, despite vast resources being expended on numerous large clinical trials, they have suffered from a lack of consistency and clinically relevant end-points or biomarkers. For example, the onset of dyskinesias has been used as end-points due to its effects on quality of life despite not being a major determinant of PD related disability. The onset of PDD is frequently used as an important milestone as it has a major impact as it frequently results in disability, institutionalization, loss of quality of life and early death. PDD is also causes an increased economic health burden.

1.3.1 MEASURING MOTOR IMPAIRMENT

The two most widely used measures of motor impairment in PD are Hoehn and Yahr staging and the UPDRS III motor score. Several other scales have been developed but their use has not been as widespread (Goetz 2003). Timed tests and use of ambulatory home-based monitors (e.g. smartwatches) are increasingly being researched to attempt to gain more objective and sensitive measures. Motor complications are uncommon early in PD which precludes their utility.

Using motor impairment as a marker of progression is complex as it responds to dopaminergic therapies, with inter-individual variation in treatment response. In attempts to account for the levodopa dose, motor examinations are usually performed consistently either at their peak response to medication or off treatment following a washout period (i.e. overnight).

Hoehn and Yahr staging was the first widely utilised measure to monitor progression (Hoehn et al. 1967). The authors developed a landmark 5 category staging system: Stage I, unilateral motor impairment; Stage II, bilateral or midline involvement without impairment of balance; Stage III, postural instability on turning or formal testing; Stage IV, requiring assistance to walk; Stage V confinement to bed or wheelchair unless aided. While a useful as a marker of key milestones, there is a lack of sensitivity to change over time, particularly in the early stages. This study was conducted before the advent of levodopa treatment, reporting a time to the most advanced state of motor impairment after 10-14 years, but this now been extended significantly. While a useful guide, it is weighted heavily toward postural stability as the index of severity and lacks sensitivity to other motor symptoms and change over time (Goetz et al. 2004).

Developed initially in the 1980's and then revised in 2008 (Goetz et al. 2008), the UPDRS is the most widely used clinical rating scale for PD. UPDRS broadly covers assessment of non-motor symptoms (UPDRS I), motor activities of daily living (UPDRS

II), motor impairment (UPDRS III) and motor complications (UPDRS IV). The UPDRS III comprehensively covers the motor symptoms associated with PD, assessing 33 individual items across 18 symptoms (several include left, right or other body distribution scores) on a 5 point severity scale from asymptomatic to severe. These assessments cover the cardinal parkinsonian signs of bradykinesia, rigidity, tremor, and postural stability, as well as other motor symptoms including gait, speech, facial expression, posture, and standing up from a chair. The UPDRS is the most widely used in PD studies and shows good clinimetric properties (Goetz et al. 2008, Bloem et al. 2016). However, this assessment remains semi-quantitative and subjective. Despite guidance and training, there remains a subjective element of interpretation by the rater. It may also be insensitive to change, particularly in those with only mild dysfunction, and suffer from floor effects in the early stages. Lastly, delivery of the UPDRS requires a trained clinician rater and is therefore relatively expensive, and usually performed in the clinic or hospital environment as a one-off test. A high level of intra-patient variability is generally seen, depending on the time of day, patient anxiety levels, and other environmental factors.

Timed tests of motor function are designed to be objective measures of simple, easily reproducible tasks. Examples include timed walking tests, hand or finger tapping tests and tests of manual dexterity (Macleod et al. 2010). When used in combination, a battery of timed motor tests may provide a more objective and sensitive measure of motor function (Haaxma et al. 2008). Delivery is again usually dependent on a trained rater. Ambulatory monitoring methods using wearable technology have the potential to supersede the ‘snapshot’ of a clinical assessment. However, while the ability to capture data from such devices is readily available, the interpretation and utility of this approach still needs to be established.

1.3.2 MEASURING COGNITIVE FUNCTION

Cognitive impairment in Parkinson's disease is heterogeneous both in severity and pattern. It is also subject to influences both PD-related and external (such as AD or vascular pathology, see paragraph 1.2.1). Numerous scales and classification systems have been used to measure cognition in PD, but there are now attempts to improve consistency between studies (Dubois et al. 2007, Litvan et al. 2012). Measuring cognitive decline over time includes a valid measure of their major cognitive domains and a functional assessment of an individual's cognitive ability to perform their activities of daily living (ADL). The impact of an individual's pre-morbid cognitive ability on future decline is not clear.

Impairments in executive function are found even at the earliest stages, with deficits in attention, explicit memory, and visuospatial function also described (Williams-Gray et al. 2009, Yarnall et al. 2014, Weintraub et al. 2015), or even before the onset of the motor syndrome (Rolinski et al. 2016). The pattern of cognitive impairment may even predict prognosis (Biundo et al. 2016). Despite the increased sensitivity and specificity of more in depth neuropsychological assessments, global cognitive scales (such as the Montreal cognitive assessment (MOCA) or mini-mental state examination (MMSE)) are often used in both research and clinical settings for convenience as they are shorter and less arduous (Dalrymple-Alford et al. 2010, Marras et al. 2014) but remain sensitive to changes over time (Biundo et al. 2016).

Although cognitive impairment in PD exists on a continuum of severity it is often divided into two categories, mild cognitive impairment (PD-MCI) and PD dementia (PDD), based on the extent to which the impairment interferes with activities of daily living. This classification system risks oversimplifying a very complex entity, but has been fundamental to clinical trials of therapies for cognitive dysfunction in PD which have been largely devoted to the treatment of PD dementia. Diagnostic criteria have been

developed by the Movement Disorders Society that help to standardise these classifications. The dementia criteria largely meet the Diagnostic and Statistical Manual of Mental Disorders (4th edition) dementia criteria.

In both PD-MCI (Litvan et al. 2012) and PDD (Emre et al. 2007), a diagnosis of Parkinson's with a gradual cognitive decline and objectively demonstrated cognitive deficits are core features. In PD dementia, cognitive deficits in more than one domain are required whereas in PD-MCI a single domain may be affected. In PD-dementia the cognitive deficits must be severe enough to impair activities of daily living whereas in PD-MCI they must not be sufficiently severe to interfere with functional independence (although minor difficulties may be present). These criteria allow for these classification to be made using global cognitive scales (level 1 criteria), accepting the lack of specificity of more in depth neuropsychological testing.

1.3.3 MORTALITY, DISABILITY AND HEALTH RELATED QUALITY OF LIFE

Parkinson's is associated with an increased mortality, but there is marked heterogeneity in reported mortality ratios. Incident cohorts are more consistent and a recent meta-analysis reporting a pooled standardised mortality ratio of approximately 1.5 (Macleod et al. 2014). While death is an important outcome, the modest effect this has on mortality, particularly in younger patients and those with early PD, makes it less useful.

Disability is defined by the World Health Organisation as "any restriction or lack of ability to perform an activity within the range considered normal for a human being due to an impairment". Disability leading to functional dependency in PD, the need for assistance in ADL, can be multi-factorial. Progression of motor and non-motor symptoms (frequently cognitive impairment), and the influence of co-morbidities, such as arthritis or visual impairment, can all affect this outcome. Rather than individual measures of disease characteristics, disability broadly reflects an individual's health related functional state and is thus immediately applicable to a real world population. Advantages of using this outcome include its relative objectivity and ease of measurement compared to, for example, quality of life. Limitation of functional performance (i.e. disability) is important to patients, their care-givers, clinicians and heath service providers and so would aid in personalised treatment planning, prognostication and clinical trials (Schrag et al. 2009).

Most studies focus on basic ADL (washing, dressing, cleaning, cooking etc.) rather than more complicated or instrumental ADL (e.g. driving or shopping). Measures of disability vary, but in PD studies this has been inferred to by a Hoehn and Yahr stage of 3 or more (i.e. requiring assistance to walk) which is essentially an assessment of motor impairment (Macleod et al. 2016) rather than a direct measure of function.

Many different disability scales have been used in PD, some PD specific while others are more general. Many of these scales have not been validated for use in PD. The Movement Disorders Society (MDS) have recommended 9 scales for use in PD, including the Schwab and England activities of daily living scale (S&E)(Shulman et al. 2016). This 11 point ordinal scale rates individuals from bedridden to independent, defining functional dependency as the need for assistance with ADL and accounts broadly for disease symptomatology (Shulman et al. 2008, Macleod et al. 2016). Health related quality of life (HRQoL) can be used to monitor progression, but the influence of external factors and relative subjectivity are disadvantages. While a useful patient-centred outcome, it lacks a clear relation to the underlying pathological processes as there are many potential external influences. Measures of HRQoL appear to be less responsive to change over time than disability scales. This is possibly because they are less responsive to subtle changes or perhaps the multi-factorial subjective changes adapt to changes over time (i.e. people become more accepting of worse disease states as their disease progresses) (Schrag et al. 2009).

1.3.4 AUTONOMIC AND OTHER CLINICAL FEATURES

Autonomic dysfunction occurs in almost all patients with PD at some stage of their disease (Chaudhuri et al. 2006), with symptoms including gastrointestinal, cardiovascular, urinary and sexual dysfunction. There is a growing understanding that autonomic features start in early PD (Khoo et al. 2013, Baig et al. 2015) or even in the pre-motor stage (Noyce et al. 2012). A disadvantage of using these disorders as markers of progression is that they are common outside of PD cases. Urinary dysfunction has recently been proposed as a marker of disease progression as it may correlate with both motor and cognitive impairment in the early stages (Picillo et al. 2017), but the high prevalence of alternative causes for these symptoms in this age group may preclude its use. Reduced variability in heart rate and orthostatic hypotension may be useful as milestones their dysfunction tends to develop in the mid-stages of PD, but as yet there is insufficient data to support their use (Maetzler et al. 2009).

Sleep disorders, such as rapid eye movement sleep behaviour disorder (RBD), could be used to monitor progression (Mollenhauer et al. 2016), but the expense of polysomnography makes this impractical, while questionnaire assessment may not be specific or sensitive enough. Excessive daytime sleepiness worsens over time with PD, but its association with higher doses of dopamine therapy is a significant confounder.

Neurospsychiatric disorders such as mood disorders are common, but their progressive relationship with PD is not clear cut. Hallucinations tend to occur later in the disease and so less useful in early PD. They are also strongly associated with higher dopamine doses, a possible confounding effect.

While there are problems with using each of these symptoms individually, they may each be able to contribute to a holistic / composite approach.

1.3.5 COMPOSITE MEASURES

As progression is multi-factorial, it may be that a composite measure of the different symptoms would be useful. This could potentially include a combination of imaging, laboratory and clinical measures. The primary aim of a measure of prognosis is to have a clinically relevant outcome which reflects the underlying neuronal loss. Given the heterogeneity in PD, this approach is advantageous in that it could account for multisystem dysfunction and thus be more sensitive in trials for disease modifying therapies. However, it may not be sensitive to benefit or change in individual symptoms. While not yet in widespread use, some research groups have started to propose candidate composite measures. There has been particular interest in composite scores of primarily dopaminergic unresponsive symptoms as they may offer a more linear course over time. Van der Heeden and colleagues have proposed the evaluation of progression using dopamine responsive measure of motor function and a composite score of 'predominantly non-dopaminergic domains' (van der Heeden et al. 2014). Using principal component analysis, they derived a composite score combining measures of cognitive impairment, psychotic symptoms, autonomic dysfunction, excessive daytime sleepiness, depressive symptoms and postural instability and gait disorder. While promising, the mixture of several assessments is time consuming and two of the domains can be side effects of dopamine therapies. The same group then refined this score by developing a more concise selection of measures derived from the same domains, the SENS-PD scale (van der Heeden et al. 2016). This score, used in combination with a motor score, such as UPDRS III, shows promise as it captures a broad spectrum of disease features.

Fereshtehnejad et al. proposed a 'global composite outcome' score, comprising of 4 domains. This included motor symptoms (UPDRS measures of functional disability and motor complications), motor signs (UPDRS III motor examination), cognition

(neuropsychological assessment) and other non-motor manifestations (including neuropsychiatric, sleep and autonomic symptoms). Again, this approach broadly covers the multi-system nature of PD.

While the weighting of such composite scores can be subject for debate, they are a promising step forward in a challenging area. Further progress could be made by the potential addition of biomarkers such as laboratory, imaging and ambulatory monitoring assessments deriving a poly-modal composite score. However, the quicker, cheaper and more clinic-ready the composite measure, the greater its potential utility.

1.4 THE NATURAL HISTORY OF PARKINSON'S DISEASE

1.4.1 HETEROGENEITY OF SYMPTOMS AND SUBTYPES

At diagnosis, there is marked heterogeneity of symptoms. As discussed in chapter 1.1, the diagnosis of PD is based primarily on the motor syndrome, with obvious differences even at this early stage. Correct delineation of these groups would infer stronger correlation of clinical, aetiological and pathophysiological factors within each group. This would likely benefit research into all these areas (Sieber et al. 2014, Fereshtehnejad et al. 2017). Despite numerous studies employing empirical and data driven methods to divide PD into more homogenous subgroups of shared clinical features, there are no widely accepted subtypes of PD.

Empirical methods have focussed on delineation using either age or motoric features. Young onset Parkinson's disease (YOPD) has been defined by different age ranges, including <40, <50 or even <55 years, which are all arbitrarily chosen. They are in general thought to have a better prognosis with a slower onset of motor and cognitive impairment but suffer more motor fluctuations (Kempster et al. 2010). However, it is difficult to disentangle the confounding effects of age.

More studies have focused on motor subtypes. For example, dividing sub-groups by the ratio of tremor compared to rigidity, bradykinesia and gait disturbance utilises the most easily stratified characteristics. Several case series and population based studies have shown that resting tremor at presentation and tremor dominance in the disease course is associated with a slower rate of progression and less cognitive impairment than predominance of rigidity, bradykinesia or gait disorder (Foltynie et al. 2002). Systematic reviews of prognostic factors have agreed that there is evidence for a postural instability and gait disorder (PIGD) or akinetic-rigid (AR) subtype being associated with a worse outcome (Marras et al. 2002, Suchowersky et al. 2006, Post et al. 2007). More recently however, longitudinal studies from the Oxford Discovery and PPMI cohorts have failed

to confirm this (Hu et al. 2014, Fereshtehnejad 2017, Schrag 2017). The clinical evidence for the existence of these sub-groups is supported by pathological confirmation (Paulus et al. 1991, Selikhova et al. 2009, Selikhova et al. 2013) and imaging studies (Eggers et al. 2011, Prodoehl et al. 2013, Rosenberg-Katz et al. 2013). Since the understanding of the multi-system nature of PD, incorporating NMS into subtyping has been an important recent step forward (Marras 2015, Chaudhuri et al. 2016). Empirical subtyping solely using NMS, such as using the presence or absence of RBD (Rolinski et al. 2013), has been shown to be predict a more severe disease state.

Data driven clustering methods allow for a 'hypothesis free' classification of subgroups. In essence, this allows the model to determine the subtypes without a priori judgement of the discriminatory variables. K-means cluster techniques have been the most frequent methods used (van Rooden et al. 2010, Erro et al. 2013, Fereshtehnejad et al. 2015, Lawton et al. 2015, Erro et al. 2016, Fereshtehnejad et al. 2017), although more sophisticated clustering methods have been used (van Rooden et al. 2011, Raykov et al. 2016). These data driven methods have identified between 2 to 5 differing subtypes of PD using both motor and non-motor symptoms. While the specifics of each subtype varied between groups, most studies identified a more benign subtype (usually associated with a younger age of onset and tremor predominance) and a more malignant subtype (usually associated with an older age of onset and a relative lack of tremor). However, the outcomes of the group characteristics depend heavily on the variables used for the analysis. Thus, two groups using the same dataset can produce quite different clustering solutions (Fereshtehnejad et al. 2017). Criticisms of previous studies include the exclusion of NMS from the dataset, including patients with more advanced PD, non-representative population samples and a lack of longitudinal follow up. Addressing these pitfalls and potentially including laboratory and imaging findings in the analysis (as some groups have started to do) may help delineate more robust subtypes.

Despite the evidence supporting the existence of subgroups, their relevance remains unclear (Marras et al. 2013). The benefits of subtyping would be to delineate differences in the underlying aetiology or disease processes, i.e. to derive distinct entities with shared clinical and pathological features coming under the broad umbrella term of PD. Some argue whether the different subtypes may in fact represent different stages in the evolution of the symptoms rather than distinct entities. Due to the lack of longitudinal follow-up published in these studies, the stability of the subgroups over time, even at the early stages is unclear. For example, a significant proportion of patients can transition from a tremor dominant (TD) to a PIGD subtype (Alves et al. 2006) over time. Thus, given that PD 'begins' many years before the onset of the motor syndrome, it is possible that these subtypes simply reflect the same multi-system disease with different symptoms progressing at different rates.

Correct delineation of subtypes would infer stronger correlation of clinical, aetiological and pathophysiological factors within each group. The challenge for future studies is to derive algorithms which can divide cohorts into robustly reproducible subtypes using readily available clinical, demographic, laboratory or imaging data.

Preliminary work on the Discovery cohort by Michael Lawton has divided baseline features into 5 groups using K-means cluster analysis. How they predict prognosis will be ascertained in longitudinal follow-up and can be used to validate prognostic factors and biomarkers (Lawton et al. 2015).

1.4.2 EVOLUTION OF SYMPTOMS OVER TIME

As discussed in this chapter, by the onset of the motor syndrome there is already a marked variance in symptomatology between individuals. Many previous studies have been small, had a short follow-up period or consisted of a disproportionate number of younger and healthier cases. Motor and cognitive impairment, thus functional disability, in PD progresses inexorably over time. The Sydney Multicenter Study, the cohort with the longest published follow-up, reported that those who survived until 20 years had a very high chance of being demented (83%) with almost half resident in nursing homes (Hely et al. 2008). At this stage, the levodopa unresponsive symptoms were the major cause of morbidity. This highlights the inevitability of multi-system decline.

Far less is known about the rate of change. A comprehensive review of existing studies was performed by Maetzler and colleagues in 2009 (Maetzler et al. 2009). Key findings included the lack of linearity in progression of motor symptoms, with bradykinesia and rigidity declining faster at the beginning of the disease. Similarly, measures of functional disability, potentially driven by this motor decline, also appear to decline more rapidly earlier. Cognitive, speech, sleep, and gait difficulties may progress in a more linear pattern in proportion to disease duration. Their lack of responsiveness to dopamine treatment is potentially an additional benefit. With all these measures, as previously discussed, it is vital to take the method of ascertaining symptom progression into account.

Since that publication, the past decade has seen the interim findings of numerous longitudinal studies being published, making headway in a previously under researched area. A summary of these studies is shown in Table 1.1.

Table 1.1 – Reported rates of change in UPDRS III, MOCA and Schwab and England score from early prevalent or incidence studies

Study	Study design*	Number of participants included in analysis	Total follow up (in months)	Age at baseline (mean, sd)	Disease duration from diagnosis (mean, sd)	UPDRS III (mean change per year, % change from baseline)	MOCA (mean change per year, % change from baseline)	Schwab and England Score (mean change per year, % change from baseline)
Campaign (Evans et al. 2011)	C, I	122	66 mean, 19.2 sd	69.5 (9.8)	0	2.2 (9%)[1]	n/a	n/a
CARPA (Velseboer et al. 2013)	H, P	129	Up to 60	66.5 (10.5)	19.8, 11.1	2.1 (12%)[1]	n/a	-2.1 (2.3%)
PPMI (Fereshtehnejad et al. 2017)	H, P	401	32.8 mean, 9.3 sd	61.1 (9.7)	6.5 (6.5)	1.9 (9%)[2]	-0.3 (1%)	-2.2 (2.4%)
DeNoPa (Mollenhauer et al. 2016)	H, I	123	25.3 mean, 2.6 sd	63.7 (9.5)	0	1.0 (4.6%)[2]	-0.3 (1%)	n/a

*Study design: C = community, H = hospital; I = incident, P = early prevalent; [1]original UPDRS (examined in on state if taking medication); [2]revised MDS-UPDRS (examined in on state if taking medication) (Goetz et al. 2008)

Importantly, the motor or functional change over time using each of these measures in absolute terms is very small per year. This exemplifies the critical need for more sensitive and objective measures of progression, as well as the need for subtypes. These outcomes would need either long follow-up or large study numbers to delineate subtle differences.

However, the included studies show good consistency across the MOCA and S&E, demonstrating that these measures of progression are relatively robust. The DeNoPa study was smaller with shorter follow up than the PPMI study, which may explain the differences, although the absolute difference was small. Encouragingly, these results are similar to the outcomes of previous studies which lacked the rigour of study design to ensure generalisability (Maetzler et al. 2009).

In the earlier stages of PD, UPDRS III and MOCA are limited in their utility as a marker of progression over a short follow-up period. While over a longer period the impairment accrues inexorably despite dopamine treatment, the changes in the short term appear minimal. S&E may be more useful in this respect, but remains less used. While not included in the table due to potential bias in study design (based on a drug trial), the NET-PD investigators found the rate of change of the UPDRS III and S&E to be sensitive enough to measure progression in a large early PD cohort, with similar findings to the above (Parashos et al. 2014).

A limitation of using these scales is that only those who are willing or able to attend follow-up appointments are included, as they depend on a clinician-rater. Thus it is likely that only the less motor, cognitively or functionally impaired will be assessed and so included in the analysis, as those with dementia or living in nursing homes are less likely to attend. For example, the reported MMSE did not change in the CARPA cohort over 5 years, but this study only reported those who attended research clinics (Broeders et al. 2013), thereby failing to account for study attrition as an important source of bias. The

use of progression milestones allows those that have withdrawn to be included if their status can be determined by other means, such as searching for medical records.

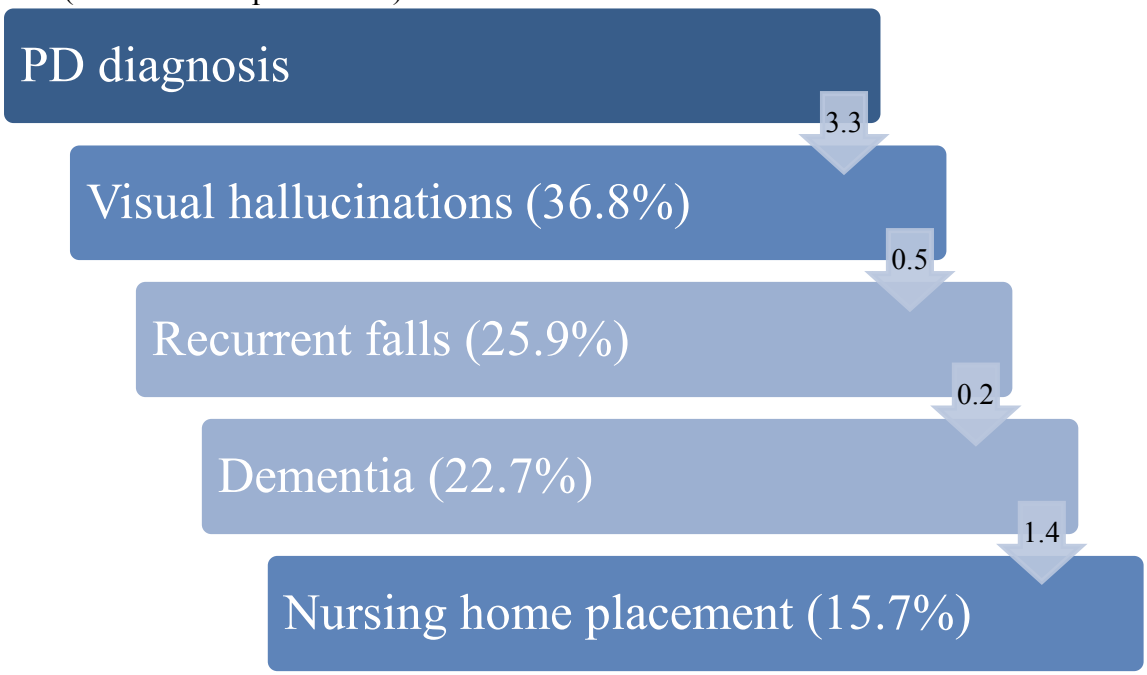
These milestones have often been conceptualised by either motor, cognitive or functional impairment. Commonly reported milestones include the onset of postural instability or falls, dementia and institutionalisation.

The Campaign cohort reported that by 5 years, 17% of patients were demented (Williams-Gray et al. 2009). At 10 years, 55% had died with 68% of survivors suffering postural instability and 46% dementia (Williams-Gray et al. 2013).The ParkWest cohort reported, at median 7 years follow-up, a common transition between milestones of visual hallucinations, falls, dementia and institutionalisation (Bjornestad et al. 2017). By 7 years, 18.4% of the 185 participants had died. Of the survivors, 23% had dementia and 16% required care in a nursing home. They proposed a model of common milestones by which PD progressed through visual hallucinations, falls, dementia and then nursing home admission (see Figure 1.6).

1.4.3 SUMMARY

Selection of progression markers are critical in the evaluation of prognostic factors. Challenges include the responsiveness to conventional treatments, heterogenous and non-linear progression rates of symptoms and individual participants and slow deterioration in a majority. Motor, cognition and disability may be able to detect progression in early PD, but a combination of continuous scales and key milestones will likely be needed.

Figure 1.6 – The proposed common pathway which most PD patients transition important milestones as suggested by the ParkWest study (Bjornestad et al. 2017). The percentage is the cumulative incidence of each milestone at 7 years. The numbers in the arrows depict the median number of years between each milestone in those who reached them (i.e. the worse performers).



1.5 AIMS AND OBJECTIVES

1.5.1 AIMS

This research study aimed to delineate factors, either clinical or biochemical, which predict prognosis in early Parkinson's disease, using the Oxford Discovery cohort. There is a critical need to develop biomarkers in PD to predict disease susceptibility, diagnose, predict prognosis and monitor progression. This study looked to identify clinical and biochemical predictive factors, which would then have the potential to:

- Inform individualised patient counselling, planning and treatment
- Perform clinical trials of appropriately matched groups with smaller groups and more rapid outcomes
- Identify patients at risk of rapid deterioration for potential treatment with neuroprotective therapies at the earliest possible stage
- Determine clinically homogenous PD subtypes, such as rapid progressors, to inform mechanistic research

The Oxford Discovery cohort comprises of a large number of community-ascertained, well-characterised patients early in their disease process with longitudinal follow up. This offers a rare opportunity for the study of clinical prognostic factors and biomarker discovery. Currently, there are other large prospective studies investigating biomarkers in PD underway in both the USA and Europe including Tracking Parkinson's (UK) and the Parkinson Progressive Marker Initiative (US and Europe). Collaboration with such projects would potentially allow the external validation of any novel findings (Heinzel et al. 2017).

1.5.2 OBJECTIVES

1. Perform systematic review of clinical assessments and serum biomarkers which predict prognosis in Parkinson's

Numerous studies have published on prognostication and biomarker discovery in PD. To determine what is known about this topic, a systematic review was conducted to identify relevant high quality studies. This area has historically suffered from a lack of quality studies with small sample sizes, short follow-up duration and a lack of consistency in outcome measures. Thus careful analysis of the literature is necessary. The biochemical factors of apolipoprotein A1, C-reactive protein, uric acid, vitamin D and epidermal growth factor were chosen a priori based on the published literature and expert opinion (see chapter 2.5). The outcome measures of motor impairment, cognitive decline and disability were based on the literature and expert opinion (see chapter 1.3) for further discussion).

2. Describe the baseline clinical and biochemical characteristics of the Discovery cohort

To ensure external validity, the demographics and clinical features of the cohort were compared to similar studies. Baseline clinical and biochemical characteristics were described and compared with a control group to delineate which factors are associated with the disease processes of PD, as these may be more closely associated with the disease process and thus prognostication. The influence of age and gender on motor and non-motor features was explored, as they are known to affect a broad range of symptoms independent of the disease.

3. Delineate clinical predictors of motor impairment, cognitive decline and disability in Parkinson's disease

Clinical features, assessed at baseline, were used to predict progression of motor impairment, cognitive decline and disability.

4. Delineate biochemical predictors of motor impairment, cognitive decline and disability in Parkinson's disease

Biochemical features, assessed at baseline, were used to predict progression of motor impairment, cognitive decline and disability.

2 SYSTEMATIC REVIEWS OF CLINICAL ASSESSMENTS AND SERUM BIOMARKERS WHICH PREDICT PROGNOSIS IN PARKINSON'S

2.1 REVIEW PROPOSAL

There is a critical need to develop biomarkers which can aid diagnosis, assessing disease severity and predicting prognosis in PD. For pre-motor diagnosis, recently published criteria from the Movement Disorder Society have examined all the available evidence to create a model to predict the onset of PD within 5 years. This has also been used retrospectively in a population based cohort with moderate success (Berg et al. 2015, Mahlknecht et al. 2016). This is encouraging, but there is still a lack of widely accepted biomarkers or combination of tests that predict prognosis. Despite many published studies, there is insufficient evidence for factors that can predict prognosis in PD. The focus of this chapter is to review the literature on clinical assessments and serum biomarkers that are proposed to predict prognosis in PD.

A recent systematic review broadly looking at any potential prognostic features found inadequate evidence to recommend the use of any biomarker in PD (McGhee et al. 2013). They also highlighted that despite the variety of biomarkers lacking evidence for utility, many could not confidently be rejected. This was despite including 183 published studies though most were cross-sectional (163) and far fewer longitudinal (20). This highlights not only the complexity of determining a biomarker for PD but also a lack of high quality studies in this important field. To quote Professor Altman: "As a consequence of the poor quality of research, prognostic markers may remain under investigation for many years after initial studies without any resolution of the

uncertainty. Multiple separate and uncoordinated studies may actually delay the process of defining the role of prognostic markers" (Altman 2001). To address this issue, cohorts with longitudinal follow-up (such as the Discovery cohort) have been designed specifically for biomarker discovery and are beginning to publish results.

The aim of this review was to appraise the evidence for clinical assessments (including demographic information) and serum biochemical indices in predicting prognosis in early PD. To interpret and synthesise the available studies, systematic reviews were performed. The serum biomarkers proposed for analysis were apolipoprotein A1 (ApoA1), vitamin D, uric acid, C-reactive protein and epidermal growth factor (EGF). These biochemical tests were chosen on a combination of expert opinion and a literature review (Parkinson's UK innovation grant K-1404). The reasoning behind this is discussed in more detail in section 2.5.1.

2.2 METHODOLOGY FOR SYSTEMATIC REVIEWS

The objectives for each of the reviews were the same, to see if the proposed factors could predict the following outcome measures:

- Decline in motor function
- Decline in cognitive function
- Functional disability (impairment of activities of daily living)

These outcomes were chosen as they help delineate clinically relevant milestones with evidence of 'definite progression' in PD (Maetzler et al. 2009). There is high quality evidence that these change with disease progression, although at the early stages the changes can be modest (see chapter 1.4.2 for more detailed discussion).

These outcomes have been shown to correlate with the extent of pathological changes seen in PD (Aarsland et al. 2005, Selikhova et al. 2009) and are functionally relevant to patients. Disability was included as a functional measure which is influenced by both motor and non-motor features.

Visual hallucinations, sleep outcomes and motor fluctuations were considered, but are (at least currently) not widely used. They also may be less sensitive to change over time, are influenced to medication changes, and may only affect a sub-population of PD patients.

While HRQoL is strongly related to motor and cognitive decline, its association to the underlying pathophysiology is not well established and thought less useful than disability (Shulman et al. 2008). Time to inception of dopamine treatment has also been used in clinical trials, as a proxy for motor deterioration. However, this is in part subjective, as individual preference and prescriber treatment patterns can cause variation, thus it only indirectly relates to motor deterioration.

To answer the study question, the following inclusion criteria were set:

1. Study population must be sporadic PD (e.g. not selected on the basis of age, family history or genetics) with the diagnosis made by clearly defined criteria, with reference made to exclude other forms of parkinsonism.
2. Longitudinal design (prospective or retrospective).
3. Follow-up assessment at least 12 months following baseline.
4. Patients initial assessment at diagnosis or early in disease course (arbitrarily limited to 3.5 years after diagnosis to match the recruitment of the Discovery cohort), no restriction was made on drug treatment.
5. The use of validated methods to assess outcome measures.

Each study was assessed on pre-defined quality criteria. The assessment tool was based on recommendations by the American Academy of Neurology (Maetzler et al. 2009) (see Table 2.1). Studies were not excluded on the basis of these criteria. Although only longitudinal studies were included in this systematic review, evidence for cross-sectional and case-control studies were discussed where relevant. These quality criteria allow for assessment of potential bias, and thus appraisal of whether the results are representative of the population studied.

Table 2.1- Quality criteria for assessing individual studies adapted from Maetzler et al. (Maetzler et al. 2009)

• Evidence level I	Prospective longitudinal study with: • Defined outcome using a validated method(s) of assessment (criterion a) • Clearly stated diagnostic criteria without restrictive medication, inclusion or exclusion criteria (criterion b) • Sufficient number of patients, adequate accounting for withdrawal / attrition (criterion c) • Presentation of relevant characteristics to assess feature progression within a study population with appropriate adjustment for potential confounders (baseline and follow-up levels, and comparison; criterion d)
• Evidence level II	Prospective longitudinal study minus one of the criteria a–d Retrospective longitudinal study fulfilling the criteria a–d
• Evidence level III	Prospective longitudinal study minus two of the criteria a–d Retrospective longitudinal study minus one of the criteria a–d Cross-sectional study that reports an association of disease duration with the feature of interest that meets criteria a–c
• Evidence level IV	Retrospective longitudinal study minus two of the criteria a–d Cross-sectional study that reports an association of disease duration with the feature of interest minus one of the criteria a–c Cross-sectional study that reports different feature stages Cross-sectional study that compares patients with control

2.3 LITERATURE STRATEGY AND SEARCH

A literature search was conducted using Ovid on the following databases MEDLINE, EMBASE, and PsychINFO for papers published between January 1990 and September 2017. These dates were chosen as research studies had just begun to use pathologically validated criteria for the diagnosis of PD at that time. The searches were limited to only human studies published in English (due to a lack of resources for translation).

Reference lists of included articles and relevant review articles were checked to identify any studies which the electronic search may have missed. The search terms are shown in Table 2.2 and Table 2.3 for clinical and biochemical reviews respectively. The search terms for the clinical predictors excluded terms in the intervention/exposure category to limit the search (the initial search retrieved 12,127 articles, many of which were not relevant after screening the first thousand abstracts).

A single reviewer retrieved the electronic search and then screened abstracts using the inclusion and exclusion criteria as detailed above. Any studies that could not be excluded on the basis of the abstract were then read in further detail.

Data was extracted using a data extraction sheet including information on study design, setting, study population, statistical analysis method, and the relationship of biomarker to outcome with the outcomes as defined above. Data extraction and quality assessment was performed without blinding as this has little effect on the overall results and would have required extra resources (Berlin 1997).

Table 2.2 – Search terms for clinical biomarkers

P = Patient, Population, Problem	AND **S** = Study type	AND **O** = Outcome	NOT **I/E** = Intervention / Exposure
Parkinson Disease/ parkinson*.ti,ab.	exp Follow-Up Studies/ follow-up*.ti,ab. prognosis/ or nomograms/ prognos*.ti,ab. disease progression/ "disease progression".ti,ab. exp Longitudinal Studies/ Longitudinal*.ti,ab.	exp Mild Cognitive Impairment/ or exp Dementia/ or exp Cognition Disorders/ cognitive*.ti,ab. dement*.ti,ab. "unified parkinson's disease rating scale".ti,ab. updrs.ti,ab. "hoehn and yahr".ti,ab. "H&y".ti,ab. exp Neuropsychological Tests/ motor*.ti,ab.	blood.ab serum.ab platelet.ab plasma.ab urine.ab cerebro-spinal fluid.ab cerebrospinal fluid.ab Neurophsyiology/.ab mri.ab

* is wildcard to denominate any letter(s); / denotes subheading; exp allows all synonyms to be included; ti includes search term in title; ab includes search term in abstract

Table 2.3– Search terms for serum biomarkers

P = Patient, Population, Problem	AND **S** = Study type	AND **O** = Outcome	AND **I/E** = Intervention / Exposure
Parkinson Disease/ parkinson*.ti,ab.	exp Follow-Up Studies/ follow-up*.ti,ab. prognosis/ or nomograms/ prognos*.ti,ab. Natural History/ "natural history".ti,ab. disease progression/ "disease progression".ti,ab. exp Longitudinal Studies/ Longitudinal*.ti,ab.	exp Mild Cognitive Impairment/ or exp Dementia/ or exp Cognition Disorders/ cognitive*.ti,ab. dement*.ti,ab. "unified parkinson's disease rating scale".ti,ab. updrs.ti,ab. "hoehn and yahr".ti,ab. "H&y".ti,ab. exp Neuropsychological Tests/ motor*.ti,ab. disability.ti.ab	Uric Acid/ Epidermal Growth Factor/ C Reactive Protein/ Apolipoprotein A-1/ Vitamin D/

* is wildcard to denominate any letter(s); / denotes subheading; exp allows all synonyms to be included; ti includes search term in title; ab includes search term in abstract

2.4 CLINICAL PROGNOSTIC FACTORS

2.4.1 PREVIOUS SYSTEMATIC REVIEWS

There have been 4 similar systematic reviews looking at prognostic factors in PD, by Marras et al. (Marras et al. 2002), Suchowersky et al. (Suchowersky et al. 2006), Post et al. (Post et al. 2007) and McLeod et al. (Macleod et al. 2016). The methods and outcomes for these reviews are detailed in Table 2.4. Marras and Suchowersky summarised that the factors that had the strongest evidence for predicting a more rapid progression (motor, disability or cognition) were older age at onset and a lack of tremor. Post and colleagues found evidence that older age at onset and a higher PIGD score led to more rapid disability and impairment, while McLeod also found a higher bradykinesia score predicted disability. All four reviews suggested that there was a lack of high quality evidence to validate or refute many of the suggested biomarkers.

Marras et al. conducted the first systematic review looking at factors predicting motor decline and disability in PD. While only one reviewer performed the data extraction, experts were consulted and clearly defined criteria for assessing the quality of the study was included. The notable absence is the exclusion of non-motor features, which at the time were not widely accepted to be a marker of disease progression. Disability is strongly influenced by motor factors (Chaudhuri et al. 2006), but is not a surrogate marker due to the effect of the NMS. Only 2 of the hospital based studies comprised a population of what were described as ‘early PD’, each was retrospective (evidence level III) (Hoehn et al. 1967, Goetz et al. 1988). By examining a non-incidence cohort with longer disease duration, the results may be biased in over-representing patients with slower disease progression (Goetz et al. 1988). Two of the included studies had less than 80% of their patients with follow up data or defined outcome (Ferraz et al. 1996, Goetz et al. 2000); 6 studies did not account for confounders in their analysis (Hoehn et al. 1967, Guillard et al. 1978, Guillard et al. 1985, Ferraz et al. 1996, Louis et al. 1999,

Jankovic et al. 2001); and 7 of the studies were imprecise as they included less than a hundred patients in their analysis (Goetz et al. 1988, Diamond et al. 1989, Diamond et al. 1990, Starkstein et al. 1992, Ferraz et al. 1996). The authors remarked on the variety of outcome measures used, heterogeneity of cohorts and lack of long term follow up with the absence of diagnostic re-appraisal to correct for misclassification due to the inclusion of non-PD conditions.

The AAN commissioned report by Suchowersky and colleagues focussed on identifying prognostic markers of motor progression, dementia and mortality. They included 7 studies, of which 2 were prospective (Contin et al. 1998, Locascio et al. 2003) while 5 were retrospective (Jankovic et al. 1990, Roos et al. 1996, Jankovic et al. 2001, Parashos et al. 2002, Diederich et al. 2003). Two studies investigated early PD (classed as level III evidence), as defined by new onset (Diederich et al. 2003) or by motor progression (Jankovic et al. 1990), and each were hospital based. The 3 largest studies (each providing evidence level III) found that a PIGD phenotype or a lack of tremor predicted poorer outcome (Jankovic et al. 1990, Roos et al. 1996, Jankovic et al. 2001). Two of these studies found older age of onset predicted worse motor outcome (Jankovic et al. 1990, Jankovic et al. 2001). Again, comment was made that there was a lack of studies which looked specifically at 'early PD' and marked heterogeneity in study design.

The paper by Post et al. was broader in its approach, including outcomes focussed on motor impairment, disability and quality of life. However, dementia/cognitive impairment was excluded. Eleven papers were rated as high quality based on their pre-specified criteria, 6 describing motor impairment, 8 disability and 2 quality of life (Starkstein et al. 1992, Hely et al. 1995, McDermott et al. 1995, Roos et al. 1996, Hely et al. 1999, Jankovic et al. 2001, Ruiz et al. 2004). Three of these studies were designed for early PD on motor progression (H&Y stage of 1-2, the DATATOP study – evidence level III) (McDermott et al. 1995), targeting de novo patients (both studies based on the

same Sydney Multi-Centre study – evidence level III) (Hely et al. 1995, Hely et al. 1999), or de novo untreated PD with a 'recent' diagnosis (evidence level IV).(Ruiz et al. 2004) The authors summarised that there was strong evidence (generally consistent evidence in high quality studies) that older age of onset (Hely et al. 1995, Jankovic et al. 2001) and a higher PIGD score (McDermott et al. 1995, Roos et al. 1996, Jankovic et al. 2001) predicted future disability. They described generally consistent findings for less motor impairment at baseline, more disability and dementia predicting future motor impairment but lower quality evidence. Similarly, higher bradykinesia score, lack of tremor, symmetrical disease onset and depression predicted more future disability with lower quality evidence.

McLeod and colleagues (Macleod et al. 2016) looked for evidence for the progression of disability in PD, including whether factors predicted the onset or severity of disability. They found 2 studies which examined factors predicting disability (evidence level III) (Guillard et al. 1985, Jasinska-Myga et al. 2012). Both studies found that bradykinesia and non-tremor were associated with a greater risk of becoming dependent. Other factors found to be associated with poorer outcome by Guillard et al. (Guillard et al. 1985) included the presence of Babinski sign and psychosis in the first year, but these were not replicated in the other cohort and the study was based prior to improvements in diagnostic accuracy criteria, as the Babinski sign would be regarded as an atypical feature for example. Jasinska-Myga and colleagues (Jasinska-Myga et al. 2012) looked at predicting future ability to work and living independently. They also found that age predicted progression to disability but they did not account for other variables during that analysis.

In summary, 4 previous systematic reviews have identified a lack of high quality studies investigating clinical features that predict prognosis in early PD. However, there was a

general consistency in identifying older age at onset or a lack of tremor / akinetic-rigid phenotype with a more rapid motor, cognitive and functional decline.

The outcome of more recent cohort studies specifically designed to determine prognostic markers have been in progress for the last decade, for which the results are starting to be published (including the population based Norwegian ParkWest, Campaign, and CARPA studies).

Table 2.4 – Summary of previous systematic reviews of clinical predictors of progression in PD

	(Marras et al. 2002)	**(Suchowersky et al. 2006)**	**(Post et al. 2007)**	**(Macleod et al. 2016)**
Languages	English French	English	English French German Spanish Dutch	English
Years included in search (databases searched)	1966- January 2002 (MEDLINE)	1997-2002 – (MEDLINE, EMBASE, CINAHL, Cochrane Database of Systematic Reviews) 1990-January 2005 – MEDLINE only (articles excluded before 1990)	1966-November 2004 (MEDLINE) 1980-November 2004 (EMBASE) 1982-November 2004 (CINAHL) 1988-November 2004 (Web of science)	1946-July 2013 (MEDLINE) 1947-July 2013 (EMBASE) 1989-July 2013 (CINAHL) 1970- July 2013(Web of Science)
Key words for search	Parkinson disease AND Progression OR Natural History	Parkinson disease Disease progression Muscle rigidity Tremor Hypokinesia Equilibrium Posture Gait	Parkinson disease Incidence Follow-up studies Prognosis Prediction Course Disability Quality of life	Summary due to complexity: Parkinson Disability Activities of daily living

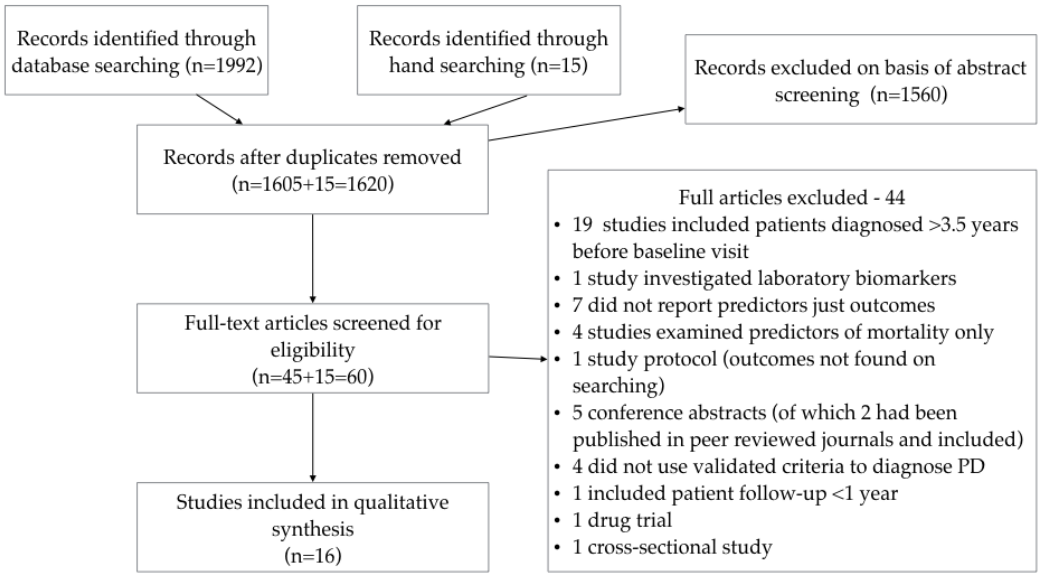
	(Marras et al. 2002)	(Suchowersky et al. 2006)	(Post et al. 2007)	(Macleod et al. 2016)
Inclusion criteria	Longitudinal data to assess factors predicting prognosis	Longitudinal data to assess putative factors	The study population consisted of patients with a diagnosis of PD Follow up of at least 6 months Full length articles or full written reports, whether published or not	Quantitative measures of disability Minimum 3 years follow-up
Exclusion criteria	Reports examining dyskinesias or motor fluctuations Reporting of only non-motor outcomes	Articles published before 1990 cause of changes in the case definition of PD	Randomised clinic trials reporting on efficacy	Cross-sectional studies Focus of parkinsonism Highly selected cohorts Drug trials without follow-up
Outcome measures	Disability Motor impairment	Motor progression by validated rating scale Motor fluctuations Dementia Quality of life Nursing home placement Death	Motor impairment (UPDRS) Disability (S&E, H&Y) Quality of life	Reaching dependency Scores of scales of disability
Number of studies identified by search	457	27	1535	21955
Included in final review	13 included studies (1 prospective cohort study, 11 retrospective cohort study, 1 case-control study)	7 (6 cohort studies and 1 clinical trial)	27 (from 147 studies reviewed against pre-defined methodological criteria and 6 additional papers from reference lists and experts)	14 publications included (9 identified by search, 5 by hand-searching)

	(Marras et al. 2002)	(Suchowersky et al. 2006)	(Post et al. 2007)	(Macleod et al. 2016)
Factors suggested to probably predict poorer outcome	**Motor progression and disability:** Greater baseline motor impairment Early cognitive disturbance / dementia **Disability:** Older age at onset Lack of tremor at onset	**Motor progression and cognitive decline:** Older age at onset Lack of tremor at onset	**Progression of disability:** Older age at onset Higher PIGD score	**Progression of disability:** Bradykinesia
Factors which possibly predict poorer outcome	**Motor progression:** Older age at onset and lack of tremor at onset (although some conflicting evidence) **Disability:** Depression, gender (male or female) had conflicting evidence	**Motor progression:** Co-morbidities (stroke, auditory deficits, visual impairments), PIGD features, and male sex. **Nursing home placement and mortality:** Older age at onset, dementia, and decreased dopamine responsiveness	**Motor progression:** Less motor impairment at baseline, more disability and dementia **Progression of disability:** Higher bradykinesia score, lack of tremor, symmetrical disease onset and depression	
Factors thought not to predict poorer outcome	**Disability:** Living in a rural area, family history (not genotyped), smoking, early dyskinesias or fluctuations, birth trauma, stress, environmental exposure and vitamin intake were thought not to predict motor progression. **Motor progression:** Gender, symmetry, ethnicity, level of education, living in a rural area, lack of tremor			

2.4.2 CLINICAL ASSESSMENTS WHICH ASSESS PROGNOSIS IN PD

2.4.3 RESULTS

Figure 2.1 – Search results for clinical assessments



Sixteen studies were identified reporting the results of 7 longitudinal cohorts, of which 6 papers were identified from hand searching (see Figure 2.1). The data extracted is shown in Table 2.5 and Table 2.6. Due to the inclusion criteria designed to allow only high quality studies, 7 of the included papers were rated evidence level I based on 4 cohorts the CamPaIGN (Williams-Gray et al. 2007), CARPA (Post et al. 2011), PINE (Macleod et al. 2016) and Norwegian ParkWest cohorts (Pedersen et al. 2013). Of these, the CamPaIGN, PINE and the ParkWest cohorts are incident population based cohorts, thus the most representative of a real-life population of Parkinson's. The CamPaIGN cohort published 2 studies reporting data after 5 years, of which the later study was included as it provided additional information not available in the earlier paper (Williams-Gray et al. 2009, Evans et al. 2011). Notably both of these studies have a higher mean age of participant at diagnosis (70.1 years in CamPaIGN and 67.5 years in ParkWest). As one might expect, the hospital based cohorts were each younger. While the population cohorts recruited from primary care, the hospital cohorts required referral from clinic.

Reflective of this potential selective bias in hospital ascertained cohorts, the UPDRS III motor score was also lower potentially due to their younger age or that patients less affected by motor disability may be more likely to enrol in hospital-based research.

The Parkinson's Progression Marker Initiative (PPMI) cohort, while designed to be representative of the earliest stages of Parkinson's in a multidisciplinary approach to biomarker discovery, has the youngest age of motor symptom onset (60.4) and age at diagnosis (61.9). The study design, while not restrictive in its eligibility criteria, is arduous with a protocol for multiple tests and assessments such as SPECT imaging, MRI and serial lumbar punctures which older participants appear less likely to volunteer for.

Time from motor symptom onset to diagnosis was similar in each study, with the mean disease duration from diagnosis 6 months or less in each study at baseline. H&Y stage was similar at baseline, with the average or median around 2.

Three of the studies had made attempts to minimise the effects of attrition either by searching for the clinical information by alternative routes (Evans et al. 2011, Broeders et al. 2013, Pedersen et al. 2013). The other studies made reference to or accounted for attritional bias in their discussion, apart from the study by Reinoso et al.(Reinoso et al. 2015).

In summary, the population based CamPaIGN, PINE and ParkWest cohorts are the least likely to be affected by selection bias. Thus they are most reflective of the true population. Of the hospital based studies, the CARPA cohort is the least likely to be affected by bias.

2.4.3.1 Motor outcomes

Five included studies examined prognostic factors for motor decline (Evans et al. 2011, Post et al. 2011, Velseboer et al. 2013, Reinoso et al. 2015, Lord et al. 2016). Male gender was reported in 3 studies as a predictor of decline (Post et al. 2011, Velseboer et al. 2013, Reinoso et al. 2015), although the highest quality study (on the CamPaIGN cohort) did not find this association (Evans et al. 2011). Evans et al. reported that age at diagnosis and higher PIGD score predicted more rapid motor impairment at 5 years (Evans et al. 2011). Velsbloer et al. split the UPDRS motor score into dopaminergic and non-dopaminergic responsive symptoms as defined by Levy, finding that male gender predicted worse dopaminergic responsive symptoms while worse cognition predicted worse non-dopaminergic symptoms at 5 years (Levy et al. 2000). Postural instability, which has a strong relationship with PIGD and non-dopaminergic symptoms, predicted early falls which is an important milestone of motor impairment (Lord et al. 2016). Reinoso and colleagues (Reinoso et al. 2015) found that there were differences over time in the ability of baseline features to predict motor outcomes. Male gender predicted more motor impairment in early to mid-phase (before 6 years) but no difference in late phase. Less motor impairment at baseline and akinetic-rigid phenotype, predicted more rapid initial progression, while age at onset and cognitive impairment predicted greater motor progression in the late phase (up to 10 years). However, these results must be viewed with caution as the study design is at risk of both selection and attritional bias. The heterogeneity of outcome assessments precluded a formal meta-analysis.

Velseboer et al. (Velseboer et al. 2016) used a composite outcome by combining both motor and cognitive outcomes, thus testing whether variables predicted a more general rapid progression. Predictor variables were derived on the CARPA cohort before being validated on the Campaign cohort. Older age, higher UPDRS III axial score, and lower

animal fluency score were each associated with a higher probability of an unfavourable outcome.

In summary, there is conflicting evidence that male gender could predict more rapid motor progression in the early to mid-phases of PD (approximately 5 years disease duration). There was one high quality study that suggested that age and worse axial impairment may predict worse motor outcome at 5 years, with a further high quality study confirming more rapid progression with a composite outcome of both motor and cognitive impairment.

2.4.3.2 Cognition

Three high quality studies were consistent with MCI (Litvan et al. 2012) predicting dementia (Broeders et al. 2013, Pedersen et al. 2013, Pedersen et al. 2017).

The CamPaIGN cohort published results from 3 and 5 year follow up with differing results. At 3 years a lack of tremor, pentagon copying errors and deficiencies on verbal fluency predicted dementia (Williams-Gray et al. 2009). However, at 5 years age at diagnosis and semantic fluency predicted dementia (Evans et al. 2011).

Similarly, different results at different outcome stages were described in the CARPA cohort. While at 3 years follow up age did not predict cognitive decline (Muslimovic et al. 2009) at 5 years both age and poor memory (Rivermead Behavioural memory test) predicted cognitive decline.

De la Riva and colleagues (de la Riva et al. 2014) found different factors predicting cognitive decline at 12 and 24 months. While overall there was a decline in MOCA score at the first and second year follow up, some participants who were deemed cognitively impaired initially improved. This could be a result of measurement error ('regression to the mean'), or possibly the effects of medication. At 2 years, age and concomitant RBD predicted worse cognitive cores. Using the same PPMI cohort, but with additional follow-up data, Schrag et al. (Schrag et al. 2017) found that older age, hyposmia, RBD,

depression and motor impairment predicted early cognitive decline. A key difference in this cohort is the younger age (mean age at baseline 61), thus a population at lower risk of cognitive impairment. Again, meta-analysis was not possible due to the heterogeneity of outcome assessment.

In summary, there is good evidence that MCI predicts dementia in PD. There was conflicting evidence for older age at disease diagnosis, potentially predicting decline at 1 and 5 years. Poor memory, visuo-spatial dysfunction, verbal fluency and RBD symptoms at baseline possibly predict cognitive impairment. Older age and axial impairment was associated with a more rapid general decline as measured by a composite outcome of both motor and cognitive impairment.

2.4.3.3 Disability

In the included studies, predictors of disability were reported on the CARPA and PINE cohorts. Older age at onset predicted more disability at both 3(Post et al. 2011) and 5 years (Velseboer et al. 2013). Baseline non-dopaminergic motor symptoms and cognitive impairment also predicted disability at 5 years (Velseboer et al. 2013). The PINE study (Macleod et al. 2016) provided strong evidence that older age, axial impairment, and a lower cognitive score were associated with a greater risk of dependency. The finding that smoking was positively associated with dependency is perhaps surprising given its inverse association with PD itself. This may reflect a greater small vessel vascular burden which may exaggerate the degenerative process.

In summary, there is good evidence that age, axial symptoms and cognitive impairment predict disability at 5 years. Smoking predicted earlier dependency in a single high quality study.

2.4.4 CONCLUSIONS FROM EXISTING LITERATURE

The results of this systematic review highlight the problems with studies investigating prognostication in PD. Despite multiple studies involving thousands of participants, there remains a lack of high quality evidence to support or refute potential clinical predictors. There is good evidence that MCI predicts PDD. There is an argument that MCI is simply a transition phase prior to the development of PD, but the variability in rate of conversion to dementia makes it an important early predictor. Consistent with the previously described systematic reviews, older age at onset and more severe non-tremor motor symptoms (such as axial symptoms) at baseline may predict disability. Older age may predict motor and cognitive decline, male gender may predict more rapid motor progression in the early phases, and cognitive decline may be predicted by RBD symptoms, verbal fluency and memory dysfunction at baseline.

The best evidence for predicting dementia came from 3 high quality cohorts that used the same assessment criteria for both the predictor and outcome. Outside of this example, there was a wide heterogeneity of clinical assessments in the included studies, and indeed across the spectrum of clinical PD research.

The major limitation of this review was the quality of the primary studies, a problem not limited to PD research (Riley et al. 2013). The criteria for inclusion were set a priori with an emphasis on selecting high quality studies. Despite this, most of the selected studies included participants with a relatively younger age at PD onset than population based estimates (i.e. younger than 70) (Linder et al. 2010, Caslake et al. 2013, Pringsheim et al. 2014). This is probably due to the inclusion of hospital based recruitment, as older patients and those with lower socio-economic status are less likely to be referred to a specialist clinic (Rybicki et al. 1995). Also, those with more rapidly progressing symptoms are more likely to withdraw or be lost to follow up. This missing data is likely to bias the study population to those less severely affected, with not all the studies

making adequate provision for this effect. In addition, despite all the included studies using validated criteria for assessment of diagnosis, with re-ascertainment, none had pathological confirmation of the diagnosis. Early diagnosis is less accurate, but even after 5 years the accuracy of diagnosis remains at around 90% (Hughes et al. 1992, Adler et al. 2014). The inclusion of participants without PD, may lead to the discovery of a marker for atypical parkinsonism rather than PD. While the PPMI study aims to improve the diagnostic accuracy of their cohort by the inclusion of functional dopamine transport scanning in their diagnostic criteria, the population they recruited appeared were significantly younger than population studies (mean age at diagnosis 61.9 years). This may be due to the number of tests in the study protocol being less appealing to older or more impaired patients (CSF sampling, imaging etc.). In addition, patients who had started dopamine replacement therapy were excluded on the basis that their UPDRS III score would have been affected by this. This could also bias the population to a less severely affected group as many patients are started on treatment at diagnosis. Their baseline results show that they are not as cognitively impaired than population based cohorts (Weintraub et al. 2015).

Based on the complexity and heterogeneity of clinical phenotype in PD, it seems unlikely that a single factor would be sensitive enough to be clinically useful in isolation. Thus, prognostic models (combinations of multiple predictors) such as motor phenotypes (eg PIGD vs TD) were investigated in multiple studies (Post et al. 2011, Broeders et al. 2013, Pedersen et al. 2013, de la Riva et al. 2014, Reinoso et al. 2015, Fereshtehnejad et al. 2017).

Limitations of the review itself include the risk of missing studies due to the search terms utilised in the search. Six of the included studies were identified through hand-searching of citations of identified articles or previous systematic reviews. This may have been a consequence of excluding certain terms from the abstract of the article, although these

terms would not have excluded the additional articles. The level of evidence used was not very discriminatory between the included studies, primarily because of the criteria for study inclusion. However, additional levels of evidence would not have been helpful or provided much more insight.

There are notable exceptions to the studies included in this review. The DATATOP study was a large placebo controlled clinical trial investigating deprenyl, tocopherol or the combination of both in early PD (evidence level 4) (Jankovic et al. 1990). This study was included as it lacked validated assessment criteria for PD, the start pre-dating their widespread use. However, this study described the TD and PIGD phenotypes, with PIGD being associated with greater disability. These were investigated as prognostic models in the included phenotypes. Although this was a large study (800 patients recruited), the results demonstrate that patients participating in clinical trials have a bias to slower progression PD (Uc et al. 2009). For similar reasons, the results of the Sydney Multi-centre study (a clinical trial comparing bromocriptine to carbidopa) were excluded, despite reporting the longest prospective cohort of PD to date. This study provided evidence that non-dopamine responsive symptoms were highly prevalent in late stages and that the vast majority of patients by this stage had developed dementia (83%) with age rather than disease duration being the more relevant predictor.

Putting the Discovery cohort in context, the most recent systematic review (Macleod et al. 2016) made suggestions for the planning of prognostic studies based on recommendations by the PROGRESS consortium (Riley et al. 2013):

- Population studies including all new cases in an area.
- Expert confirmation of diagnosis using validated diagnostic criteria.
- No exclusion criteria (other than those related to accuracy of diagnosis).
- Prospective follow-up of long-term outcomes, using valid and reliable outcome measures.

While this would give the best chance of truly representing the PD population, the expense and resources required for a large cohort study of this nature is prohibitive. The Discovery cohort is a hospital based, prospective cohort of early PD which is designed to study the natural history of PD, prognostic factors and biomarkers. While the study population (for reasons given above) will be at risk of selecting a younger and less severely affected population, the benefits of the larger size will increase the chance of finding a useful biomarker due to the increase in power. With that caveat, replication between this and the other population based studies as a form of validation will enhance the external validity of the results. Other advantages include being able to compare findings with the Tracking PD cohort (due to the similar design of the study in population, assessment and size) and having a control group to help control for the increasing co-morbidities associated with age.

Table 2.5 – Data extraction sheet for clinical assessments study design and outcome

Paper (by cohort and year published)	**Study design, length of follow-up and setting**	**Study population including medication status, diagnostic and exclusion criteria**	**Clinical assessment**	**Outcome**	**Statistical analysis method**	**Relationship of clinical assessment to outcome**
(Williams-Gray et al. 2007) CAMPAIGN cohort	Prospective longitudinal, followed up for mean 3.5 years. Population based, UK, single area.	Newly diagnosed PD, UKBB criteria,(Gibb et al. 1988) usual treatment, atypical parkinsonism excluded	Age at diagnosis, motor phenotype, verbal fluencies, pentagon copying (scored 0-2).	**Cognition:** Dementia (DSM IV criteria)	Backwards stepwise method using multivariate regression to predict cognitive decline (MMSE) on all potential predictors selected by bivariate analysis on change of cognition. Bivariate analyses were then performed to see if they predicted dementia.	**Cognition:** Dementia: Non-tremor dominant (RR 4.1, 95% CI 1.0,17.8), pentagon copying (RR 5.2, 95% CI 1.9,14,1), semantic fluency defecit (RR 9.5, 95% CI 1.9,47.5)

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Evans et al. 2011) CAMPAIGN cohort	Prospective longitudinal, followed up for mean 5.2 years. Population based, UK, single area.	Newly diagnosed, PD, UKBB criteria,(Gibb et al. 1988) usual treatment, atypical parkinsonism excluded	Age at diagnosis, gender, motor phenotype, Verbal IQ, verbal fluencies, pentagon copying (scored 0-2), LEDD.	**Motor:** H&Y stage 3, dyskinesia **Cognition:** Dementia (DSM IV criteria)	Cox regression adjusting for age at diagnosis, sex, average medication dose, presenting motor phenotype and MAPT genotype. For the dementia analysis, baseline IQ, fluencies and pentagon copying were included.	**Motor:** H&Y 3: age at diagnosis (RR 1.05, 95% CI 1.02,1.08, p<0.001), TD vs PIGD (RR 0.42, 95% CI 0.24,0.72, p=0.002). Dyskinesia: LEDD (RR 1.002, 95% CI 1.001,1.003, p=0.02). **Cognition:** Dementia: Age at diagnosis (RR 1.12, 95% CI 1.06,1.19, p<0.001), semantic fluency (RR 3.69, 95% CI 1.07,12.7, p=0.04).

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Muslimovic et al. 2009) CARPA study group	Prospective longitudinal, followed up for 3 years. Hospital based, Dutch, single centre.	De novo, incident PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment, diagnostic criteria, age<85, dementia excluded, atypical parkinsonism excluded, life expectancy <1 year, fluency in Dutch.	Age at onset, disease duration, 6 motor domains of UPDRS III.	**Cognition:** Cognitive decline as per a multivariate comparison method.	For hierarchical multivariate regression analysis, age at onset and disease duration were entered as independent variables in the first step. In the second step, axial impairment at baseline was added to the model.	**Cognition:** None of the predictor variables were significant
(Post et al. 2011) CARPA study group	Prospective longitudinal, followed up for 5 years. Hospital based, Dutch, single centre.	Early PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment, diagnostic criteria, age<85, dementia excluded, atypical parkinsonism excluded, life expectancy <1 year, fluency in Dutch.	Age at onset, sex, disease duration, UPDRS III, comorbidity, cognitive function, and affective symptoms	**Motor:** UPDRS III **Disability:** S&E ADL, Academic Medical Center Linear Disability Scale	Linear mixed effects models	**Motor:** Male gender predicted faster motor decline. **Disability:** Older age at onset predicted more disability

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Broeders et al. 2013) CARPA study group	Prospective longitudinal, followed up for 5 years. Hospital based, Dutch, single centre.	Early PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment, diagnostic criteria, age<85, dementia at baseline excluded, atypical parkinsonism excluded, life expectancy<1 year, fluency in dutch.	Age, years of education, age at onset of PD, UPDRS motor score, bradykinesia, axial symptoms, Levy B, Hoehn & Yahr scale, LEDD, battery of neuropsychological tests	**Cognition:** Cognitively impaired as defined by multivariate normative comparison across a battery of neuropsychological tests	Hierarchical logistic regression analysis (stepwise) comparing testing demographics, baseline clinical and neuropsychological variables with cognitively impaired with not impaired at 5 years. Goodness of fit was assessed with Hosmer and Lemeshow test.	**Cognition:** Age of onset (OR=4.34; 95% CI = 1.22–16.11; p 0.02) and Rivermead Behavioral Memory Test immediate recall (OR=7.78; 95% CI= 2.10 – 28.86; p=0.002).
(Broeders et al. 2013) CARPA study group	Prospective longitudinal, followed up for 5 years. Hospital based, Dutch, single centre.	Early PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment diagnostic criteria, age<85, dementia excluded at baseline, atypical parkinsonism excluded, life expectancy<1 year, fluency in dutch.	PD-MCI (MDS task force criteria)	**Cognition:** PDD (MDS criteria) (Dubois et al. 2007)	Modifiable Reliable Change Index (mRCI) to determine change in score corrected for retest effects. Multivariate ANCOVA was used for comparison, adjusting for gender, age and years of education with group as an independent variable	**Cognition:** Dementia: All patients who developed PDD had PD-MCI at a previous assessment

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Velseboer et al. 2013) CARPA study group	Prospective longitudinal, followed up for 5 years. Hospital based, Dutch, single centre.	Early PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment diagnostic criteria, age<85, dementia excluded, atypical parkinsonism excluded, life expectancy<1 year, fluency in dutch.	Age at onset, male gender, baseline cognitive dysfunction, UPDRS III symptoms,	**Motor:** UPDRS III (Levy A&B subscales), H&Y **Disability:** S&E ADL, Academic Medical Center Linear Disability Scale	Linear mixed effect model	**Motor:** UPDRS III total: Male sex (effect 0.99; CI 0.17,1.81; p=0.02) and cognitive dysfunction (effect 1.33; CI 0.30,2.35; p=0.01); Levy A: Male sex (effect 0.78; 0.17,1.39, p=0.01). Levy B: Cognitive dysfunction (effect -3.05; -5.16,-0.93, p=0.005); age at onset (effect -0.01; -0.02,-0.01, p=0.001). **Disability:** S&E: Levy B (effect -0.67; -1.05,-0.29 , p=0.001); ALDS: cognitive dysfunction (effect -0.23;-0.42,-0.05, p=0.02), higher age at onset (effect -0.23; -0.42,-0.05, p=0.02).

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Velseboer et al. 2016) CARPA (discovery cohort) and CamPaIGN (validation cohort) (discovery cohort demographics presented here)	Prospective longitudinal, followed up for 5 years. Hospital based, Dutch, single centre.	Early PD, clinical data available for those who had withdrawn, Gelb(Gelb et al. 1999) usual treatment diagnostic criteria, age<85, dementia excluded, atypical parkinsonism excluded, life expectancy<1 year, fluency in dutch.	Age, gender, cognition (MMSE and verbal fluencies), mood (HADS and BDI), motor score (UPDRS III) and co-morbidity (cumulative illness rating scale)	**Cognitive and motor composite:** Dementia or the onset of postural stability were considered poor outcomes	Model performance was reported as Harrel c-statistics for discriminative ability and model calibration by local regression	**Cognitive and motor outcome:** Older age, higher UPDRS III axial score, and lower animal fluency score were all associated with a higher probability of an unfavourable outcome. Area under the receiver operating characteristic curve of 0.85 (95% CI 0.77–0.93) and a well-calibrated model with a calibration slope of 1.13 and no significant lack of fit (Hosmer-Lemeshow test: p=0.39).

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Pedersen et al. 2013) Norwegian ParkWest study	Prospective longitudinal, followed up for 3 years. Population based, Norway, multi-centre.	De-novo, incident PD, all withdrawn patients had dementia status available, dementia excluded, atypical parkinsonism excluded.	PD-MCI (MDS task force criteria) Neuropsychological tests of sub domains	**Cognition:** PDD (MDS criteria) (Dubois et al. 2007)	Relative risks with 95% CI for MCI conversion to PDD. Wilcoxon signed rank test was used to compare neuropsychological variables over time.	**Cognition:** RR for dementia in patients with MCI vs non-MCI at baseline was 39.2 (95% CI, 5.2-296.5; $p<0.001$); older age, impairment in verbal memory and attention were associated with conversion from MCI to PDD.

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Bjornestad et al. 2016) Norwegian ParkWest study	Prospective longitudinal, followed up for 5 years. Population based, Norway, multi-centre.	De-novo, incident PD, all withdrawn patients had dementia status available, dementia excluded, atypical parkinsonism excluded.	Age, gender, cognition (MMSE), mood (MADRS), motor score (UPDRS III)	**Disability:** Loss of independence (standardised interview assessing ADL and care needs)	Kaplein-Meier survival analysis and cox logistic regression	**Disability:** Higher age, shorter symptom duration, increasing motor severity, and presence of mild cognitive impairment at PD diagnosis independently predicted independence loss during follow- up. Falling (RR 4.8, $p<0.001$), hallucinations (RR 4.4, $p<0.001$), or dementia (RR 4.2, $p<0.001$) were associated with a higher risk of institutionalisation compared to home-dwelling patients

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Pedersen et al. 2017) Norwegian ParkWest study	Prospective longitudinal, followed up for 5 years. Population based, Norway, multi-centre.	De-novo, incident PD, all withdrawn patients had dementia status available, dementia excluded, atypical parkinsonism excluded.	PD-MCI (defined by level I MDS criteria)	**Cognition:** PD dementia using MDS criteria (based on clinical interview, MMSE and neuropsychological tests with functional impairment attributable to cognitive dysfunction)	Logistic regression modesl adjusted for age, gender and education	**Cognition:** The conversion rate to dementia was 59.1% in patients with persistent PD-MCI at 1 year vs 7.2% in those with normal cognition during the first year (adjusted odds ratio 16.6, 95% CI 5.1–54.7, p<0.001).
(Reinoso et al. 2015)	Prospective, longitudinal, 3-10 year follow up. Hospital based, single centre, Singapore.	Early PD within 2 years of diagnosis, NINDS diagnostic PD criteria, excluded atypical PD and patients who had DBS.	Gender, Age at onset (young <50), phenotype (akinetic-rigid, mixed, TD), cognitively impaired (MMSE<24), motor score (less impaired on UPDRS III <30)	**Motor:** UPDRS III	Linear mixed effect model analysis for early (1-1.5 years), mid (1.5-6.5 years) and late (>6.5 years)	**Motor:** Male gender predicted more motor impairment in early to mid phase (before 6 years) but no difference in late phase. Less motor impairment at baseline and akinetic-rigid phenotype, predicted more rapid initial progression. Age and cognitive impairment, predicted greater motor progression in the late phase up to 9 years.

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Macleod et al. 2016) PINE study	Prospective, longitudinal, followed up for 5-12 years from diagnosis. Population based, UK, single centre.	Incident PD, UKBB criteria, drug induced parkinsonism and atypical parkinsonian disorders were excluded.	Age, gender, social deprivation, co-morbidity, cognition (MMSE), motor scores (axial and bradykinesia sum scores from the UPDRS III) and co-morbidity	**Disability:** Schwab and England ADL score (<80%)	Kaplan-meier analysis and cox regression	Hazard ratios for loss of independence: Older age at diagnosis (hazard ratio for 10-year increase 2.23, 95% CI 1.66-2.98), greater smoking history (hazard ratio for 10-pack-year increase, 1.15, 95% CI 1.04-1.26), more severe axial impairment (hazard ratio for 5-point increase in sum of axial items from UPDRS (1.78, 95% CI 1.30-2.44), and lower MMSE score (hazard ratio 0.88, 95% CI 0.79-0.98) were independently associated with a higher risk of dependency

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(Lord et al. 2016) ICICLE-GAIT	Prospective, longitudinal, followed up for 3 years. Population based, UK, single centre.	De-novo incident PD, UKBB criteria, dementia at onset or dementia with Lewy bodies, drug-induced parkinsonism, vascular parkinsonism, progressive supranuclear palsy, multiple system atrophy, cortico-basal degeneration, or poor command of English were excluded.	Age, gender, motor score and phenotype (UPDRS III), cognition (MOCA and CANTAB) orthostatic hypotension and depression (GDS)	**Motor:** Falls (diary recordings)	Chi-squared automatic interaction detection, t-tests, and multivariable cox-regression	Hazard ratios for predictors of fall onset: Gait speed (3.4, 95% CI 1.6 to 7.5), stance time (3.3, 95% CI 1.4 to 7.8), and Hoehn & Yahr III stage (2.8, 95% CI 1.4 to 5.7). The hazard ratio for risk factors combined was 7.8 (95% CI 2.8 to 21.8).

Paper (by cohort and year published)	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Clinical assessment	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome
(de la Riva et al. 2014) PPMI	Prospective, longitudinal, 2 year follow up. Hospital based, international, multi-centre, Europe, Australia, and the USA.	Early, untreated PD, typical clinical syndrome and DAT scan positive, diagnosis within 2 years, dementia excluded.	Male gender, education, age, depression, UPDRS III, hyposmia, PIGD phenotype, RBD (questionnaire based).	**Cognition:** MOCA	Linear mixed-effects models adjusting for co-variates of gender, education, age, depression, UPDRS III, hyposmia, PIGD phenotype, RBD.	**Cognition:** Age (effect -0.08, p=0.003); RBD (effect -0.37; p=0.001)
(Schrag et al. 2017) PPMI study	Prospective, longitudinal, 2 year follow up. Hospital based, international, multi-centre, Europe, Australia, and the USA.	Early, untreated PD, typical clinical syndrome and DAT scan positive, diagnosis within 2 years, dementia excluded.	Age, gender, education, age, depression (GDS), MDS-UPDRS III, hyposmia, , RBD (questionnaire based).	**Cognition:** MOCA	Regression models and ROC analysis	**Cognition:** Older age, hyposmia, RBD, depression and motor impairment were independent predictors of motor decline, predicting cognitive impairment together with an AUC of 0.76 (95% CI 0.68-0.83).

Table 2.6 - Data extraction sheet for population features and evidence level

Paper (by cohort and year published)	**Study recruitment period**	**Disease duration from diagnosis (years) at baseline**	**Recruited / Losses to follow-up / total patients included in analysis**	**Mean age at motor symptom onset / diagnosis (years) / baseline**	**Baseline motor scores**	**Evidence Level with criteria met**
(Williams-Gray et al. 2007) CAMPAIGN cohort	2000-2002	0.5	309 referred (239 consented - 41 alternate diagnoses, 15 lost to follow up and 14 died prior to baseline) / 159 recruited / 14 died, 10 dementia, 7 lost to follow-up, 1 major stroke, 1 prevalent / 126 included in analysis	Mean age at motor symptom onset 67.8 / mean age at diagnosis 70.1 (sd 9.8) / mean age at baseline 70.6	UPDRS III mean 25.9, H&Y median stage 2	I (a,b,c,d)
(Evans et al. 2011) CAMPAIGN cohort	2000-2002	0.5	309 referred (239 consented - 41 alternate diagnoses, 15 lost to follow up and 14 died prior to baseline) / 159 recruited / 14 died, 10 dementia, 7 lost to follow-up, 1 major stroke, 1 prevalent, 4 alternative diagnosis on follow-up / 122 included in analysis	Mean age at motor symptom onset 67.8 / mean age at diagnosis 70.1 (sd 9.8) / mean age at baseline 70.6	UPDRS III mean 25.9, H&Y median stage 2	I (a,b,c,d)

Paper (by cohort and year published)	Study recruitment period	Disease duration from diagnosis (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores	Evidence Level with criteria met
(Muslimovic et al. 2009) CARPA study group	2002-2005	Incident	116 recruited / 17 refused retesting, 5 died, 3 deteriorated for other health conditions, 2 unable to be retested for severe confusion / 89 followed-up for 3 years	Mean age at motor symptom onset 63.2 /mean age at diagnosis and baseline 64.7 (9.7)	UPDRS III mean 16.5, H&Y mean 1.7 (sd 0.7)	II (a,b,d)
(Post et al. 2011) CARPA study group	2002-2005	0.3	133 recruited / 6 had PDD, 1 had MSA, 10 lost to follow-up (6 dead, 4 voluntary withdrawal) / 116 followed-up for 3 years	Mean age at motor symptom onset 64.6 (sd 10.4) /mean age at diagnosis 65.9 (sd 10.4) / mean age at baseline 66.2 (10.4)	UPDRS III mean 17.2, H&Y median stage 2 (IQR 1-2)	I (a,b,c,d)
(Broeders et al. 2013) CARPA study group	2002-2005	0.4	133 recruited / 4 had diagnosis changed, 30 lost to follow-up (13 dead, 16 voluntary withdrawal, 1 untraceable) / 56 followed-up for 5 years	Mean age at motor symptom onset 64.8 (sd 10.5) /mean age at diagnosis 66.1 (sd 10.5) / mean age at baseline 66.5 (10.5)	UPDRS III mean 17.1, H&Y median stage 2 (IQR 1-2)	II (a,b,d)
(Broeders et al. 2013) CARPA study group	2002-2005	0.4	133 recruited / 4 had diagnosis changed, 30 lost to follow-up (13 dead, 16 voluntary withdrawal, 1 untraceable) / 93 followed-up for 3 years and 56 followed-up for 5 years	Mean age at motor symptom onset 64.8 (sd 10.5) /mean age at diagnosis 66.1 (sd 10.5) / mean age at baseline 66.5 (10.5)	UPDRS III mean 17.1, H&Y median stage 2 (IQR 1-2)	II (a,b,d)

Paper (by cohort and year published)	Study recruitment period	Disease duration from diagnosis (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores	Evidence Level with criteria met
(Velseboer et al. 2013) CARPA study group	2002-2005	0.4	133 recruited / 4 had diagnosis changed, 32 lost to follow-up (25 dead, 7 lost) / 97 included for analysis	Mean age at motor symptom onset 64.8 (sd 10.5) /mean age at diagnosis 66.1 (sd 10.5) / mean age at baseline 66.5 (10.5)	UPDRS III mean 17.1, H&Y median stage 1.5-2 (IQR 1-2)	II (a,b,d)
(Velseboer et al. 2016) CARPA (discovery cohort) and CamPaIGN (validation cohort) (discovery cohort demographics presented here)	2002-2005	0.4	133 recruited / 4 had diagnosis changed, 22 excluded (5 missing data, 17 H&Y>2 at baseline) / 111 included for analysis	Mean age at motor symptom onset 64.6 (sd 10.1) /mean age at diagnosis 65.2 (sd 10.1) / mean age at baseline 65.6 (10.1)	UPDRS III mean 17.1, H&Y median stage 1.5-2 (IQR 1-2)	I (a,b,c,d)
(Pedersen et al. 2013) Norwegian ParkWest study	2004-2006	Incident	196 recruited / 14 had diagnosis changed / 182 included in analysis at 5 years	Mean age at motor symptom onset 65.2 (calculated) / mean age at diagnosis / mean age at baseline 67.5 (sd 9.3)	UPDRS III mean 22.9 (sd 11.0) H&Y mean 1.9 (sd 0.6)	I (a,b,c,d)
(Bjornestad et al. 2016) Norwegian ParkWest study	2004-2006	Incident	212 recruited / 18 had diagnosis changed, 5 not drug naïve / 189 included in analysis at 5 years	Mean age at motor symptom onset 65.4 (calculated) / mean age at diagnosis 67.2 / mean age at baseline 67.5 (sd 9.3)	UPDRS III mean 23.4 (sd 11.3) H&Y mean 1.9 (sd 0.6)	I (a,b,c,d)

Paper (by cohort and year published)	Study recruitment period	Disease duration from diagnosis (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores	Evidence Level with criteria met
(Pedersen et al. 2017) Norwegian ParkWest study	2004-2006	Incident	196 recruited / 14 had diagnosis changed, 7 withdrawn by first follow-up visit / 175 included in analysis at 1 year	Mean age at motor symptom onset 65.2 (calculated) / mean age at diagnosis / mean age at baseline 67.5 (sd 9.3)	UPDRS III mean 22.9 (sd 11.0) H&Y mean 1.9 (sd 0.6)	I (a,b,c,d)
(Reinoso et al. 2015)	2002-2012	NS	576 recruited / loss of follow up NS / 576 included in analysis unclear disease durations, unclear how missing data was handled	Mean age at motor symptom NS /mean age at diagnosis 63.7 (sd 10.4) / mean age at baseline NS	UPDRS III mean 21.0 (sd 9.4), H&Y mean 2.13 (0.7)	III (a,b)
(Lord et al. 2016) ICICLE-GAIT	2009-2011	Incident	121 recruited / 2 had diagnosis changed, 26 had already had falls, 2 declined to participate further, 14 lost to follow-up / 77 analysed	Mean age at motor symptom onset NS / mean age at diagnosis 68.3 / mean age at baseline 68.5	MDS-UPDRS III mean 23.6 (11.7)	II (a,b,d)
(Macleod et al. 2016) PINE study	2002-2006	Incident	355 recruited / 156 had an alternative diagnosis / 198 analysed	Mean age at motor symptom onset (approximation) 71.4 / mean age at diagnosis 72.5 (10.4) / mean age at baseline 72.5 (10.0)	UPDRS III 25.1 (11.6)	I (a,b,c,d)

Paper (by cohort and year published)	Study recruitment period	Disease duration from diagnosis (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores	Evidence Level with criteria met
(de la Riva et al. 2014) PPMI	2010-2013	0.5	423 recruited / loss to follow up NS, follow-up ongoing / 261 with 1 year follow up, 96 with 2 years follow up included for analysis	Mean age at motor symptom onset 60.4 (calculated) /mean age at diagnosis 61.9 (calculated) / mean age at baseline 62.4 (9.7)	MDS-UPDRS III mean score 20.9 (sd 2.9), H&Y median 1 (IQ range 1-2)	II (a,c,d)
(Schrag et al. 2017) PPMI study	2010-2013	0.4	423 recruited / 3 had missing data, 82 had not yet been followed-up / 318 included for analysis	Mean age at motor symptom onset NS /mean age at diagnosis 60.9 (calculated) / mean age at baseline 61.2 (9.8)	MDS-UPDRS III median score 20 (IQR 14-26)	II (a,c,d)

2.5 BIOCHEMICAL PROGNOSTIC FACTORS

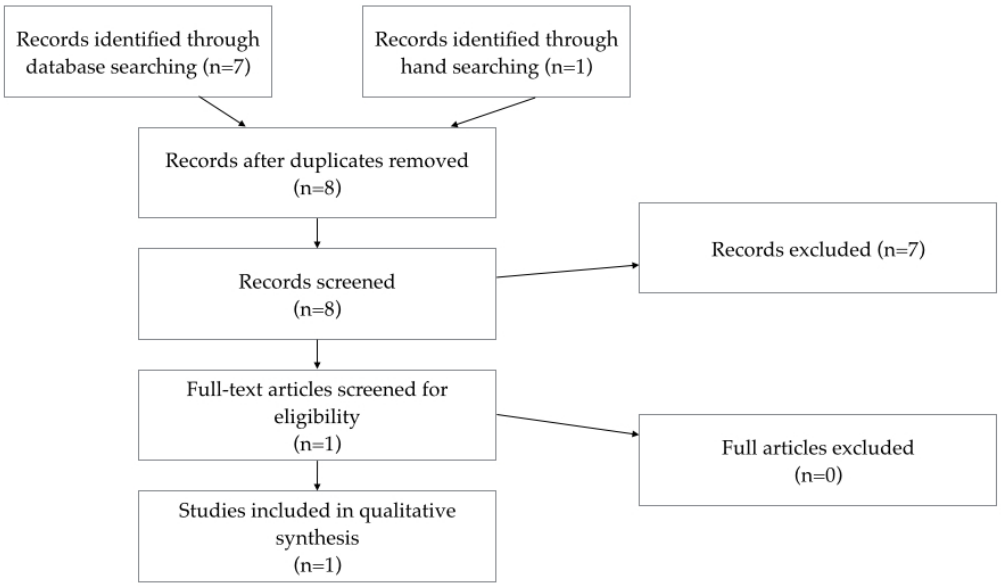
2.5.1 PREVIOUS SYSTEMATIC REVIEW AND BIOMARKER SELECTION

McGhee et al. performed a broad systematic review across all putative biomarkers for disease progression, including serum biomarkers (McGhee et al. 2013). Across all the included studies (n=183), only 20 were longitudinal, of which none examined serum biomarkers (51 cross-sectional studies were identified). Only 3 reported studies examining blood based biomarkers had more than 100 patients in their sample. In addition, they reported that the studies (all the biomarker studies rather than specifically the blood based) had a low recruitment age (mean 63.5, sd 5.6) and were not often early PD (median disease duration 5.7 years, IQR 3.6-7.4). They highlighted that there was difficulty with the available data, despite the number of studies, in selecting which candidates merited further investigation and which were very unlikely. The authors also reported that the reasons for selecting a candidate biomarker could be tenuous, with little link to the underlying pathophysiology of PD. The use of a large early PD cohort of well-characterised patients in a longitudinal study, such as the Discovery cohort, would allow rigorous external evaluation of the baseline biomarkers proposed as recommended by the authors.

Four of the most promising serum biomarkers for this innovative study were chosen based on recent reviews and current evidence (McGhee et al. 2013, Chahine et al. 2014): Vitamin D, Apolipoprotein A1 (ApoA1), Uric Acid and Epidermal Growth Factor (EGF). Following pilot results (discussed in more detail in chapter 5), the EGF study was curtailed in favour of C-reactive protein (CRP). Each of these biomarkers is available commercially for potential translation into clinical practice. There remains a lack of evidence for the utility of each these biomarkers as they lack studies of sufficient power and methodological quality for widespread use or to be confidently dismissed.

2.5.2 APOLIPOPROTEIN A1

Figure 2.2– Search results for Apolipoprotein-A1



2.5.2.1 Results

As shown in Figure 2.2, there was only one study (evidence level II) which investigated the use of plasma ApoA1 as a prognostic biomarker by Swanson and colleagues (Swanson et al. 2015). This was performed on plasma obtained from the PPMI study (see tables 2.7 and 2.8 for the extracted data). The mean age at onset was 63 (SD 9) and mean UPDRS III motor score 20.4 (SD 7.9), which are younger and less motor impaired than comparable population based incident studies (as discussed in chapter 2.4.4). The analysis of the other subgroup did not reveal a relationship with time to initiation of therapy.

In this medium sized study of the PPMI cohort (split into 2 subgroups), they failed to find evidence of its use as a prognostic marker after a follow up period of one year. Following stratification of the ApoA1 levels into tertiles, the highest and lowest tertiles were not associated with a significant difference in motor decline as measured by UPDRS III. They also reported that longitudinal measurement of ApoA1 was not associated with UPDRS III over time in linear mixed-effects models adjusted for age and

gender (the figures were not reported). However, the follow up time was short, and they are planning to follow up the participants for a longer period.

While they did demonstrate that earlier age of PD onset and greater motor severity were associated with lower ApoA1 levels, there was no evidence that ApoA1 is useful for predicting motor severity at 1 year. However, this result should be viewed with caution due to the short follow up length.

2.5.2.2 Conclusions

ApoA1 was initially identified as a potential biomarker by a screening method for candidate plasma proteins in a case control study (a multiplex immunoassay was used) (Qiang et al. 2013). Subsequent analysis of the clinical data found that lower levels correlated with the earlier onset of PD symptoms and higher motor severity score (UPDRS III). They replicated these findings of in an independent cohort, this time using a different technique (ELISA). Using an enriched cohort of people at high risk of developing PD (the PARS study (Siderowf et al. 2012)), they found an association with DAT deficit and lower ApoA1 levels. Thus, they demonstrated cross-sectional evidence of lower ApoA1 corresponding to both motor and imaging outcomes. Following further studies, the same group (Swanson et al. 2015) also performed a meta-analysis of studies of ApoA1 including 1018 PD patients from 5 different cohorts, using 3 different methods (multiplex immunoassay, ELISA and a high-throughput immunoturbidity assay) in patients who were not fasted (Qiang et al. 2013, Berlyand et al. 2014, Swanson et al. 2015). Using the combined cohort, they performed a linear regression model adjusted for age and gender, finding associations with age at onset (coefficient $\beta = 0.86$, $p<0.001$) and UPDRS III (coefficient $\beta = -1.57$, $p<0.001$). The authors also found lower levels of ApoA1 in PD compared with controls and other forms of neurodegeneration (amyotrophic lateral sclerosis and fronto-temporal dementia) in the same study. Independently, lower plasma levels have been associated MCI and Alzheimer's disease

(Song et al. 2012), and also with cognitive decline independent of amyloid deposition (Choi et al. 2016). Indirect evidence for circulating ApoA1 being altered in PD patients comes from lower levels being found in post-mortem CSF (Maarouf et al. 2012).

The biological basis of the association of ApoA1 with PD is not yet well explained. ApoA1 is the major component of HDL and facilitates reverse transport of cholesterol, removing excess cholesterol from peripheral tissues to the liver for excretion.(Millan et al. 2009) Low levels of ApoA1 have been associated with cardiovascular disease (Rader et al. 1994). Cardiovascular risk in itself is associated with worse motor and cognitive performance in PD (Malek et al. 2016), and Apoa1 containing on circulating HDL may have a beneficial role in cerebral vessels, providing indirect benefit to brain cells (Wellington et al. 2016). It may also play a role in beta amyloid deposition (Song et al. 2012), which is associated with cognitive decline in PD (Giasson et al. 2003, Irwin et al. 2013) (although these cohort studies in PD have not shown a correlation with cognition). In summary, it may be that higher levels of ApoA1 may confer some form of neuroprotection, neurodegeneration lowers ApoA1 levels or some common process is responsible for either. Levels can be modified by lifestyle and pharmacological methods, with indirect evidence that the regular use of a statin may confer protection against PD (Gao et al. 2012, Berlyand et al. 2014). Thus, replication of these findings may prove not only to be significant as a biomarker but also as a potential avenue of treatment. There is currently a lack of evidence for its utility as a biomarker, but growing indirect evidence of its potential.

Table 2.7 – Data extraction sheet for ApoA1 studies design and outcome

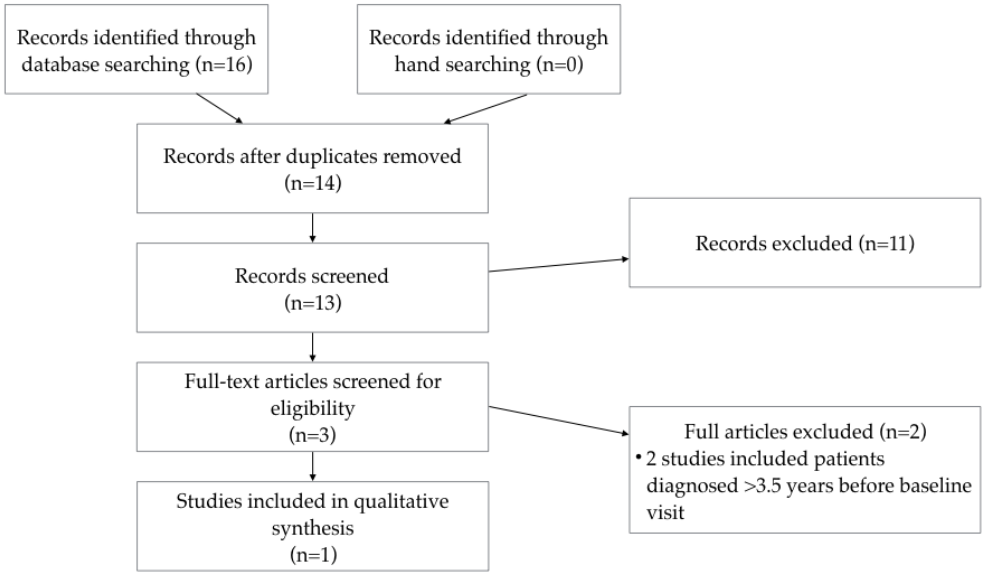
Paper	**Study design, length of follow-up and setting**	**Study population including medication status, diagnostic and exclusion criteria**	**Serum biomarker**	**Outcome**	**Statistical analysis method**	**Relationship of clinical assessment to outcome**	**Evidence Level with criteria met**
(Swanson et al. 2015)	Prospective, longitudinal, 1 year follow up. Hospital based, international, multi-centre, Europe, Australia, and the USA.	Early, untreated PD, DAT scan positive, diagnosis within 2 years, dementia excluded.	ApoA1	Motor: MDS-UPDRS III	Linear mixed-effects models adjusting for co-variates of age and gender was performed on the UPDRS; logistic regression and Cox's proportional hazards models were used for initiation of therapy adjusting for age and gender.	No relationship found between baseline ApoA1 levels and motor decline. The difference in annual change in UPDRS III score between the highest and lowest tertile of plasma ApoA1 was 0.19 (95% confidence interval: -3.75, 4.11).	II (a,b,d)

Table 2.8 - Data extraction sheet for ApoA1 studies population features

Paper	**Study recruitment period**	**Disease duration (years) at baseline**	**Recruited / Losses to follow-up / total patients included in analysis**	**Mean age at motor symptom onset / diagnosis (years) / baseline**	**Baseline motor scores**
(Swanson et al. 2015)	2010-2013	Median 0.6 range (0-3)	423 recruited / loss to follow up NS, follow-up ongoing, 37 excluded on plasma quality control, plasma unavailable in some / 154 included in analysis	Mean age at motor symptom onset NS /mean age at diagnosis 63 (9) / mean age at baseline 63 (8)	MDS-UPDRS III mean score 20.4 (sd 7.9), H&Y mean 1.5 (sd 0.5)

2.5.3 EPIDERMAL GROWTH FACTOR - RESULTS

Figure 2.3 – Search results for EGF



2.5.3.1 Results

There was only one study (evidence level II), as shown in Figure 2.3, investigating serum EGF as a prognostic marker in early PD by Pellecchia et al. (Pellecchia et al. 2013), see Table 2.9 and 2.10 for details. The two excluded studies demonstrated that lower levels of EGF predicted cognitive decline over time in established rather than in early PD. Specifically, Chen-Plotkin et al. (Chen-Plotkin et al. 2011) found that lower plasma EGF levels predicted poorer performance on a cognitive scale with the lowest levels predicting conversion to PDD range scores. The same group repeated the study on a different cohort, demonstrating that low levels of EGF were more likely to convert to PDD (Lim et al. 2015). The exclusion of 25 patients who either refused or did not attend appointments for follow up were not accounted for in the analysis. In addition, the low age of participants (mean 60, sd 8.3) and low UPDRS III (mean 14.5, sd 6.7). Both these points suggests that those who attended clinic may have less impairment than the true PD population.

In this small study (n=40), Pellecchia et al. found that EGF levels were inversely correlated to semantic fluency ($R^2 = 0.147$; $p = 0.03$ (β coefficient not given)) and color naming task of Stroop color-word test ($R^2 = 0.121$; $p = 0.04$ (β coefficient not given)) in linear regression models adjusting for age, gender, UPDRS and dopaminergic medication. Overall cognitive function was not reported and multiple testing in this study leads to a risk of type 1 error. EGF levels were higher in the PD population than the control population, in-keeping with the hypothesis of EGF being part of a neuroprotective pathway with levels increased as a compensatory mechanism to prevent further cellular damage.

The evidence suggests that lower EGF levels may predict alteration of function in posterior (impaired color perception) and frontal (semantic fluency) cerebral areas, possibly as an impaired neuroprotective response. However, this is a small study with possibly an atypical PD population and so the clinical significance of these associations is unclear.

2.5.3.2 Conclusions

EGF was selected as a candidate biomarker following screening of plasma proteins in a cohort of PD with evidence that it predicted cognitive decline (Chen-Plotkin et al. 2011). This group then published a further study, aimed at investigating its utility in predicting prognosis, using a cohort of PD across different stages (n=236) and a group of patients at high risk of developing PD (n=35) (Lim et al. 2016). Availability of data varied (from n=165 at 12 months to n=10 at 60 months) but low baseline plasma EGF predicted poorer longitudinal cognition (mixed effects model using age, sex and baseline MOCA as co-variates: coefficient $\beta=0.86$, $p<0.001$) but not motor decline. They also found that low plasma EGF levels correlated with visuo-spatial dysfunction in the highest risk participants in the at risk cohort (linear regression model adjusting for age, sex and

dopamine transporter binding levels: coefficient β=0.51, p=0.01). Although the results from this enriched prodromal cohort are similar to the study by Pellechia et al., this needs to be viewed with caution as this was a subgroup analysis in only a small number or patients. Additionally, they found that lower EGF levels may predict conversion from amnestic MCI to Alzhiemer's disease (lowest tertile of baseline EGF on Cox proportional hazards model adjusting for age, sex and baseline MMSE: HR=1.87, 95% CI 1.41-2.48, p<0.001).

Animal models have shown that trophic factors have the potential to slow neurodegeneration in PD (Behrstock et al. 2006) and human trials have also been attempted (Allen et al. 2013). Neurogenesis in the adult brain, triggered by dopamine in the subventricular zone, has been shown to be mediated by EGF (O'Keeffe et al. 2009, O'Keeffe et al. 2009). Thus, it is plausible that higher circulating levels may reflect a level of neuroprotection.

In summary, this single study provides evidence that EGF has the potential to predict cognitive decline.

Table 2.9– Data extraction sheet for EGF studies design and outcome

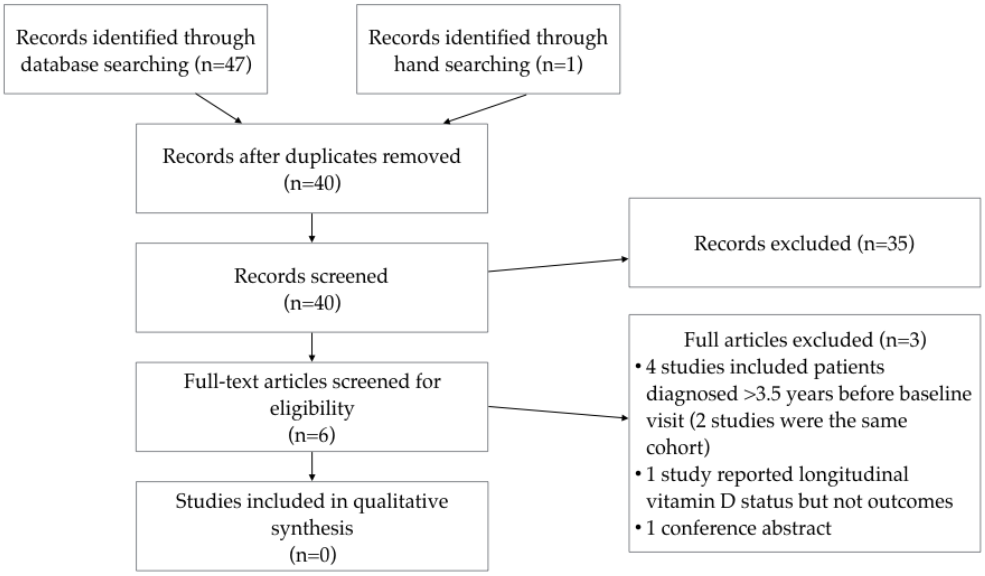
Paper	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Serum biomarker	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome	Evidence Level with criteria met
(Pellecchia et al. 2013)	Prospective, longitudinal, 2 years follow up. Hospital setting, single centre, Italy.	Early drug naïve PD, UKBB criteria, major depression, abnormal brain imaging, dementia, centrally acting drugs.	EGF	Cognition: Battery of neuropsychological tests	Linear regression models adjusting for the co-variates of age, gender, UPDRS and dopaminergic medication.	EGF correlated with semantic fluency (R^2 = 0.147; p = 0.025) and color naming task of Stroop color-word test (R^2 = 0.121; p = 0.044).	II (a,b,d)

Table 2.10 - Data extraction sheet for EGF studies population features

Paper	Study recruitment period	Disease duration (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores
(Pellecchia et al. 2013)	NS	Incident	65 recruited / 25 withdrawn or missed appointments / 40 included in analysis	Mean age at motor symptom onset 58.5 (sd 8.3) /mean age at diagnosis and baseline 59.7 (sd 8.3)	Mean UPDRS III 14.5 (sd 6.7)

2.5.4 VITAMIN D - RESULTS

Figure 2.4 – Search results for Vitamin D



2.5.4.1 Results

The literature search (shown in Figure 2.4), including hand searching through recent systematic reviews examining the relationship between vitamin D and Parkinson's (Zhao et al. 2013, Lv et al. 2014, Rimmelzwaan et al. 2016), revealed a single longitudinal study investigating if vitamin D predicted prognosis in early Parkinson's. Based on the ICICLE cohort, this conference abstract reported that PD patients had lower levels of vitamin D than controls and that this difference was maintained at 18 months (Sleeman et al. 2016). They did not find an association between vitamin D status and either cognitive or motor outcomes. The abstract itself provided insufficient data to be extracted into the table, but 129 PD patients were compared to 72 controls, with the serum extracted at diagnosis.

2.5.4.2 Conclusions

Vitamin D plays a role in bone health and growing evidence increasingly demonstrates it has a role in normal brain function. Lower levels have been associated with a variety of neurodegenerative diseases (particularly multiple sclerosis and Alzheimer's disease), stroke, autoimmune conditions, mental health disorders, cancer and cardiovascular disease. The active form of vitamin D (1,25-dihydroxyvitamin D_3 (calcitrol)) is synthesised and regulated in a multi-step process, initiated by photosynthesis in the skin or absorbed from dietary sources. The primary physiological function of vitamin D is regulating calcium and phosphate transport and bone mineralisation but it also mediates cell-signalling and transcription of target genes (Deeb et al. 2007, Harms et al. 2011). The 1,25-dihydroxyvitamin D_3 receptor (VDR) and 1α-hydroxylase (the enzyme that generates 1,25-dihydroxyvitamin D_3) are both found in neuronal and glial cells, with high concentrations of 1α-hydroxylase in the substantia nigra (Eyles et al. 2005). In addition, vitamin D may have a neuro-protective effect by promoting anti-oxidants (Garcion et al. 2002) and neurotrophins (Smith et al. 2006). Alternative mechanisms suggested include neuronal calcium regulation, immunomodulation, enhanced nerve conduction, and detoxification mechanisms (Yeshokumar et al. 2015). Indirect evidence is drawn from the potential association of VDR polymorphisms with PD, although the studies to date have been inconsistent (Butler et al. 2011, Lee et al. 2014, Zhang et al. 2014).

These provide a plausible biological basis for its association with PD. Animal models have shown benefit of supplementation and a single placebo controlled trial suggested stabilisation of PD stage (Suzuki et al. 2013).

The most recent systematic review by Rimmelzwaan and colleagues (Rimmelzwaan et al. 2016) identified 8 recent case-control studies which found lower vitamin D levels in PD patients compared to controls in 7 studies (Sato et al. 1997, Sato et al. 2005, Abou-

Raya et al. 2009, Topal et al. 2010, Evatt et al. 2011, K et al. 2011, van den Bos et al. 2013) and no difference in a single study (Petersen et al. 2014). A meta-analysis of 6 studies with sufficient data to be included (639 PD patients and 1255 controls), the mean serum vitamin D was lower in PD than in controls (mean -11.6μg/ml; 95% CI -12.23,-10.86).

Two prior systematic reviews with meta-analyses were conducted by Lv (Lv et al. 2014) (7 studies identified (Sato et al. 1997, Sato et al. 2005, Abou-Raya et al. 2009, Knekt et al. 2010, Evatt et al. 2011, Ding et al. 2013, van den Bos et al. 2013)) and Zhao (Zhao et al. 2013) (identified 5 studies (Sato et al. 1997, Sato et al. 2005, Abou-Raya et al. 2009, Knekt et al. 2010, Evatt et al. 2011)) and revealed similar results. Lv and colleagues found a mean difference of -16.88 (95% CI -33.5,-0.23) in 1008 PD cases and 4536 controls, while Zhao and colleagues found a standardised mean difference of -1.33 (95% CI -2.44,-0.21) including 434 PD cases and 3451 controls. All three meta-analyses described significant heterogeneity (I^2 = 98-99%, $p<0.001$). Only 2 studies included more between 100-300 PD patients (Sato et al. 2005, Peterson et al. 2013, van den Bos et al. 2013), with 1 study including 388 PD cases (Ding et al. 2013) highlighting the small size of cohorts. There was also considerable overlap in the absolute levels of Vitamin D between the cases and controls in each cohort, despite PD cases repeatedly found to have a lower group mean.

Early PD patients (defined as H&Y <2.5) were reported in 4 studies (Sato et al. 1997, Evatt et al. 2011, Ding et al. 2013, van den Bos et al. 2013), with one finding no difference in vitamin D compared to controls (20 PD patients and 33 controls) (Sato et al. 1997). A population based study of newly diagnosed PD (ICICLE-PD) found that even in the earliest stages of PD, patients had lower vitamin D (Sleeman et al. 2016). Similar rates of deficiency were found between PD patients treated or untreated with dopaminergic medication (Evatt et al. 2011, Ding et al. 2013). Vitamin D may be related

to the severity of PD symptoms at baseline including total UPDRS (Ding et al. 2013), motor impairment (Sato et al. 1997, Sato et al. 2005), cognitive impairment and mood (Peterson et al. 2013), but evidence is conflicting (Evatt et al. 2011, Sleeman et al. 2016). The Harvard Biomarker study also included follow up data (evidence level II) (Ding et al. 2013), but their recruitment had not defined early PD consistent with the inclusion criteria for the systematic review. Their results were consistent with the others, finding that Vitamin D levels were lower in PD patients (17.6% considered deficient compared to 9.3% of controls). They also found that lower levels of vitamin D correlated with total UPDRS (linear regression model using age, sex, race and vitamin D supplementation as co-variates: coefficient β= -0.16, p=0.01) and disease duration (coefficient β= -1.73, p=0.004) at baseline. In addition, lower vitamin D predicted a higher total UPDRS score at mean follow up of 2 years (partial fixed effect model coefficient β= -0.13, p=0.01). Similar results were also found with the more active vitamin D_3 when considered separately.

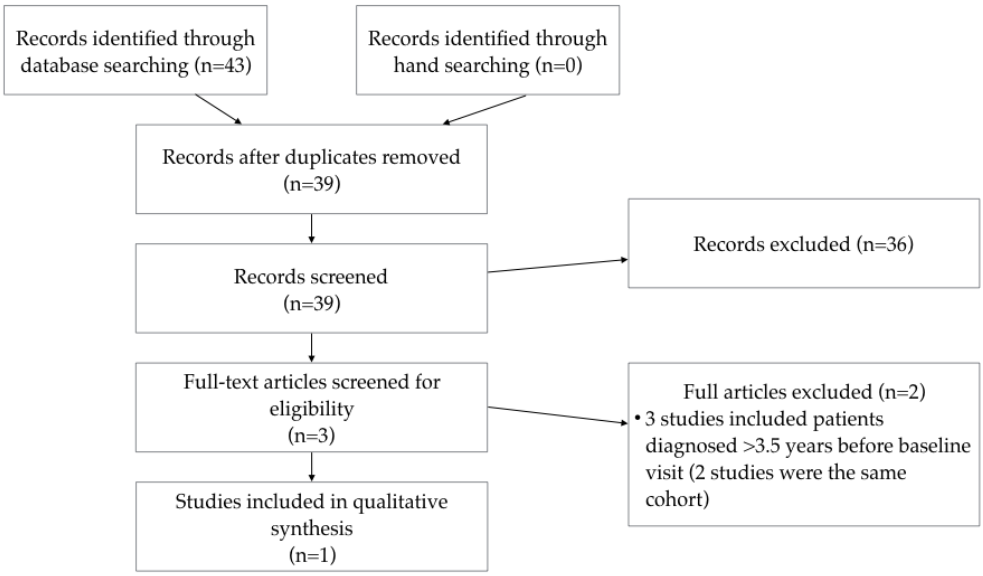
A more recent study also reproduced the findings of hypovitaminosis in vitamin D, with a larger cohort (478 PD cases and 431 controls) (Wang et al. 2015). A more advanced PD cohort than the Harvard Biomarker study (Ding et al. 2013), there were higher rates of deficiency (36%) and insufficiency (71%) in-keeping with other studies. They considered dietary (vitamin D_2) sources and those mainly from sunlight exposure (vitamin D_3) separately, and found that both were inversely associated with PD. Evatt and colleagues (Evatt et al. 2011) investigated whether vitamin D levels changed over time. Contrary to expectations of progressively lower vitamin D levels, the levels did not decline and showed a mild increase. This provides some evidence that the association is not caused by the disease process driving immobility thus reduced sun exposure. Reduced dietary intake may also be directly linked to the disease process itself as reduced gastric motility and appetite are both common.

The Mini-Finland health survey (Knekt et al. 2010) suggested that high vitamin D status provides protection against PD based on the 50 incident cases found on following 3173 aged between 50-79 disease free at baseline, followed up for up to 29 years. The relative risk between the highest and lowest quartiles of vitamin D was 0.33 (95% CI, 0.14-0.80), adjusting for sex, age, marital status, education, alcohol consumption, leisure-time physical activity, smoking, body mass index, and month of venesection. In a similar study design, an analysis of the Atherosclerosis Risk in Communities Study cohort identified 67 incident cases in 15792 participants aged 45-64 who were disease free at baseline with a median follow up period of 17 years (Shrestha et al. 2016). With this younger and much larger population, they did not find that vitamin D reduced the risk of developing PD. The lower proportion of cases identified in this later study is may reflect the younger age of the population. Again, the small number of cases identified (despite the large size of the study) may leave it under-powered to detect small differences. Also, this latter study set in the USA had much higher levels of vitamin D compared to the Finnish study which may have attenuated the findings.

In summary, there is high quality and reproducible evidence that vitamin D is lower in PD cases compared to controls. There is conflicting evidence of its relation to disease severity at baseline, with a single longitudinal study in early PD failing to show an association. Numerous environmental, behavioural, and physiological factors that can influence circulating vitamin D levels (known and unknown) making assessment and comparison difficult. Whether these findings relate to the underlying pathogenesis as cause or consequence, or indeed whether this correlation is due to an alternative mechanism is not clear. Even as a risk factor for the disease, it may not necessarily affect disease progression once the pathophysiology is more advanced.

2.5.5 C-REACTIVE PROTEIN - RESULTS

Figure 2.5 – Search results for C-Reactive Protein



2.5.5.1 Results

The literature search, including hand searching (see Figure 2.5), found a single a longitudinal study investigating if CRP predicted prognosis in early Parkinson's based on the ICICLE-PD study (Williams-Gray et al. 2016). This study provides highest quality evidence for the potential of using immune markers to predict prognosis (see tables 2.11 and 2.12). They investigated a battery of 11 serum immune markers (including CRP), which they combined (using principle component analysis) into 3 components of inflammatory state at baseline (2 comprised of pro-inflammatory markers and one was anti-inflammatory). Each of the 3 components predicted one of: worse cognition at baseline (pro-inflammatory); more rapid motor progression (pro-inflammatory including CRP); or slower motor progression (the anti-inflammatory component). This supports the hypothesis of baseline immune markers predicting progression, and also suggests a complex relationship with PD. Notably, CRP was no different between cases and controls. Despite predicting progression in combination with

other pro-inflammatory markers, CRP in isolation was not sufficient to predict prognosis.

2.5.5.2 Conclusions

CRP itself is a peripheral marker of inflammation, synthesised in hepatocytes as a response to pro-inflammatory cytokines. Levels fluctuate with transient rises in response to inflammation whether driven by infection or another cause. The relationship between Parkinson's and inflammation is not clear, but inflammation has also been implicated in other forms of neurodegeneration such as Alzheimer's disease.(Tan et al. 2007) Evidence for this relationship comes from an increase in inflammatory activity found at the neuronal level, an association with genetic susceptibility and a potential risk reduction mediated by anti-inflammatories.

Post-mortem studies in PD (McGeer et al. 1988, Loeffler et al. 2006, Toulorge et al. 2016) and animal models (Brochard et al. 2009) demonstrate evidence of increased inflammatory activity, with increased number and activation (priming) of microglial cells. Infiltration of peripheral immune cells and the role of astrocyte activation are not clear but may mediate an inflammatory process (Toulorge et al. 2016). Imaging studies using [(11)C]-PK11195 positron emission topography show increased microglial activation in vivo (Edison et al. 2013). This may be because release of pro-inflammatory agents may be harmful for neurones. A proposed mechanism of how this pathway may accelerate neurodegeneration, is that once the microglia are primed, they may subsequently have an exaggerated response to systemic inflammation increasing neuronal damage (Perry et al. 2014).

Genome-wide association studies have linked the human leucocyte antigen (HLA) region with PD, further evidence of a role for inflammation (Saiki et al. 2010, Nalls et al. 2011). Suppression of inflammation could therefore present a target for disease modifying therapy. Non-aspirin non-steroidal anti-inflammatory drugs may reduce the

risk of developing PD with ibuprofen having the largest potential effect (Gagne et al. 2010, Gao et al. 2011, Rees et al. 2011), potentially by supressing the inflammatory component of neuronal damage. This evidence comes from observational studies, with alternative explanations including that the symptoms being treated by NSAID's confer a protection against PD. Another alternative is that there is a change in personality or pain modulation by the disease itself, leaving an individual to need more analgesics.

Inflammatory markers are therefore hypothesised to predict prognosis in PD as a potential marker of processes which accelerate neuronal loss, rather than a direct inflammatory response to the process itself. But it could also in itself be a response to the abnormal α-synuclein triggering a systemic response (Allen Reish et al. 2015).

CRP is the most extensively investigated inflammatory marker of vascular risk, although this has not proven useful in risk stratification for cerebrovascular disease (Kaptoge et al. 2010). Vascular risk and leucokariosis predict a more rapid progression in early PD (Malek et al. 2016), thus CRP elevation may reflect an increased neurovascular burden. Small cross-sectional studies have suggested that CRP could be useful in predicting more advanced symptoms in PD (Hassin-Baer et al. 2011, Zhang et al. 2011, Sawada et al. 2014). Umemura and colleagues (Umemura et al. 2015) specifically addressed whether CRP could be used as a biomarker of motor decline in a large PD cohort (n=406) across all disease stages. They had excluded patients on steroids or with known inflammatory or infective conditions and found a positive association between CRP and motor decline.

In summary, inflammation has been linked to the pathogenesis of PD. The use of peripheral markers to predict prognosis is a promising field, although the reasons as to why remain unclear. A combination of markers rather than CRP may be more accurate.

Table 2.11 – Data extraction sheet for CRP studies design and outcome

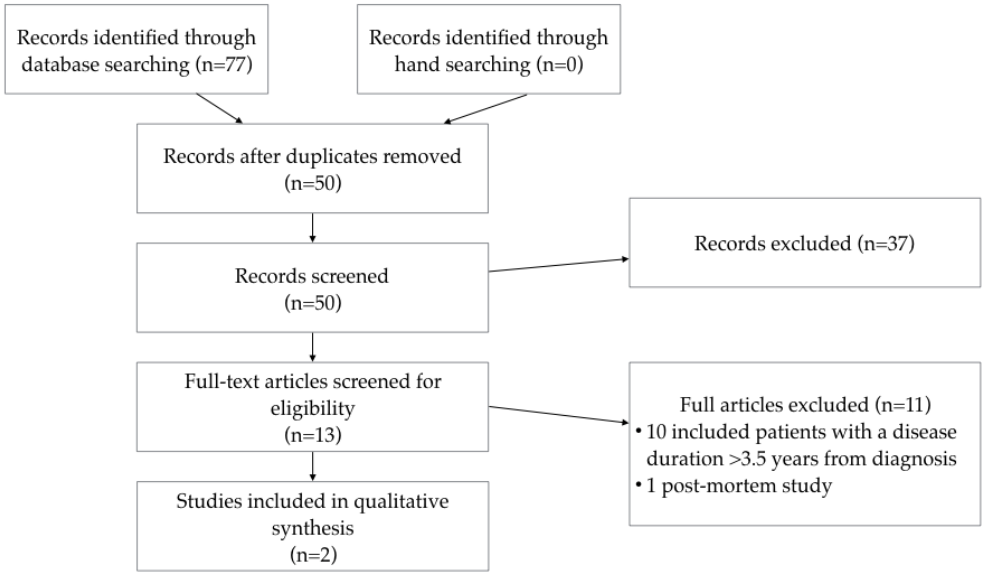
Paper	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Serum biomarker	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome	Evidence Level with criteria met
(Williams-Gray et al. 2016)	Prospective, longitudinal, 3 years follow up. Hospital setting, single centre, UK.	Newly diagnosed PD, UKBB criteria(Hughes et al. 1992), usual treatment, atypical parkinsonism excluded	CRP	Motor: MDS-UPDRS III Cognitive: MMSE	Repeated-measure analysis of variance, multiple linear regression		I (a,b,c,d)

Table 2.12 - Data extraction sheet for CRP studies population features

Paper	Study recruitment period	Disease duration (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores
(Williams-Gray et al. 2016)	2009-2011	0.6	682 referred / 262 recruited / 11 excluded for change in diagnosis on follow up, 21 missing serum samples / 230 included in analysis	Mean age at motor symptom onset NS /mean age at diagnosis 65.9 (sd 9.5) / mean age at baseline 66.4 (sd 9.5)	Mean MDS-UPDRS III 27.9 (sd 11.5)

2.5.6 URIC ACID - RESULTS

Figure 2.6 – Search results for uric acid



2.5.6.1 Results

Two studies fulfilled the criteria (evidence level II) to be included (see Figure 2.6 and Tables 2.13 and 2.14) (Moccia et al. 2015, Mollenhauer et al. 2013). In the smaller study (n=69) by Moccia et al., none of the patients reported co-morbidities or drugs that would affect uric acid levels. Higher uric acid levels were associated with slower progression of motor symptoms after 2 years, as assessed by the UPDRS III. However, this correlation was unadjusted for age and gender, both of which can affect uric acid levels. Participants who had not started levodopa therapy also had higher uric acid levels, which is suggestive of better motor function but not conclusive. They also reported that higher uric acid levels were also associated with fewer self-reported non-motor symptoms, including attention/memory complaints (p=0.05). The self-reporting of attention or memory problems may predict cognitive decline (Erro et al. 2014), but is not a validated cognitive outcome for cognitive status.

Mollenhauer and colleagues reported the initial 2 year findings of the DeNoPa cohort, part of the protocol included measuring serum uric acid levels at baseline and follow-up visits in cases and controls. They found no differences between cases and controls nor baseline and repeated uric acid levels after 2 years. There was no association between uric acid and recognised outcomes at 2 years, although there was an association with LEDD (effect size not reported).

One smaller study provides evidence that uric acid could possibly predict motor progression, while another larger study did not replicate this finding. Both studies were well designed, using early drug naïve participants.

2.5.6.2 Conclusions

The most common symptomatic complication of hyperuricaemia is gout, but epidemiological studies have also shown that there may be a positive relationship with obesity, metabolic syndrome, hypertension, renal and cardiovascular disease (Feig et al. 2008). Conversely, studies suggest that elevated levels are protective against neurological disorders including Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and PD (Auinger et al. 2010, Kim et al. 2015, Pakpoor et al. 2015). These findings in epidemiological studies are underpinned by putative pathophysiological pathways, but remain controversial.

There is evidence for the utility of uric acid as a biomarker for risk, diagnosis and prognosis in PD. Lower uric acid levels have been linked with a higher risk of developing PD in large prospective incidence studies (Davis et al. 1996, de Lau et al. 2005, Weisskopf et al. 2007, Chen et al. 2009, Jain et al. 2011). This association is repeatedly reported to be less or absent in women (O'Reilly et al. 2010, Gao et al. 2016). Higher uric acid levels have also been associated with a longer duration of REM sleep behaviour disorder without conversion to PD, suggesting a protective role (Martin et al. 2013). However, an association between gout (a proxy for a hyperuricaemic state) is not

clearly associated with a lower risk of developing PD as case control and cohort studies reveal conflicting results (Pakpoor et al. 2015, Ungprasert et al. 2015).

Cross-sectional studies have repeatedly shown that uric acid is lower in PD cases compared to controls (Annanmaki et al. 2007, Andreadou et al. 2009, Winquist et al. 2010, Sun et al. 2012, Zhang et al. 2012, Jesus et al. 2013, Vieru et al. 2016), with recent meta-analyses confirming this association, but advising caution due to the limited studies available (Shen et al. 2013, Wen et al. 2017). Lower levels are associated with worsening disease severity (Ikeda et al. 2011) and a greater rate of decline (Schwarzschild et al. 2008, Ascherio et al. 2009, Shen et al. 2013).

The evidence for uric acid being neuroprotective could relate to the role of uric acid as an anti-oxidant and free radical scavenger, which may reduce the oxidative stress causing dopaminergic loss in PD (Church et al. 1994, Toulorge et al. 2016). Oxidative stress is a thought to represent a common pathway by which several proposed aetiological pathways of Parkinson's converge. This is potentially implicated in the pathogenesis of PD via the enhanced oxidation of dopamine, misfolding and aggregation, mitochondrial dysfunction, cell cycle reactivation, apoptosis and excitotoxicity (Cipriani et al. 2010, Chen et al. 2012).

Despite multiple previous attempts at trying to use anti-oxidant therapies as a disease modifying agent for PD, the results have been disappointing (such as co-enzyme Q10, Vitamin E and mitoquinone) (Hart et al. 2009). It remains unclear whether these trials failed due to targeting the wrong mechanism, the lack of efficacy of the proposed anti-oxidant or inadequate trial design (eg inadequate patient group selection and choice of outcomes which have been mainly motor). This remains an ongoing target for disease modifying therapies, with a recent clinical trial (phase III) using inosine to elevate uric acid has now started (June 2016) following a successful tolerability trial (Schwarzschild

et al. 2014). Uric acid therefore remains of interest for its potential target as a therapy as well as a biomarker.

In summary, the association of uric acid with disease severity demonstrates good potential as a biomarker, but currently lacks replication in a high quality longitudinal study to allow generalizability. This study would also help define the association between uric acid levels and women, an important link as previous studies have either shown a correlation in men or both sexes together but not women on their own.

Table 2.13 – Data extraction sheet for uric acid studies design and outcome

Paper	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Serum biomarker	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome	Evidence Level with criteria met
(Moccia et al. 2015)	Prospective, longitudinal, 2 years follow up. Hospital setting, single centre, Italy.	Early drug naïve PD, UKBB criteria, major depression, abnormal brain imaging, dementia, centrally acting drugs.	Uric Acid	Motor: UPDRS III, inception of treatment Cognitive: Self reported attention and memory problems	ANOVA with post hoc Bonferroni analysis was performed on uric acid categorized into tertiles for self-reported attention and memory problems, adjusting for age, sex and LEDD. Spearman's rank correlation co-efficient was used to assess relationship between serum uric acid levels and change in UPDRS III. Two-tailed t-test to compare difference in uric acid between patients who started or had not started dopamine treatment.	Higher uric acid levels were related to a lower change in UPDRS III scores (adjusted R^2 = 0.24; p=0.04). Lower uric acid levels were found in patients not requiring levodopa (5.4 vs 4.7 mg/dl, p=0.04).	II (a,b,d)

Paper	Study design, length of follow-up and setting	Study population including medication status, diagnostic and exclusion criteria	Serum biomarker	Outcome	Statistical analysis method	Relationship of clinical assessment to outcome	Evidence Level with criteria met
(Mollenhauer et al. 2016)	Prospective, longitundinal, 2 years follow-up. Hospital setting, single centre, Germany.	Early drug naïve PD, UK BB criteria	Uric acid	Motor: UPDRS III, H&Y staging, LEDD	Linear mixed effects models, unadjusted	No relationship between uric acid and primary outcome measures, although an association with higher LEDD	II (a, b, c)

Table 2.14 - Data extraction sheet for uric acid studies population features

Paper	Study recruitment period	Disease duration (years) at baseline	Recruited / Losses to follow-up / total patients included in analysis	Mean age at motor symptom onset / diagnosis (years) / baseline	Baseline motor scores
(Moccia et al. 2015)	2008-2009	<2 years	65 recruited with all included in the analysis	Mean age at motor symptom onset NS /mean age at diagnosis and baseline 59. (sd 8.3)	Mean UPDRS III 15.6 (sd 7.7)
(Mollenhauer et al. 2016)	2008-2012	New diagnosis*	269 recruited / 15 lost to follow-up / 123 analysed	Mean age at motor symptom onset 61.6 /mean age at diagnosis and baseline 63.7 (sd 9.5)	Mean UPDRS III 21.5 (sd 11.6)

*Disease duration from diagnosis not specified, but soon after diagnosis.

2.5.7 CONCLUSIONS ON EXISTING LITERATURE

There is a lack of high quality studies reporting long term follow up results of serum biomarkers. Multiple low quality or cross-sectional studies may falsely identify an association between these biomarkers and prognosis. Vitamin D and uric acid particularly have been the subject of numerous studies, with conflicting evidence and precious resources used in the absence strong conclusions. Given the numerous studies, a single study of high quality, even if negative, would help direct future studies. While there is pilot evidence for the use of EGF, ApoA1 and CRP, their results require further replication.

3 METHODS

3.1 RECRUITMENT OF THE DISCOVERY COHORT

The Discovery cohort began recruitment in September 2010, comprising 4 groups: (i) patients with Parkinson's disease (PD) assessed within 3.5 years of diagnosis; (ii) first-degree relatives of patients with PD; (iii) patients with rapid eye-movement sleep behaviour disorder (RBD); and (iv) control subjects. This thesis focussed on prognostication in the PD group, using the control group for comparison and excluding the other two groups. Participants were prospectively recruited primarily from the Thames Valley population in the UK. The office for national statistics estimates our study population at 2.2 million based on the 2011 census (rising to almost 2.4 million in the mid-2014 estimate).

Following the start of this study, a meta-analysis reported a pooled estimate of the incidence rate for PD as 14.6 (12.2-17.3 95% CI) per 100,000 per year, noting a high heterogeneity between the included studies (study estimates varied from 8.4-18.3) (Caslake et al. 2013). During the 5 year recruitment period, incident cases for a total of 8.5 years would have been eligible, as those with a diagnosis up to 3.5 years before the start date were still possible.

Based on these figures we would approximate that there would be between 2300-3200 eligible cases of PD in the study region.

Potential participants were referred from hospital clinics in the region, primary care and were also able to individually register their interest in participation through the OPDC website, following which eligible patients were invited to attend research clinic. A video detailing the research of the OPDC cohort was used to promote recruitment, and has had over 4,900 views since 2012 (https://www.youtube.com/watch?v=FOtWoFcMBCI).

For locations of the sites, please see Figure 3.1. Additional information about PD-Discovery can be found on the study website: http://opdc.medsci.ox.ac.uk.

Each participant was sent a questionnaire prior to attending a clinic appointment where the assessments were conducted by both a study neurologist and research nurse or clinical psychologist. The self-assessment questionnaire was reviewed in clinic by the clinician to check the participant's understanding of the questionnaire. Validated questionnaires were used to assess a broad range of motor, non-motor and physical characteristics (see Table 3.3 for the assessed questionnaires by group). At the clinic appointment, following written and verbal informed consent, a blood sample was taken from each participant. The sample was then spun in a centrifuge before extracting and freezing the serum.

The baseline visit was termed Visit 1 with follow-up visits planned every 18 months from recruitment. The PD group were offered a clinic follow-up visit every 18 months, or the option of a telephone interview. Control participants were offered a telephone follow-up only. The participant pathway is shown in Figure 3.2, detailing the differences in follow up for each group. The assessments at each encounter are discussed later in this chapter.

Figure 3.1 - Location of Discovery clinics for PD and control recruitment across the Thames Valley UK.

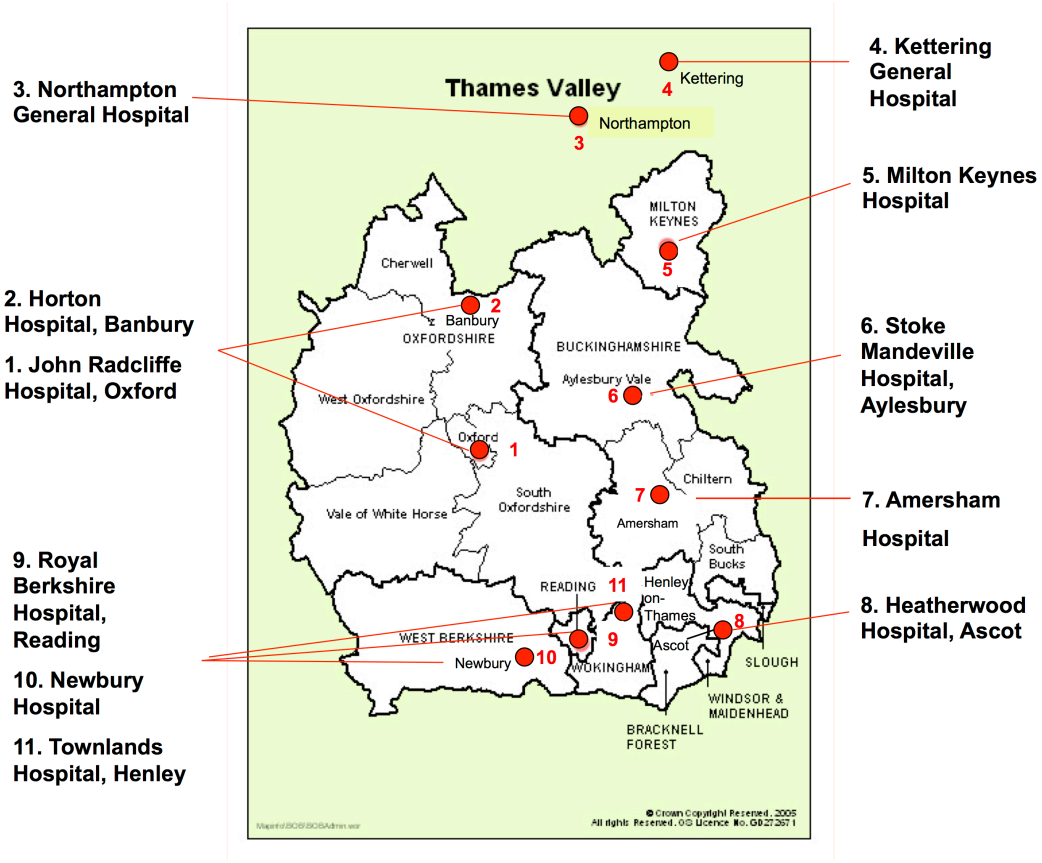
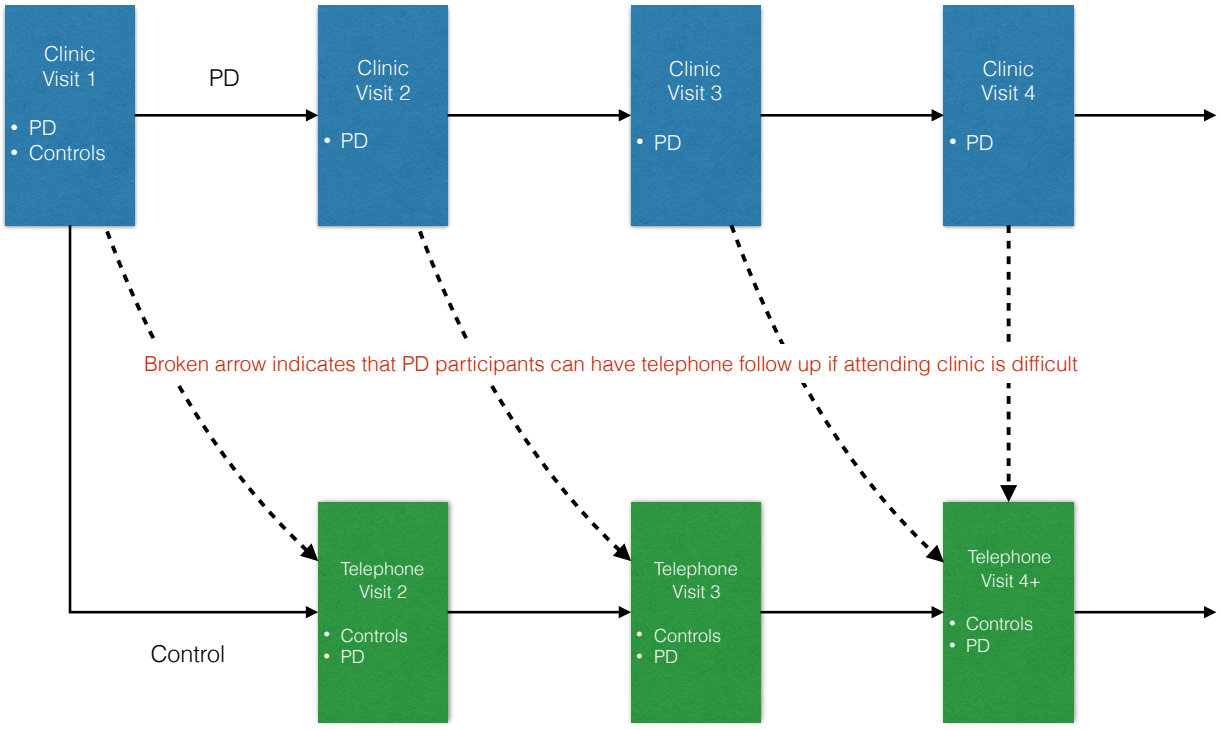


Figure 3.2 – Patient pathway following recruitment



3.1.1 INCLUSION AND EXCLUSION CRITERIA

3.1.1.1 All groups:

Inclusion criteria for all groups were:

- Aged > 18 years inclusive.
- Fluent in English.

Exclusion criteria for all groups were:

- Significant co-morbidity (medical or psychiatric illness) that would interfere with completing initial and follow-up assessments.
- A lack of capacity to make informed decisions on research participation, including dementia or psychosis.
- Active pregnancy.

3.1.1.2 Parkinson's disease group:

PD cases were recruited from neurology and elderly care clinics in participating hospitals. Neurologists, PD specialist nurses, geriatricians and GP's from participating hospitals were asked to identify and refer all cases within 3 years of diagnosis. Eligible patients could also self-refer for recruitment through registration on the OPDC website. Additional support from the Dementia and Neurodegenerative Diseases Network (DeNDRoN) helped disseminate information and encourage recruitment. All the participating clinicians were regularly contacted to ensure screening of incident cases. Eligible cases were then contacted by post to invite them to participate in the study.
All patients who were referred were pre-screened for a diagnosis of Parkinson's disease using GP and hospital correspondence where available. Each participant had their diagnosis reviewed by a movement disorders neurologist or fellow with specific training for PD assessment during their clinic visit.

Group specific inclusion and exclusion criteria:

1. All cases satisfying the UK PD Brain Bank Criteria for a diagnosis of PD were eligible for inclusion.
2. Cases with more than one relative with a diagnosis of PD, who otherwise fulfil the criteria, were still included.
3. Cases reviewed in clinic more than 3.5 years from their diagnosis were excluded (in line with other UK based studies). This allows a 6 month window from referral to the baseline clinic visit for those referred close to the 3 year referral cut-off.
4. Atypical parkinsonian features were routinely screened at each visit using the National Institute of Neurological Disorders and Stroke Parkinson's tool. The physician was then asked to rate the percentage likelihood of PD with all cases excluded from subsequent analysis if they had a <90% probability of PD at their latest clinic visit.
5. Patients who developed dementia within 12 months of the onset of their symptoms were excluded as having dementia with Lewy-body disease.

3.1.1.3 Control group:

The control population were recruited from spouses and friends of patients taking part in the study, as well as the general public through advertising. Spouses and friends were actively recruited as they may share both known and unknown risk factors for the PD cases.

Group specific inclusion and exclusion criteria:

- Control cases were clinically assessed to ensure they did not have PD
- Participants underwent an enumerated family history questionnaire to exclude first-degree relatives of patients with PD.

3.1.2 GENETIC SCREENING

All participants were screened for G2019S and R1441C gene mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene and N370S and L444P mutations in glucocerebrosidase (GBA) using methodology described elsewhere (Zokaei et al. 2014). In brief, genomic DNA was extracted from blood samples using the AutoPure LS (QIAGEN). PCR reactions were carried out with AmpliTaq Gold DNA polymerase (Applied Biosystems). Mutations were then confirmed by sequencing. The sequencing reaction was performed according to BigDye Terminator v3.1 Cycle Sequencing protocol (Applied Biosystems). Following a clean up step, the sequencing read was performed on a 3700 DNA Analyser (Applied Biosystems) sequencing platform. This process was performed by members of the Professor Wade-Martins group in the Department of Physiology, Anatomy and Genetics, Oxford University.

3.2 CLINICAL ASSESSMENTS

Basic characteristics of each participant (such as date of birth, ethnicity, social situation, medication past and present etc.) were recorded including symptoms at the start of the condition. Disability was assessed using the Schwab and England Activities of Daily Living scale (S&E ADL). Participants were asked to rate their ability to complete their ADL on an 11-point scale from 'essentially normal' to 'bed ridden'. Health related quality of life (HRQoL) was assessed with EQ-5D questionnaire. The 5 domains of mobility, self-care, usual activities, pain and mood each on a 3 point scale which is then converted into a summary index score for comparison. Socio-economic position was defined as a composite score of wealth and education. Details of the individual parameters are listed in Table 3.3.

3.2.1 MEASURES OF MOTOR FUNCTION

Motor function was assessed in all the groups using the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) II and III, the clinical global impression of change to gauge levodopa response, Hoehn and Yahr staging, Purdue peg-board test, Flamingo balance test and timed get up and go during the clinic visit.

3.2.1.1 Movement Disorder Society Unified Parkinson's Disease Rating Scale parts II and III

The MDS-UPDRS II is a self-assessed questionnaire examining the motor aspects of experiences of daily living (M-EDL). The scale uses a 5-point scale (0-4), each score relating to clinically accepted description: absent, slight, mild, moderate or severe motor symptom (this scale is the same for all 4 parts of the UPDRS). This covers a variety of functional motor activities including self-care, gait and activities. Of note, for the purposes of this study, hypersialorrhoea and dysphagia, while included in the MDS-UPDRS II, were considered as non-motor symptoms.

The MDS-UPDRS III is a motor scale based on a physical examination. The motor score part III examination includes assessments of rigidity, bradykinesia, tremor, gait and postural instability with other features (Goetz et al. 2008). This uses the same 5 point severity scale as above. All patients were asked to take their usual medication for the clinic and were examined in the 'on' state. The only exception is at the second visit where the participants were asked to withhold their medication (if they were on levodopa) for a washout period prior to clinic, and were then examined before and after taking it. PD patients were classified into three motor phenotypes (postural instability and gait difficulty (PIGD), tremor dominant (TD) and indeterminate) based on their MDS-UPDRS III motor score (Stebbins et al. 2013).

3.2.1.2 Clinical global impression of change

The clinical global impression of change (CGIC) is a 7-point scale of symptom severity or treatment response. It is rated by the clinician based on their assessment of symptoms and signs. In this study, it was used to rate the patient's response to treatment (not worsening over time) as very much worse, much worse, minimally worse, no change, minimally improved, much improved and very much improved.

3.2.1.3 Hoehn and Yahr staging

Hoehn and Yahr staging is a 5 point scale to measure the severity of PD motor symptoms: unilateral involvement only; bilateral involvement without impairment of balance; mild to moderate bilateral disease with impaired postural reflexes (needing assistance to recover from pull test); severe disability but still able to walk or stand unassisted; and wheelchair bound or bedridden unless aided (Hoehn et al. 1967).

3.2.1.4 Purdue pegboard test

The Purdue pegboard test assesses manual dexterity and bimanual co-ordination (Vingerhoets et al. 1997). It uses a flat board with two parallel rows of 25 peg holes

running longitudinally down the centre. Pins and washers are loaded into cups at the top of the board. The patient is seated a comfortable distance from the board and asked to perform 4 tasks, after being given the opportunity to practice. They are asked to enter as many pins as they can (starting with the top hole) with each hand individually, then together, for a 30 second period each. The total number of pins inserted (sum of all three scores) is recorded. They are then asked to perform a 60 second assembly task of placing the pin in the hole followed by a washer, collar and second washer in turn. The number of assembled items are counted.

3.2.1.5 Flamingo balance test

The flamingo test assesses balance by timing how long the participant can stand on one leg (Tsigilis et al. 2002). The participant stands facing some fixed point on a wall in front of them and is asked to stand on their preferred leg for up to 30 seconds (maximum duration). They are asked to keep their standing leg straight and lift their other leg off a few inches from the ground. If the participant is unable to perform the task or if the task is clearly dangerous as they are unable to stand unaided, then a score of '0' is given.

3.2.1.6 Timed get up and go test

The timed get up and go test (TUG) is a measure of functional mobility (Shumway-Cook et al. 2000). The participant is sat down resting their back and hips against the back of the chair and resting their arms on the arm rest. If needed, they are permitted to use their usual walking aid and this is recorded. The subject is then timed walking 3m toward a marker, touching the line, returning to the chair and sitting down again.

3.2.1.7 Levodopa equivalent dose

Medication use was recorded allowing the calculation of the levodopa equivalent daily dose (LEDD) (Tomlinson et al. 2010). See Table 3.1 for most common medications.

Table 3.1 – Levodopa equivalent dose table (Tomlinson et al. 2010)

Drug class	**Drug**	**Total levodopa equivalent daily dose (mg)**
Levodopa	Levodopa	100
	Controlled release levodopa	133
COMT inhibitors	Entacapone	LD × 0.33
	Tolcapone	LD × 0.5
Non ergot-derived dopamine receptor agonists	Pramipexole	1mg salt
	Ropinirole	5
	Rotigotine	3.3
Ergot-derived dopamine receptor agonists	Lisuride	1
	Bromocriptine	10
MAOB inhibitors	Selegiline 10mg (oral)	10
	Selegiline 1.25mg (sublingual)	1.25
	Rasagiline	1
Other	Amantadine	100

COMT, catechol-O-methyl transferase; MAOB, monoamine oxidase type B.

3.2.2 MEASURES OF NON-MOTOR SYMPTOMS

Validated questionnaires were used unaltered to assess non-motor symptoms (NMS) as the composition of the clinical research forms. This leads to some duplication of features in the data collected. Where there was more than one assessment for a symptom, the assessment judged to be the better test to address the hypothesis (such as being more sensitive or specific) is included. For example, both the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MOCA) assess cognition, but the MOCA is more sensitive in detecting cognitive decline in PD (Hu et al. 2014).

In this chapter, for simplicity, all the items assessed by the MDS-UPDRS (regardless of their NMS domain) are described together as they use the same rating scale.

3.2.2.1 Movement Disorder Society Unified Parkinson's Disease Rating Scale parts I and II

The following NMS were assessed by MDS-UPDRS I: hallucinations and psychosis; apathy; sleep problems; pain and other sensations; urinary problems; and fatigue. Questions from MDS-UPDRS II assessing dysphagia and hypersialorrhoea were also used as NMS. The scale was rated on a 5 point scale (0-4), each score relating to clinically accepted description: absent, slight, mild, moderate or severe motor symptom (this scale is the same for all 4 parts of the UPDRS). As the patients are relatively early in the natural history, the results were dichotomised (0 versus, ≥ 1).

3.2.2.2 Neuropsychiatric

Anxiety was assessed using the Leeds Anxiety and Depression Scale (LADS). This questionnaire uses a 4 point scale (0-3) asking 14 questions to assess mood (7 for anxiety and 7 for depression) (Snaith et al. 1976).

Depression was assessed with the Beck Depression Inventory-II. This comprises of 21 statements describing somatic and affective symptoms of depression. The participant is asked to rate each statement on a 4 point scale (0-3) (Beck et al. 1961).

Cognitive dysfunction was assessed using the MOCA and fluencies. The MOCA is a 30 point screening tool taking approximately 10 minutes to complete (Hu et al. 2014). Initially designed to assess for mild cognitive impairment, it assesses across the following cognitive domains: attention and concentration, executive function, memory, language, visuo-constructional skills, conceptual thinking, calculations and orientation. An extra point is given to individuals who have 12 years or fewer of formal education for a possible maximum of 30 points.

Visuo-spatial tasks included cube copying, pentagon copying and clock copying. Clock copying was marked 0-3 as per the MOCA scoring guidance. Pentagon copying was marked dichotomously (as per the Mini-mental state examination scoring system) and also on a 3 point ordinal scale (0-2) as proposed by Ala et al. (Ala et al. 2001). Cube copying was scored as per the MOCA and also by counting the number of mistakes on the connecting angles (connection errors) and parallel lines (Maeshima et al. 1997). A maximum score of 2 was given for each, with a point lost for each mistake.

Phonemic (using the starting letters F, A and S) and semantic fluencies (using animal and boys' names) were recorded for 60 seconds for each topic. The totals were then scaled to adjust for age.

The shortened Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP-S) was used to screen for impulsive compulsive disorders (ICD). At the first visit, participants were asked if they had experienced the symptoms at anytime, while at follow up visits they were asked if they were experiencing them within the preceding 4 weeks.

3.2.2.3 Gastrointestinal

The constipation questionnaire as based on the Honolulu-Asia Ageing Study (HAAS) assesses bowel movement and laxative use over the preceding week. Constipation was determined as <1 daily bowel movement or use of laxatives (Abbott et al. 2003).

3.2.2.4 Sleep

Rapid Eye Movement Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) is a 14 point screen for RBD. A threshold of ≥6 for a positive screen was used in the early PD group and ≥5 in the non-PD groups. The use of differing thresholds accounts for all the PD group scoring a point for the presence of a neurological disorder.

Daytime somnolence was assessed with the Epworth Sleepiness Scale (ESS). This asks the participant to rate on a 4 point scale of 0-3 how likely they would be to doze off or fall asleep in 8 situations.

3.2.2.5 Autonomic

Orthostatic hypotension was used as a measure of autonomic dysfunction. Blood pressure was measured (using a BP machine) after the patient lies flat for 3 minutes then again after standing upright for 2 minutes. Any unexpected results were re-checked with a manual BP cuff. A systolic drop of blood pressure (BP) of ≥20mmHg or a diastolic drop of ≥10mmHg were deemed positive (lying then standing blood pressure after 2 minutes).

3.2.2.6 Sensory

Pain was assessed with the EQ5D (Question 5). This is a 3 point scale of no, moderate or extreme pain or discomfort. Moderate or extreme pain were deemed positive (EuroQol 1990).

Hyposmia was assessed with the 16-item Sniffin stick test (See Figure 3.3). For each scent, participants were required to identify one of 4 choices which they thought was

most likely to be correct. They were asked to make a choice regardless of whether they could identify the smell or not. The scores were dichotomised at 10th percentile according to normal values for age and sex (Hummel et al. 2007).

Example of a typical question includes:

1

• ORANGE	☐	• STRAWBERRY	☐
• BLACKBERRY	☐	• PINEAPPLE	☐

Figure 3.3 – Sniffin sticks



3.2.2.7 Sexual function

The National Institute of Neurological Disorders and Stroke Parkinson's tool (NINDS) was used to assess the presence or absence of sexual dysfunction (in either gender) and erectile dysfunction.

3.2.2.8 Personality traits and addictive behaviours

Personality was assessed using the Big Five Inventory (BFI-44) (John et al. 2008). This is a self-rated questionnaire that uses a 5-point scale to rate 44 short phrases of character to evaluate the five factor model of personality: extraversion; neuroticism; agreeableness; openness; and conscientiousness (Soto et al. 2009). Each characteristic is then scored with the sum total of each personality domain used as the measure. Table 3.2 describes the big five in terms of their correlated adjectives as described by the revised NEO personality inventory (Costa et al. 1992, John et al. 2008). Despite the relative brevity of this measure, it has demonstrated good internal consistency and retest reliability and has been validated against longer Big Five measures (John et al. 2008). The addictive behaviours assessed were smoking, alcohol and caffeine use, each scored using the Mini Environmental Risk questionnaire for PD (MERQ-PD). Current and past use for each was assessed, with consumption before the diagnosis of PD used for pre-morbid assessment.

Table 3.2 - This table compares the Big Five dimensions personality used in this study with the facets of personality as described by the revised NEO personality

Big Five Dimensions	**Facet (and correlated trait adjective)**
Extraversion vs. introversion	Gregariousness (sociable) Assertiveness (forceful) Activity (energetic) Excitement-seeking (adventurous) Positive emotions (enthusiastic) Warmth (outgoing)
Agreeableness vs. antagonism	Trust (forgiving) Straightforwardness (not demanding) Altruism (warm) Compliance (not stubborn) Modesty (not show-off) Tender-mindedness (sympathetic)
Conscientiousness vs. lack of direction	Competence (efficient) Order (organized) Dutifulness (not careless) Achievement striving (thorough) Self-discipline (not lazy) Deliberation (not impulsive)
Neuroticism vs. emotional stability	Anxiety (tense) Angry hostility (irritable) Depression (not contented) Self-consciousness (shy) Impulsiveness (moody) Vulnerability (not self-confident)
Openness vs. closedness to experience	Ideas (curious) Fantasy (imaginative) Aesthetics (artistic) Actions (wide interests) Feelings (excitable) Values (unconventional)

Table 3.3 – Summary table of parameters

DOMAIN	SCALE / CLINICAL ASSESSMENT
Motor symptoms	
Movement Disorder Society-revised Unified Parkinson's Disease Rating Scale Parts II & III (Goetz et al. 2008)	The scale was rated on a 5 point scale (0-4), each score relating to clinically accepted description: absent, slight, mild, moderate or severe motor symptom (this scale is the same for all 4 parts of the UPDRS). Questions from MDS-UPDRS II assessing dysphagia and hypersialorrhoea were considered to be non-motor symptoms. As the patients are relatively early in the natural history, the results were dichotomised (0 versus, ≥ 1) where needed for analysis.
Hoehn and Yahr staging (Hoehn et al. 1967)	Hoehn and Yahr staging is a 5 point scale to measure the severity of PD motor symptoms: unilateral involvement only; bilateral involvement without impairment of balance; mild to moderate bilateral disease with impaired postural reflexes (needing assistance to recover from pull test); severe disability but still able to walk or stand unassisted; and wheelchair bound or bedridden unless aided.
Purdue Peg Board test (manual dexterity) (Vingerhoets et al. 1997)	The total number of pegs inserted into the board using each hand individually and then both hands over a total of 90s. This was followed by an assembly task. The data was stratified into quintiles for comparison, with the worst performing quintile considered poor performers.
Flamingo balance test (Tsigilis et al. 2002)	The participant is timed on their ability to stand on one leg for up to 30s. Where the patient was unable to perform the task, they were given a score of 0. The data was stratified into quintiles for comparison, with the worst performing quintile considered poor performers.
Timed get up and go test (Shumway-Cook et al. 2000)	The participant is timed walking 6m from and back to a seated position in a chair. This test was dichotomised at the 90^{th} percentile (including those who were unable to perform this task).
Non-motor symptoms (NMS)	
Movement Disorder Society-revised Unified Parkinson's Disease Rating Scale Parts I & II	The following NMS were assessed by MDS-UPDRS I: hallucinations and psychosis; apathy; sleep problems; pain and other sensations; urinary problems; and fatigue. Questions from MDS-UPDRS II assessing dysphagia and hypersialorrhoea were also used as NMS. The scale was rated on a 5 point scale (0-4), each score relating to clinically accepted description: absent, slight, mild, moderate or severe motor symptom (this scale is the same for all 4 parts of the UPDRS). As the patients are relatively early in the natural history, the results were dichotomised (0 versus, ≥ 1) where needed for analysis.

DOMAIN	SCALE / CLINICAL ASSESSMENT
Neuropsychiatric	
Depression and anxiety assessment	Anxiety was assessed using the Leeds Anxiety and Depression Scale (LADS). This questionnaire uses a 4 point scale (0-3) asking 14 questions to assess mood (7 for anxiety and 7 for depression). A threshold of >7 scores positive in either anxiety or depression where needed for analysis (Snaith et al. 1976). Beck Depression Inventory II: using scores of 0-13 to denote minimal depression, scores of 14-19 to denote mild depression, scores of 20-28 denote moderate depression, and scores of 29-63 to denote severe depression (Beck et al. 1961).
Cognition	Montreal Cognitive Assessment tool (MOCA): using screening cut-off scores of 22-23 to denote possible mild cognitive impairment (MCI) and <22 for possible dementia (Hu et al. 2014). Semantic and phenomic fluencies were dichotomised at the 20th percentile to determine poor performers.
Impulse control behaviours	The shortened Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP-S): using a positive response in any category as a positive screen for impulse control behaviours (Weintraub et al. 2009).
Gastrointestinal	
Constipation symptoms	The constipation questionnaire as based on the Honolulu-Asia Ageing Study: <1 daily bowel movement or use of laxatives were deemed constipated (Abbott et al. 2003).
Sleep	
REM sleep behaviour disorder	Rapid Eye Movement Sleep Behaviour Disorder Screening Questionnaire (RBDSQ): using a threshold of ≥6 for a positive screen in the early PD group and ≥5 in the relatives and control groups. The use of differing thresholds accounts for all the PD group scoring a point for the presence of a neurological disorder (Nomura et al. 2011).
Daytime somnolence symptom	Epworth Sleepiness Scale (ESS): using a threshold of ≥10 as a positive screen (Johns 1991).
Autonomic	
Orthostatic hypotension assessment	Blood pressure was measured after the patient lies flat for 3 minutes then again after standing upright for 2 minutes: a systolic drop of blood pressure (BP) of ≥20mmHg or a diastolic drop of ≥10mmHg were deemed positive (Freeman et al. 2011).
Sensory	
Pain	EQ5D: Question 5: A 3 point scale of no, moderate or extreme pain or discomfort. Moderate or extreme pain were deemed positive (EuroQol 1990).
Hyposmia assessment	Sniffin sticks odour identification test dichotomised at 10th percentile according to normal values for age and sex (Hummel et al. 2007): Males and females age 16-≤36: 11 or lower; Males aged >36-≤55: 12 or lower; females aged >36-≤55: 11 or lower; Males and females age >55: 9 or lower.

DOMAIN	SCALE / CLINICAL ASSESSMENT
Sexual Dysfunction	
Sexual dysfunction	Questionnaire from PD-DOC study / NIH common data elements: present or absent.
Erectile dysfunction	Questionnaire from PD-DOC study / NIH common data elements: rated very poor, poor, fair, good or very good. Very poor and poor were considered positive for erectile dysfunction.
Total NMS Score	
Total NMS score – PD group	The sum total of 18 NMS domains assessed were used, excluding erectile dysfunction as gender specific: cognition; hallucinations and psychosis; apathy; fatigue; anxiety; depression; ICB; orthostatic hypotension; urinary dysfunction; saliva and drooling; chewing and swallowing; constipation; pain; hyposmia; sleep problems; daytime somnolence; RBD; and sexual dysfunction. Where a single item was missing from a case, that symptom was imputed as negative and the case included in the total score.
Addictive behaviours	
Mini Environmental Risk Questionnaire for PD Baseline (MERQ-PD B)	Assessment for exposure to commonly accepted risk factors for PD was used to assess current caffeine, alcohol and smoking consumption, while premorbid consumption was also assessed as prior to PD diagnosis or in the past if no diagnosis of PD.[12] For total exposure total pack years of smoking were split into 3 categories of never smoked; 1-10 pack years history; and ≥10 pack years history. The total units of alcohol consumed in a week was calculated and split into 3 categories: 0 units per week; 1-14 units per week; 15+ units per week (based on UK alcohol guidelines). The number of caffeine beverages consumed in a day for each individual was calculated and split into 3 categories: 0-3 cups per day, 4-5 cups per day and 6 or more cups per day (to represent no or minimal consumption, moderate and heavier consumption).
Activities of daily living	
Schwab and England ADL scale	Participants are asked to rate their ability to manage their ADL on an 11 point scale from essentially normal to a bedridden state.
Quality of life	
EQ-5D-3L	The 5 domains of mobility, self-care, usual activities, pain and mood each on a 3 point scale which is then converted into a summary index score for comparison.(EuroQol 1990) The summary index was then stratified into quintiles for analysis.
Socio-economic position	
Years in education	Total years in education including school and higher education whether full or part-time. For inclusion in analysis, this was stratified into quintiles due to non-normality.
Accommodation owned at diagnosis	Dichotomised to 'accommodation rented' scored as 0 versus 'accommodation owned or have a mortgage' scored as 1.
More than 3 Bedrooms in accommodation at diagnosis	Dichotomised to '≤3' scored as 0 versus '>3' scored as 1.
Vehicles owned at diagnosis	Dichotomised to '≤1' scored as 0 versus '>1' scored as 1.

3.3 MEASURES OF PROGRESSION

Defining progression is complex (see chapter 1 for discussion), but while there is no 'gold standard' method, the most commonly used parameters are motor impairment and cognitive decline. Functional disability, a clinically relevant patient-centered outcome, increases with disease duration and can reflect the patient's disease state and individual circumstances. Thus, for the purposes of this study, the following measures were chosen:

1. **Motor impairment: UPDRS III motor score.**

   This is the most commonly used scale for motor impairment and comprehensively covers the breadth of PD associated motor features.

2. **Cognitive impairment: Montreal cognitive assessment.**

   While a global score of cognition lacks the specificity of in depth psychological assessment, the MOCA has been validated for use in PD and remains sensitive to change over time. While MMSE is more commonly used, the MOCA is thought more sensitive.

3. **Functional disability: Schwab and England activities of daily living scale.**

   This 11 point scale clearly defines the level of dependency which is a potential advantage over the UPDRS II, although this is used more commonly.

3.4 METHODS FOR SERUM BIOMARKER ANALYSIS

Venepuncture was performed at the clinic visit using BD Vacutainer SSTII tubes. 5ml of blood was collected in each tube, inverted 5 times (5 complete turns of the wrist, 180 degrees and back) and let to stand for 10 minutes at room temperature for the clot to form. The samples were then spun in a centrifuge for 10 minutes at 3700 RPM/1300g in a fixed angle centrifuge Hettich EBA 20 Centrifuge. The serum was then pipetted into cryovials using the plastic Pasteur pipette and stored on dry ice for transport to the -80^{O}C freezer. These tubes were then frozen at -80^{O}C for long term storage, prior to analysis. Analysis was performed by Greg Touslon, under the Supervision of Dr Reza Morovat, in the Oxford University Hospitals Foundation Trust laboratories. A brief description of the techniques is outlined below.

3.4.1 ANALYSIS OF SERUM 25-(OH) VITAMIN D

Serum 25(OH)-VitD was measured using a one-step immunoassay (Abbott diagnostics, Illinois USA) using a routine automated immunochemistry analyser, the Abbott Architect i2000. The methodology used was as recommended by the manufacturer's instructions (Abbott Architect system kit inset Ref 3L52 49-8941/R02) and utilised the reagents provided by the manufacturer.

Calibration of the assay was performed in line with the manufacturer's instructions and assay performance was assessed using three levels of serum matrix matched quality control material (Technopath, Ballina, Ireland). All quality control values were within 2 standard deviation from the laboratory running mean and deemed acceptable prior to samples being run.

3.4.2 ANALYSIS OF SERUM URIC ACID

Serum uric acid was measured using a two-part enzymatic assay (Abbott diagnostics, Illinois USA) using a routine automated general chemistry analyser, the Abbot Architect C16000. The methodology used was as recommended by the manufacturer's instructions (Abbott Architect system kit inset Ref 3P39 304647/R02) and uses reagents provided by the manufacturer. The assay utilises a two-part reaction whereby uric acid is first oxidised, producing hydrogen peroxide, which then converts the reagent into quinoneimine dye, which can then be measured as a change in light absorbance at 604nm.

Calibration of the assay was performed using a two-point calibration in line with the manufacturer's instructions. Assay performance was assessed using two levels of serum matrix matched quality control material (Technopath, Ballina, Ireland*)*. All quality control values were within 1 standard deviation from the laboratory running mean and deemed acceptable prior to samples being run.

3.4.3 ANALYSIS OF SERUM C-REACTIVE PROTEIN AND APOLIPOPROTEIN-A1

Serum CRP was measured using an immunotubidometric assay (Abbott diagnostics, Illinois USA) using a routine automated general chemistry analyser, the Abbot Architect C16000. The methodology used was as recommended by the manufacturer's instructions (CRP Abbott Architect system kit inset Ref8G65-21 30-4143/R1; ApoA1 Ref 9D92-21 3-4624/R02) and used reagents provided by the manufacturer. An optical sample blank measurement is then performed by the analyser prior to the addition of anti-human CRP. This excess of antibody with sample substrate present causes an increase in sample turbidity due to the formation of insoluble immune complexes. The degree of immunocomplex formation is proportional to the concentration of sample CRP, measured via a reduction in light transmission.

Calibration of the assay was performed in line with the manufacturer's instructions. Assay performance was assessed using two levels of serum matrix matched quality control material (Technopath, Ballina, Ireland*)*. All quality control values for CRP were within 1 standard deviation from the laboratory running mean and deemed acceptable prior to samples being run.

Assay performance for ApoA1 was assessed using two levels of serum matrix matched quality control material obtained from Technopath. While this assay is intended for diagnostic use, it is not currently deployed as a routine hospital laboratory test. As such, a limited verification of the ApoA1 assay was performed to ascertain if the assay performance was similar to that reported by the manufacturer. Due to limited reagent availability, this was limited to gauging assay linearity, inter-batch precision and interference due to haemolysis. The ELISA used to measure ApoA1 is reliable for relative quantitation, rather than absolute quantitation, permitting the comparison of sample values within a run, but allowing less confidence between runs.

3.4.4 EVALUATION OF SERUM EPIDERMAL GROWTH FACTOR ASSAY AND PLATELET COUNT DETERMINATION

The simple step™ EGF ELISA used was obtained from Abcam (Cambridge, UK). All reagents used were provided by the supplier and used according to the methodology outlined in the kit insert (Abcam ab179888). Each well of a 96 well plate was coated with an affinity-tag labelled immobilization antibody which binds a capture antibody. An antibody cocktail is added to sample, containing a capture antibody and a HRP-conjugated detector antibody. Both the capture and detection antibodies bind sample EGF (at different sites) and the resulting immunocomplex is bound to the well surface via the immobilization antibody. Following incubation, wells were washed, substrate solution was added and following a second incubation period a stop solution was added

to each well. Following a short 5 minute room temperature incubation the optical density was measured using a plate reader.

3.5 METHODS TO DETECT PHENOTYPIC HETEROGENEITY

3.5.1 EMPIRICAL SUB-CLASSIFICATION

The most commonly used empirically defined subtypes are by either motor phenotype or by age. The motor phenotype can be divided into tremor dominant and non-tremor dominant (often postural instability gait disorder (PIGD) or akinetic-rigid types) sub-groups, while age is divided into early versus late onset (usually using ages 40 or 50 as the arbitrary cut-off point).

Dividing sub-groups by the ratio of tremor compared to rigidity, bradykinesia and gait disturbance utilises one of the most visibly obvious and easily stratified characteristics. They have been investigated either at onset or as the predominant symptom as the disease progresses. The most widely used method of subgrouping has been a calculation derived from the UPDRS, whereby the sum of the 'tremor' items are divided by the 'PIGD' items creating a ratio which classifies patients into ≤1.0 as PIGD; 1.0-1.5 as indeterminate; and >1.5 as tremor dominant (Stebbins et al. 2013). Alternative methods for dividing patients into tremulous, akinetic-rigid and mixed groups have used expert opinion or variations in ratios of 'tremor' scores and 'akinetic-rigid' scores (Marras et al. 2013). For empirical classification in this study, the formulas developed by Stebbins et al. were used for comparison of non-motor symptoms and for predicting motor and cognitive decline.

3.5.2 DATA DRIVEN METHODS OF SUB-CLASSIFICATION

Clustering techniques use algorithms to divide data into groups based on the similarity of their characteristics. These allow for the classification of subgroups of patients without

knowledge of the different causes and types of disease. However, the outcomes of the group characteristics depend heavily on the variables used for the analysis. The approach can be supervised, semi-supervised and unsupervised, varying in the level of assumptions or knowledge prior to analysis. The unsupervised approach in this context is the most useful as it allows the discovery of new patterns in the data without an a priori hypothesis.

In subtyping PD, K-means cluster analysis has been the most common technique used, although alternatives such as more sophisticated techniques such as Bayesian probabilistic modeling have been used (Raykov et al. 2016). A K-means cluster analysis was performed on the Discovery cohort, lead by Michael Lawton on behalf of the research group (Lawton et al. 2015).

K-means cluster analysis divides the data set into a defined number of partitions (k number of clusters) containing randomly chosen patients, and the centres of the clusters were computed based on the values of the patients. As the variables have different scales, the variables were standardised first. The cluster centre was calculated by then minimizing the squared distance of each mean to its' centre. As each sample is assigned to the nearest cluster centre, a new partition is generated allowing for the re-calculation of the cluster centre. This iterative process is repeated for the further samples until the optimal value of the cluster centre is determined or the cluster membership stabilizes. The major drawback of this algorithm is that it is sensitive to the selection of the initial partition. The risk is that it may converge to a locally optimal solution which is not representative of the sample population. To counteract this, a number of alternative starting points are used.

3.6 STATISTICAL ANALYSIS

3.6.1 REGRESSION MODELS

Regression models can be used to assess the relationship between multiple independent variables and an outcome / dependent variable. In this study, logistic regression is most commonly used, with linear regression employed where appropriate.

Multivariable linear regression requires a number of key assumptions which are not required in logistic regression: a linear relationship between the dependent variable and the independent variables; the independent variables are not highly correlated with each other (multi-collinearity); the variables are normally distributed; and the variance in error terms is are similar across the independent variables (homoscedasticity).

Binomial logistic regression (referred to as logistic regression) does not require all these assumptions and is used to model dichotomous outcomes, but multi-collinearity can cause problems with the model estimation. Ordinal logistic regression can be used to model ordered categorical outcomes, but requires the additional assumption of proportional odds, that each independent variable has an identical effect at each cumulative split of the ordinal dependent variable. Where ordinal logistic regression was used, the Wald test of proportional odds was used to test for the proportional odds assumption.

The outcomes of the logistic regression and ordinal logistic models were reported as odds ratios. In logistic regression, the interpretation of the odds ratio refers to the relative change in the odds of the outcome in response to a unit increase in the independent variable. In ordinal logistic regression, the interpretation of the odds ratio refers to the relative change in the odds for a one category change in the outcome, assuming the same effect across all categories, in response to a unit increase in the independent variable.

Although a threshold of 0.05 was considered the level of significance, due to multiple testing, p-values between 0.05-0.001 were interpreted with caution as they may reflect a type I error.

3.6.2 MIXED-EFFECTS LINEAR REGRESSION

Linear mixed-effects models were used to analyse longitudinal data in this study. By the time of the data download, participants had attended up to a maximum of 4 follow-up appointments. Thus there were insufficient data points to determine non-linear relationships, so a linear relationship was assumed. Particular benefits of this approach include that it allows for time to be continuous (participants were recruited and re-attended at different times) and the number of follow-up appointments to differ. This model also allows for generalisations for non-normal data.

The use of random intercepts and random slopes allows one to account for both fixed effects (the population average) and random effects (individual specific deviations for the average intercept and slope). The models were centred on mean age at diagnosis to make the intercept more interpretable for each model. Thus the interpretation of the intercept reflects the value of the outcome variable at the mean age of diagnosis with a disease duration of zero. The interpretation of the slope co-efficient is the rate of change over time, i.e. the predicted change in the value of the outcome variable for an increase in the disease duration of one year.

If one has time to event data (e.g. date of death) then one can use survival analysis regression techniques such as Cox's proportional hazard models. However as the date of symptom onset is unclear by the available method collection at the time of analysis (the dates of dementia are being collected), this was not felt to add more information.

3.6.3 POWER CALCULATIONS FOR BIOCHEMICAL ANALYSIS

Allowing for an estimated incidence rate of dementia of 10% at a mean disease duration since diagnosis of 3.5 years (Williams-Gray et al. 2007) and an overall prevalence of dementia of between 24-31% (Aarsland et al. 2010), it would be expected that by 3 years of follow-up (disease duration 3-6 years) that between 80-120 of the sample of 800 PD patients would have dementia though with longer follow-up this should increase up to 220. This study was powered on the basis of predicting PDD in the PD cohort.

The biomarker was transformed into a standardised z-score. Allowing for a 10% loss to follow-up, the cohort of 800 could detect between a 0.29 to 0.35 standardised difference between the converted dementia and non-demented groups at 80% power and 5% significance depending on whether 72 or 108 cases converted in the cohort of 720 subjects.

4 BASELINE CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE DISCOVERY COHORT

This chapter describes the socio-demographic and clinical features of the cohort at the first clinical assessment, including results of the serum biochemistry analysis. The baseline clinical features, including the presence of common mutations, are compared to published incidence studies to assess its external validity. Effects of age and gender are also discussed as they may influence progression. Comparisons were made with a control group to delineate any features associated with the disease process of PD. The prevalence, impact and treatment of non-motor symptoms in early PD and relatives compared to controls has already been published in a separate study (Baig et al. 2015).

4.1 RECRUITMENT

Recruitment for the Discovery project is ongoing. The final dataset used for this thesis was downloaded on 16/06/2016. Baseline clinical data from 1,240 participants (945 PD and 295 controls) were available for analysis. In the study investigating biochemical predictors of PD progression, some serum samples were not available for the biochemical analysis and thus were not included (see Chapter 4.7).

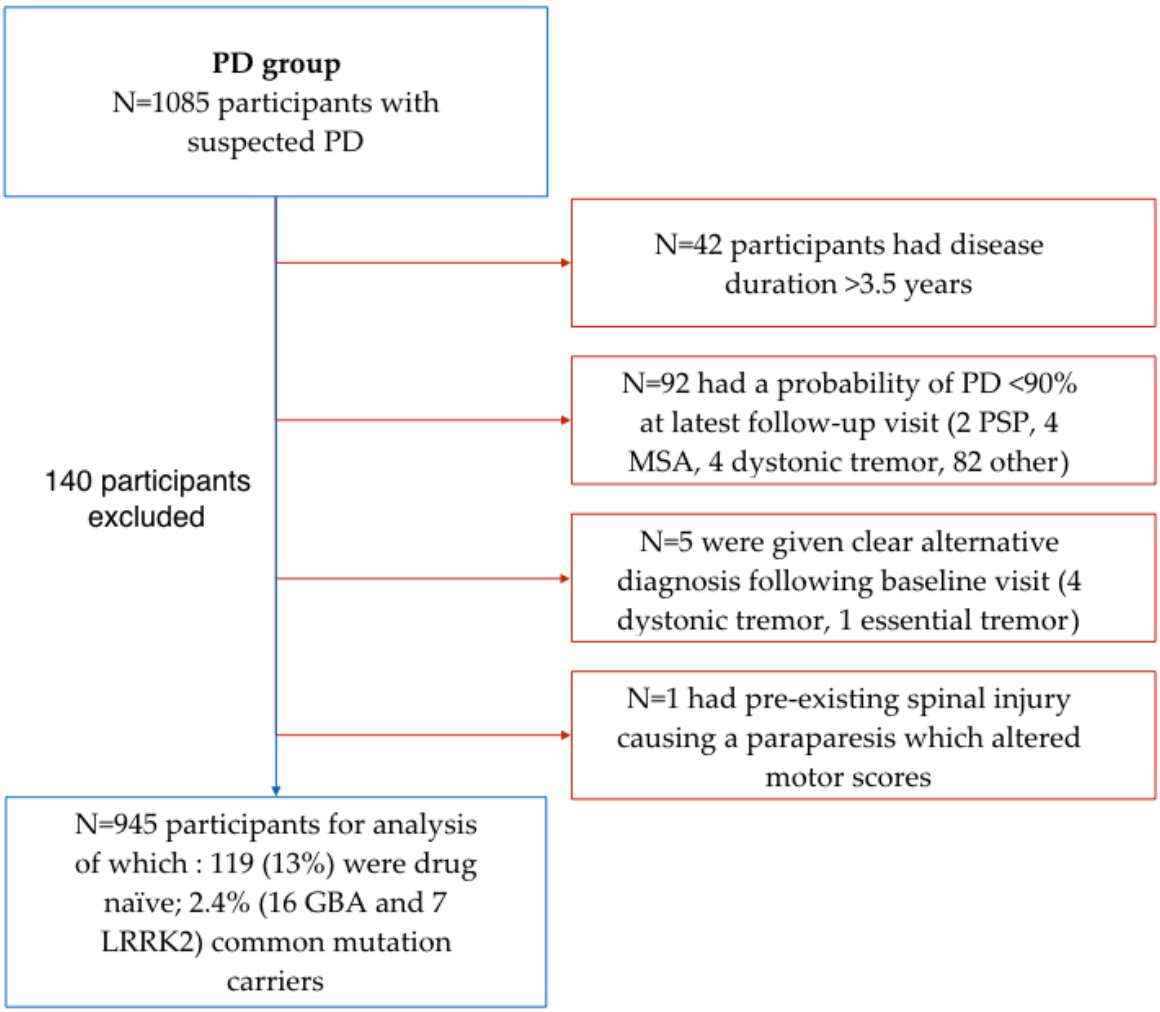
Figure 4.1 shows the included and excluded participants in the PD group. Based on the estimated 2,300-3,200 new or recent onset PD cases in the study region during the recruitment period (see chapter 3.1), 1,085 referred suspected PD cases are likely between 34% to 47% of the total patient population. Even as a rough estimate, this is a relatively large sample of the possible total population but may under-represent certain types of patients e.g. elderly frail patients, ethnic minorities.

4.1.1 EXCLUDED CASES

By 16/06/2016, 140 participants had been excluded from the PD group (see Figure 4.1 for reasons). The 82 cases which were excluded as not being typical for PD (without a clear alternative diagnosis) are continuing under review. These cases may include those with suspected vascular or drug induced parkinsonism, alternative tremor disorders and atypical parkinsonian disorders, but a clear diagnosis has not yet been reached. Those that have been withdrawn will have their clinical records checked to determine for any alternative diagnoses, but this is not available at the time of writing. Each participant consented to their clinical data being retrieved from health systems, including their death certificate, in future. In the PD group, 120 participants had confirmation of their diagnosis with DAT imaging in addition to their clinical diagnosis.

All controls who attended the clinic were included. The presence of parkinsonism or first degree relatives with PD were excluded by history and examination.

Figure 4.1 – Flowchart of participants included in the PD group



4.1.2 MISSING DATA

There was no missing data for age, gender and diagnosis date. Symptoms recorded by the clinician at diagnosis was missing in up to 31% of cases, most often due to missing clinical letters at regional sites. Missing data from the clinical assessments was generally small at less than 3% for each variable, except for BDI (5.8%), QUIP (4.6%), and RBDSQ (4.8%) which each had at least one data point missing. The number of subjects with complete data for the different analyses were all greater than 80% of the total sample.

4.2 SOCIO-DEMOGRAPHIC, DIAGNOSTIC AND RISK FACTORS AT BASELINE

4.2.1 AGE, GENDER, ETHNICITY AND SOCIO-ECONOMIC POSITION

Table 4.1 - Demographics

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (OR, 95% CI, p-value)
Age (mean, range (SD))	67.2, 32-90 (9.5)	65.0, 28-88 (10.2)	1.14, 1.04-1.25, p=0.006[1]
Gender (female n (%))	335 (35.5)	151 (51.2)	0.54, 0.42-0.71, p<0.001[2]
Ethnicity (non-white n (%))	20 (2.1)	6 (2.1)	1.23, 0.48-3.15, p=0.66[3]
First-degree relatives with PD (n (%))	147 (5.6)	0	∞
Accommodation owned, (n (%))	860 (91.8)	277 (94.9)	0.59, 0.33-1.04, p=0.07[3]
More than 3 Bedrooms in accommodation, (n (%))	403 (44.0)	151 (52.4)	0.72, 0.55-0.94, p=0.02[3]
More than 1 vehicle owned, (n (%))	459 (50.4)	158 (55.1)	0.87, 0.66-1.16, p=0.35[3]
Number of years in formal education (median, interquartile range)	14 (11-16)	16 (12-17)	0.78, 0.71-0.86, p<0.001[3, a]

Where included, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years) in each model; [1]Logistic regression model adjusting for gender; [2]Logistic regression model adjusting for age; [3]Logistic regression model adjusting for age and gender; [a]Educational years were split into quintiles for addition to the regression model.

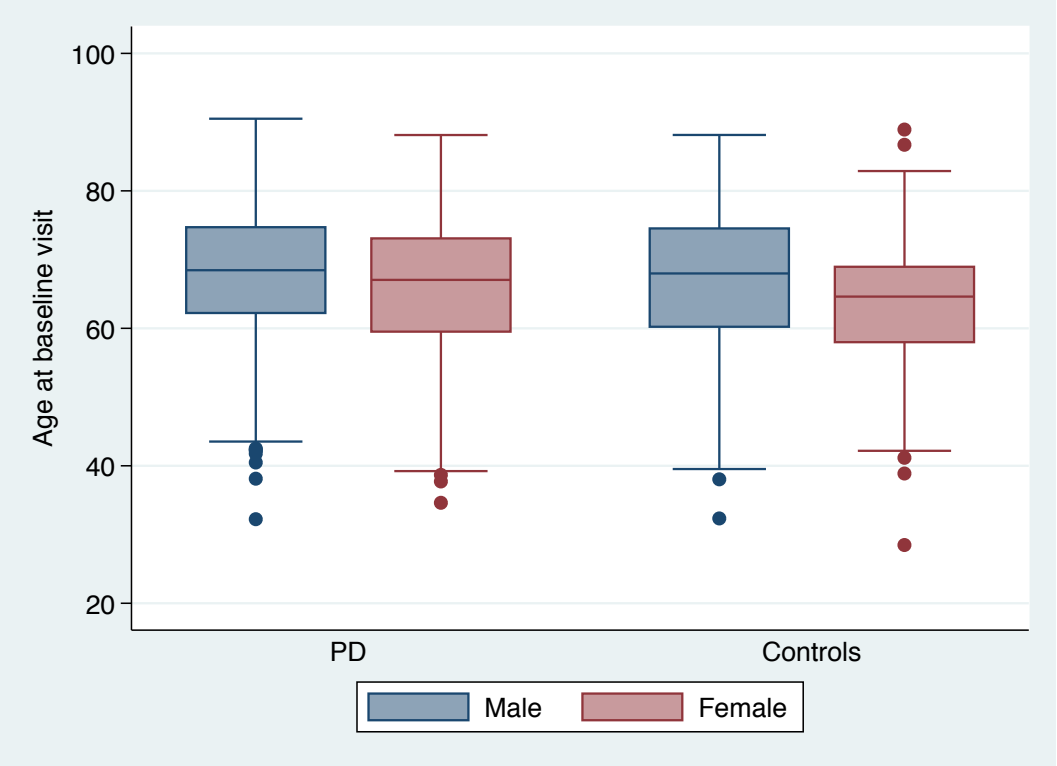
4.2.1.1 Age of recruited subjects

The mean age at diagnosis of 66.0 in the PD group is similar, but a little younger, than two published UK based incident cohorts which have ranged from 66.3 (Khoo et al. 2013) to 70.1 (Williams-Gray et al. 2009). As recruitment relied on clinician (or in a few cases self-referral), it is possible that some of the more elderly PD patients in the sampled population may have elected not to participate due to frailty or co-morbidity.

This potential case ascertainment bias could lead to recruitment of younger and less aggressive cases. Given the similarity in age with these incident cohorts, the sampled population seems reasonably representative.

The age of each group is shown graphically in Figure 4.2. The PD group was older than the control group at recruitment (p=0.006), although there was considerable overlap in the ages of the participants in each of the groups.

Figure 4.2 – Ages of the groups split by gender: the women in each group were overall slightly younger than the men, with the control group slightly younger than the cases.



4.2.1.2 Gender

The male to female ratio of approximately 2:1 in the PD group is consistent with population studies (Taylor et al. 2007). The reasons for this are not well understood, but oestrogens are thought to play a protective role, with longer lifetime exposure to oestrogens (endogenous or exogenous) associated with a reduced risk of PD and, at least initially, a milder phenotype (Picillo et al. 2017). The PD group had fewer women than the controls ($p<0.001$). The control population is more consistent with the sampled unaffected population, close to an even balance of men and women. This difference persisted despite an effort to actively recruit more male controls to match the population of cases by ceasing female control recruitment before male.

4.2.1.3 Ethnicity

The low percentage of non-white participants recruited likely reflects the local population ethnography (97% white British on the 2011 UK census) and generally low recruitment rates of ethnic minorities in health research. There were no significant differences in ethnicity between groups.

4.2.1.4 Socio-economic status

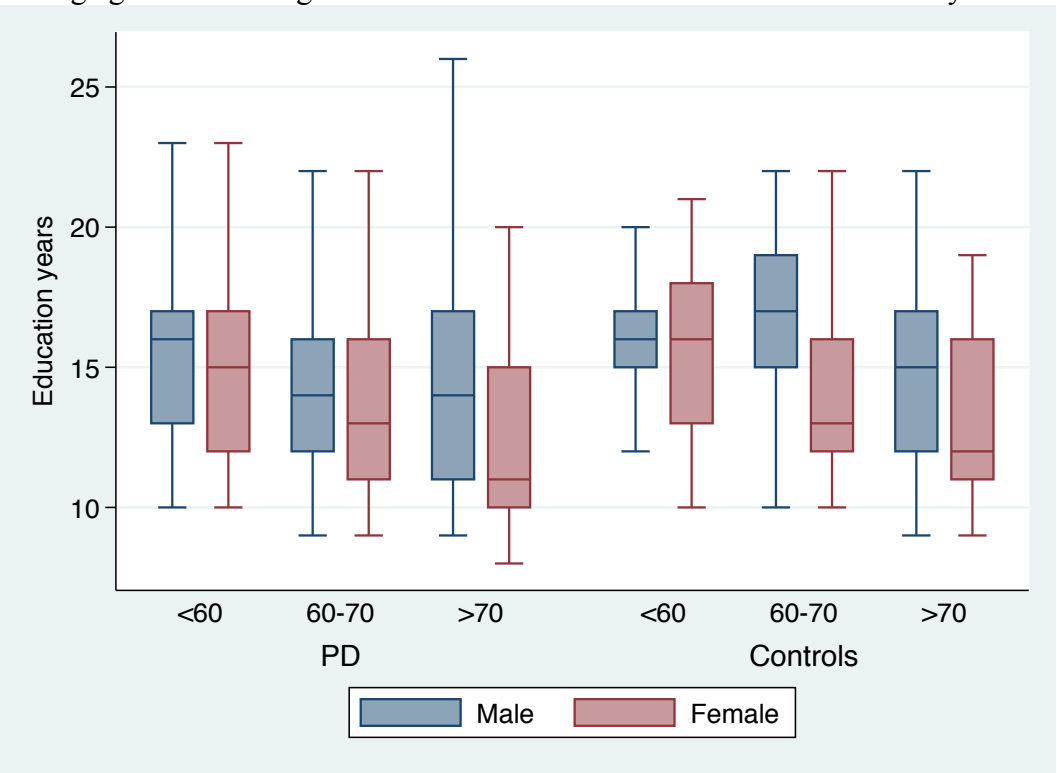
Socio-economic status was determined by a combination of proxies for wealth (type of accommodation and vehicle ownership) and education level (using years in formal education). Recruitment was predominantly from the Thames valley, a relatively wealthy region of the UK, a factor which is partly offset by regional recruitment across a range of hospitals. The sample population had higher home ownership rates than the population average (73-78% in the over 45's, English Housing Survey 2012-2013 by the Office for National Statistics). While the PD group had similar home ownership rates and vehicle ownership as the controls, they had less bedrooms ($p=0.02$). The average number of bedrooms per household in England and Wales was 2.7 (2011 UK Census), lower than

the mean number of bedrooms in the cohort (3.4) and the PD group itself (3.4). There were an average of 1.4 cars or vans per household in the South East (2011 UK Census), while the average in the cohort was 1.7.

Compulsory education in the UK is currently for ages between 5-18 years. However, from 1918 to 1944 it was 5-14, before it was extended to 15 and then to 16 in 1972. Thus, given the average year of birth in the cohort was 1950, the compulsory years of education would be 9-10 years. Across the cohort, the average years in formal education was 14, higher than the average of 12.3 in the UK (UNESCO Institute for Statistics 2013). 69.0% of the cohort population attended higher education (defined as further education after school either part or full time), although they were not asked specifically whether a qualification was achieved. This is far higher than the population average of 39% (including A-level qualifications or higher). This may reflect a highly educated local populations in an area with many skilled occupations and professions compared to few labouring or manufacturing jobs. An alternative explanation is that those with more years in higher education may be more willing to take part in research.

Access to higher education has improved substantially over time, and this is reflected in the younger participants spending more time in education (see Figure 4.3). This is particularly noticeable in the female participants, as for many years their access to higher education was restricted. The median years of education in women over 70 was 12 years, the lowest of each age and gender category.

Figure 4.3 – The number of years in formal education split by group, age and gender: increasing age and female gender were associated with less formal education years



The PD group had fewer years formal education than the control group ($p<0.001$), despite adjusting for age and gender. This is unlikely associated with the disease process and may be associated with a selection bias. Patients with PD may have more vested interest in participating in this study than control subjects, and so those with less resources, such as wealth and education may have been more motivated to participate in the study than similarly placed controls.

4.2.1.5 Summary

The age and gender ratio of the PD group is broadly reflective of a typical population, but had a higher socio-economic status than the average UK population. The comparison control group comprised of more women than the PD group and was slightly younger. The control group were more educated and wealthier, reflective of a higher socio-economic status.

4.2.2 PARKINSON'S FEATURES AT DIAGNOSIS

4.2.2.1 Age and gender differences at onset

As described in Table 4.2 and shown in Figure 4.4, the recruited female participants tended to be about 2 years younger than the men in the PD group when recruited (p=0.01). They were younger at symptom onset (p=0.01) and at diagnosis (p=0.03). While it is evident that gender is important in PD, illustrated in the gender ratio of those affected, the effects of gender on phenotype are not well understood and there is conflicting evidence in the literature. The difference found here, while significant, is small. Population studies have found either no difference or that men are affected at a younger age (Georgiev et al.). The data here suggests that older women may be less likely to volunteer than younger women, leading to them being under-represented in the over 70 age group compared to the men (31%) and being over-represented in the younger age group (42%). An alternative explanation, is that Parkinson's in women progresses more rapidly than in men, hence they present at a younger age.

4.2.2.2 Disease duration and diagnostic delay

The PD cohort were recruited within a maximum of 3.5 years of their diagnosis, with more than half seen within a year of diagnosis (median 1.0 years, IQR 0.4-2.0). Women were recruited at a similar interval as men (p=0.18). Many of the patients recruited later in their disease were at the start of the recruitment period, as the centres were initially able refer their patients who had been recently diagnosed, rather than new cases.

The average delay from reported motor symptom onset to diagnosis (1.7 years) compares favourably to published studies (ranging from 1.3 to 2.3 years, see Table 2.6). There was a trend that the diagnostic delay was longer in women than men (p=0.007), but the absolute difference was small at an average of 2.4 months. This may relate to more difficulty in clinicians making the diagnosis in women or that initial symptoms may be

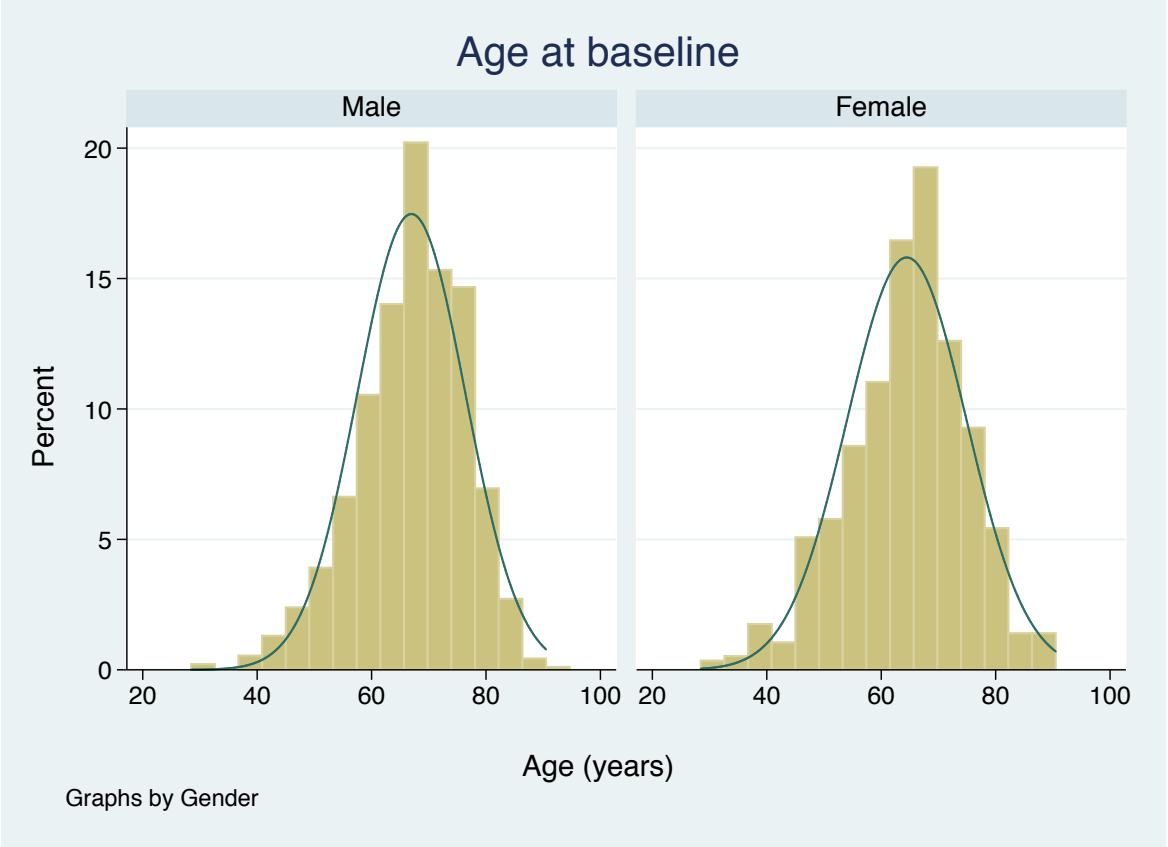
more subtle in women. The diagnosis may not have been suspected as the disease is less common in women. An alternative is that, given these data were collected retrospectively, women may over-estimate the length of their symptoms compared to men. While in some countries, women have more barriers to healthcare this is unlikely to be a problem in the UK.

Table 4.2 – Age and gender stratification of PD group demographics at baseline

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)	**Stratification by gender**		Effect of female vs male gender (OR, 95% CI, p-value)
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
Gender (n (%))								
Females	335 (35.5)	86 (42.2)	129 (36.2)	120 (31.2)	0.89, 0.81-0.97, p=0.01[1]			
Age (years) (mean (sd)):								
Age at motor symptom onset	64.3 (9.6)					65.0 (9.4)	63.0 (10.4)	0.73, 0.58-0.93, p=0.01[2]
Age at diagnosis	65.9 (9.6)					66.6 (9.2)	64.8 (10.2)	0.76, 0.60-0.97, p=0.03[2]
Age at baseline	67.2 (9.5)					67.8 (9.2)	66.1 (10.1)	0.74, 0.58-0.93, p=0.01[2]
Disease duration (years) (mean (sd))[a]:								
Disease duration at baseline since diagnosis	1.2 (0.9)	1.2 (0.9)	1.3 (1.0)	1.2 (0.9)	1.04, 0.96-1.13, p=0.32[3]	1.2 (1.0)	1.3 (0.9)	1.18, 0.93-1.49, p=0.18[4]
Disease duration at baseline since symptom onset	2.9 (1.9)	2.8 (1.8)	2.9 (1.8)	2.9 (2.0)	1.03, 0.95-1.11, p=0.51[3]	2.8 (1.8)	3.1 (2.0)	1.47, 1.16-1.87, p=0.001[4]
Delay from symptom onset to diagnosis	1.6 (1.7)	1.7 (1.7)	1.6 (1.5)	1.7 (1.8)	0.98, 0.91-1.06, p=0.67[3]	1.6 (1.6)	1.8 (1.8)	1.39, 1.09-1.76, p=0.007[4]

Where included, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [1]Logistic regression (the odds ratio is for a one category change of age from baseline); [2]Ordinal logistic regression; [3]Ordinal logistic regression model adjusting for gender; [4]Ordinal logistic regression model adjusting for age (the odds ratio is for a one category change of age from baseline); [a]Each disease duration was stratified into quintiles and entered as a 5-level ordinal variable in the regression model

Figure 4.4 – Distribution of age at diagnosis split by gender: the distribution of age at onset was similar for each gender, although women were slightly younger at onset



4.2.2.3 Symptoms reported at diagnosis

At diagnosis, unsurprisingly the most common symptom reported by the referring clinician was bradykinesia (87.2%), while patients most commonly reported a tremor at symptom onset (78.3%). This is consistent with a previous primary care database study of 55,000 subjects, which found that tremor was the most commonly reported prediagnostic symptom by patients five years before a PD diagnosis was reached (Schrag 2014). There was broadly agreement between the patient reported and clinician reported results, but a third of the information from the clinician was missing. Table 4.3 shows the information reported by the clinician, where available, as this was a contemporary record and less likely to be affected by recall bias. This would also be a clinical opinion based on patient symptoms and examination findings so will likely be more sensitive to the full range of phenomenology.

Rigidity was less frequently reported at onset when the participant was older ($p<0.001$). Whether this reflects a true difference in subtype associated with age, or an inclination to attribute stiffness to the natural ageing process is not clear. Postural instability or balance problems were more frequently associated with female gender ($p<0.001$) and possibly an association with older age ($p=0.03$). This is a feature, when found on examination, often associated with a more aggressive clinical course and early cognitive impairment. However, it is likely that patients develop symptoms before their stability is impaired to the extent that it is elicited on examination. There seemed to be an inverse association between rigidity and age ($p<0.001$), potentially consistent with reports of older patients being more likely to present with a tremor, although tremor itself did not show a clear association with age.

By the time of assessment, all included participants had developed bradykinesia. It may be that it was omitted from being specifically mentioned in the clinical record or a difference in interpretation of the term ‘parkinsonism’ by the assessor. On this basis, the

association of bradykinesia and female gender should be viewed with caution, but it may be more evident in this group.

More patients reported their symptoms on the right side (49.7%), although they may be more likely to notice symptoms in their dominant hand (87.6% of the cohort were right handed). 69.1% of participant reported their dominant hand as the side of onset. There were no significant differences between the side of symptom onset between the genders. Older age was also associated with a higher likelihood of symmetrical parkinsonism, albeit still uncommon in even the older age group (11.1%). This is an atypical feature of PD, more often associated with mimics.

4.2.2.4 Summary

The PD features at diagnosis are as expected in an early cohort, with only subtle gender differences detected although there was a higher frequency of balance problems reported in women. Older age was associated with increased symmetry and less rigidity. These results must be viewed with caution, however, as information from the reporting clinicians was incomplete.

Table 4.3 – Age and gender stratification of PD symptoms at diagnosis

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[2]
		<60	60-70	>70		Male	Female	
	n=675	n=142	n=267	n=266		n=443	n=232	
Symptoms reported by clinician at diagnosis (n (%)):								
Resting tremor or tremor of the hands at rest	532 (78.8)	117 (82.4)	196 (73.4)	219 (82.3)	1.04, 0.93-1.16, p=0.52	345 (77.9)	187 (80.6)	1.15, 0.82-1.61, p=0.43
Rigidity or stiffness of the body on movement	493 (74.6)	114 (80.3)	200 (76.1)	179 (69.9)	0.79, 0.71-0.87 p<0.001	316 (72.6)	177 (78.3)	1.33, 0.98-1.81, p=0.07
Bradykinesia or slowness of movement	579 (87.2)	119 (83.8)	237 (90.1)	223 (86.1)	0.95, 0.85-1.07, p=0.41	371 (85.1)	208 (91.2)	1.17, 0.83-1.67, p=0.37
Postural instability or balance problems	103 (15.9)	18 (13.0)	37 (14.3)	48 (19.1)	1.13, 1.01-1.27, p=0.03	53 (12.4)	50 (22.6)	1.83, 1.33-2.53, p<0.001
Side of symptom onset reported by patient (n(%)):								
Right	461 (49.7)	94 (47.2)	184 (52.4)	183 (48.5)	0.98, 0.90-1.07, p=0.66	306 (51.0)	155 (47.4)	0.86, 0.66-1.13, p=0.28
Left	393 (42.4)	100 (50.3)	141 (40.2)	152 (40.3)	0.93, 0.85-1.01, p=0.10	241 (40.2)	152 (46.5)	1.27, 0.97-1.67, p=0.09
Symmetric	73 (7.9)	5 (2.5)	26 (7.4)	42 (11.1)	1.42, 1.18-1.71, p<0.001	53 (8.8)	20 (6.1)	0.73, 0.42-1.24, p=0.23

Where included, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [1]Logistic regression model adjusting for gender (the odds ratio is for a one category change of age from baseline); [2]Logistic regression model adjusting for age

4.2.3 PARKINSON'S MEDICATION AT BASELINE

4.2.3.1 Prescribing patterns

The majority (87%) of the PD group were treated with levodopa, a dopamine agonist or an MAOB-I (see Table 4.4). Of these, 75% were on monotherapy and. the most likely treatment given was levodopa monotherapy (45% of treated patients). This is typical in early PD as, by the time of diagnosis, many patients benefit symptomatically from therapy.

Seventeen (1.8%) patients in the PD group were treated with anti-cholinergic medication, of which 9 (1.0%) were using this as monotherapy. Only 13 treated patients (1.6%) were taking catechol-O-methyl transferase inhibitors and 7 (0.9%) were prescribed amantadine.

Table 4.4 – Prescribing patterns at baseline (treated patients only)

	Monotherapy	Dopamine agonist with MAOB-I	Levodopa with MAOB-I	Levodopa with dopamine agonist	Levodopa, dopamine agonist and MAOB-I
Number of treated patients (n (%))	615 (75.2%)	47 (5.7%)	68 (8.3%)	72 (8.8%)	16 (2.0%)
Disease duration in years (median (interquartile range))	0.9 (0.4-1.7)	2.1 (1.4-2.8)	1.4 (0.7-2.1)	2.0 (1.3-2.8)	2.7 (2.2-3.0)
Levodopa equivalent daily dose (median (interquartile range))	300 (150-320)	280 (250-400)	400 (300-500)	570 (450-770)	633 (525-700)

Table 4.5 - Medication at baseline stratified by age and gender

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
Untreated PD (n(%))	127 (13.4)	27 (13.2)	59 (16.6)	41 (10.7)	0.94, 0.82-1.07, p=0.33[1]	90 (14.8)	35 (11.0)	0.74, 0.49-1.13, p=0.16[1]
Participants prescribed medication (n (%))[1]:								
Levodopa	523 (55.3)	71 (34.8)	168 (47.2)	284 (73.7)	1.69, 1.53-1.87, p<0.001[1]	342 (65.8)	181 (60.7)	1.02, 0.77-1.36, p=0.89[2]
Dopamine agonist	280 (29.6)	97 (47.6)	125 (35.1)	58 (15.1)	0.55, 0.49-0.62, p<0.001[1]	166 (31.9)	114 (38.3)	1.19, 0.86-1.63, p=0.30[2]
MAOB-I	234 (24.8)	81 (39.7)	93 (26.1)	60 (15.6)	0.67, 0.60-0.75, p<0.001[1]	156 (30.0)	78 (26.2)	0.77, 0.56-1.07, p=0.12[2]
LEDD[a] (median (IQR))	325 (193)[c]	280[c] (120-420)	300[c] (150-400)	300[c] (250-425)	1.13, 1.04-1.23, p=0.003[3]	300 (160-450)	300 (150-400)	0.90, 0.71-1.14, p=0.39[3]
CGIC[b] (treated PD group only) (n (%)):								
• Improvement	647 (81.3)	152 (87.4)	229 (79.8)	266 (79.4)	0.89, 0.78-1.01, p=0.06[4]	400 (79.2)	247 (84.9)	1.41, 0.96-2.08, p=0.08[4]
• No change	128 (16.1)	20 (11.5)	46 (16.0)	62 (18.5)		91 (18.0)	37 (12.7)	
• Worse	21 (2.6)	2 (1.2)	12 (4.2)	7 (2.1)		14 (2.8)	7 (2.4)	

Where included, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years), levodopa equivalent daily dosage and disease duration from diagnosis were analysed as quintiles; [a]Levodopa equivalent daily dosage; [b]Clinical global impression of change, CGIC was analysed as a 3 level ordinal variable with a change of 'worse' used as the base variable; [c]Treated patients only [1]Logistic regression model adjusting for gender and disease duration from diagnosis (the odds ratio is for a one category change of age from baseline); [2]Logistic regression model adjusting for age and disease duration from diagnosis; [3]Ordinal logistic regression model adjusting for gender and disease duration from diagnosis (the odds ratio is for a one category change of age from baseline); [4]Logistic regression model adjusting for age and disease duration from diagnosis

Prescribing patterns vary based on patient and clinician preference, but the use of levodopa as the most commonly prescribed drug is typical. Levodopa remains the most effective medication for the motor symptoms of PD. For some years, although refuted by the recent PD-MED study (Gray et al. 2014), there was a belief that a delay in prescribing levodopa would delay motor complications. Thus younger patients would often be started on alternatives. This treatment pattern is reflected in Table 4.5, with MAOB-I and dopamine agonists prescribed less frequently in preference for levodopa in older patients as the concern for delaying motor fluctuations is less relevant. Older patients may be prescribed higher doses, despite accounting for the duration of their disease. Prescribing patterns were similar between male and female participants. This treatment pattern is similar to a survey of prescribing patterns published by Parkinson's UK in their National Audit report 2011. In this survey, in newly diagnosed patients, levodopa was prescribed in 48%, dopamine agonists in 24% and MAOB-I in 14% (this did not include the addition of a second agent). Levodopa was the most common, with dopamine agonists and MAOB-I the second and third most common respectively. The slightly larger proportion of patients treated with each medication in this cohort likely reflects the addition of second line agents in this early rather than newly diagnosed cohort.

Most (81%) of patients who were treated experienced improvement. The significance of this is unclear. Historically the responsiveness of parkinsonism to treatment was considered practically diagnostic (94% responded in a large clinic-pathological study (Hughes et al. 1993)), data on levodopa responsiveness is limited and increasingly an awareness of 'poor responders' are recognised (Constantinescu et al. 2007). There is also difficulty in an objective measurement, as it relies on the judgement of the clinician and may be tempered by the expectations of the patient.

As expected, combination therapies were more likely in those with a longer disease duration. LEDD correlates strongly with disease duration (linear regression adjusting for age and gender, coefficient 93.0 $p<0.001$). The use of a MAOB-I in combination with the subsequent addition of a dopamine agonist or levodopa is common as this is thought by some clinicians to reduce the risk or delay the potential complications of higher dosages. Similarly, the concurrent prescription of levodopa and dopamine agonist is thought by some to have synergistic value, attempting to achieve the maximum symptom control while using lower doses of the individual drugs. Only a few patients were on triple therapy, who tended to be younger (mean age 59.4) and had a longer disease duration at baseline.

4.2.3.2 Summary

Pharmacological management of the early PD cohort appears typical of current UK practice.

4.2.4 CO-MORBIDITIES, VASCULAR AND OTHER RISK FACTORS FOR PARKINSON'S

4.2.4.1 Risk factors for PD

While the aetiology remains unclear, epidemiological studies have linked several environmental factors to an increased risk of Parkinson's (see Table 4.6). Despite numerous studies, these have often been conflicting, leading to inconclusive results. Potential associations may be complex, with an increased risk occurring only in genetically susceptible individuals, thus confounding unselected population samples. In this cohort, as the information about risk factors was collected retrospectively, there remains a risk of recall bias. In addition, the numbers are too small to delineate modest associations.

Table 4.6 – Possible risk factors for Parkinson's

Risk factors for PD	**PD** (n=945)	**Controls** (n=295)	**PD vs Controls**
	n (%)	n (%)	(OR, 95% CI, p-value)[1]
Occupational exposure to pesticides, chemical solvents or heavy metals	256 (27.3)	64 (21.7)	1.18, 0.86-1.63, p=0.31
Domestic exposure to pesticides	298 (31.6)	104 (35.3)	0.83, 0.63-1.10, p=0.20
Head injury causing loss of consciousness or concussion	184 (19.5)	53 (18.0)	1.08, 0.76-1.52, p=0.66
Current use of L-type calcium channel blocker	106 (11.2)	28 (9.5)	0.99, 0.63-1.55, p=0.97
Exposure to non-steroidal anti-inflammatory medication	643 (68.0)	200 (67.8)	1.09, 0.82-1.45, p=0.56
Exposure to oral contraceptives or hormone replacement therapy	180 (53.7)	86 (57.0)	0.92, 0.62-1.35, p=0.66[2]

[1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years) in each model; [2]Logistic regression adjusting for age, females only

A recent systematic review found that 13 of the 23 included case-control studies identified pesticides were associated with an increased risk of developing PD (Freire et al. 2012). Farming and rural residence was not associated with an increased risk. Recruitment for the Discovery cohort were not in heavily agricultural areas. Occupational exposure was similar between cases and controls. Due to the problems in quantifying pesticide exposure and a lack of standardisation in data collection, it is difficult to compare between studies. However, it is reassuring that the rates of exposure were overall similar between groups, which would be against the PD cohort comprising of individuals who had a high risk of exposure to neurotoxins.

Head injury (with or without concussion) has been associated with a modest but significant increased risk of PD in a recent meta-analysis (Noyce et al. 2012) and a large hospital based retrospective population study (Gardner et al. 2015). However, the data is conflicting. Two recent large retrospective case control studies, (based in Denmark) only found this association in the few months preceding diagnosis, thought to be a consequence of mobility problems associated with the development of PD rather than a risk factor (Rugbjerg et al. 2008, Kenborg et al. 2015).

The rates of reported head injury in this cohort were similar in both the PD cases (19.5%) and controls (18.0%). These are comparable, although slightly lower, to a US based cohort (assessed by interview with a similar question): 24.4% of PD cases and 20.6% of controls reported a previous head injury (Tanner et al. 2009).

L-type calcium channel blocker use has been suggested to be protective against or ameliorate the effects of Parkinson's (Ritz et al. 2010, Pasternak et al. 2012). There was no difference in the use of these drugs between groups in this cohort, with further sensitivity analysis (excluding amlodipine as not centrally acting) showing no differences either (although only 7 controls and 33 PD patients were taking them).

Non-steroidal anti-inflammatory drugs have been proposed to reduce the risk of developing or delay the onset of Parkinson's by suppressing an inflammatory process, but this lacks robust evidence and remains unproven (Rees et al. 2011). However, there were no differences in prior or current NSAID use across the groups, implying that this would not affect any underlying processes.

Exposure to exogenous hormone therapy with either oral contraception or hormone replacement therapy was no different in either group when considered together or separately (not shown).

The most consistent risk factor for PD is non-smoking, and possibly reduced caffeine and alcohol consumption. This data is considered in chapter 4.2.5 in more detail.

4.2.4.2 Co-morbidities

Self-reported past medical history was similar between cases and controls, with only minor variations (see Table 4.7) aside from an increased prevalence of cardiovascular or cerebrovascular disease, and angina in the PD group compared to controls (OR 1.92 and 3.87 respectively, 95% CI 1.16-3.16 and 1.66-9.03). The differences between the rates of cardiovascular and cerebrovascular disease between PD and controls is largely explained by this difference in angina between the groups. Other self-reported vascular risks or events, for example rates of hypercholesterolaemia and myocardial infarction, were similar. However, these findings relate to the patient-reported history of comorbidities, rather than direct measurement of cardiovascular risk factors, for example through serum cholesterol measurement. A recent study combining 2909 early PD patients from the Oxford Discovery and UK Tracking Parkinson's study found over 60% of PD patients had high or medium cardiovascular risk meriting statin usage, and that statins were underused in PD patients with concomitant vascular risk (Swallow et al. 2016). PD patients may be less aware of any concomitant increase in their vascular risk factors,

particularly if General Practitioners are less inclined to investigate or treat. Population studies would ideally be better placed to investigate whether a link exists.

Weight was similar between the two groups, of interest 44% of both groups were overweight (BMI >25 m^2/kg) and 24% were obese (BMI >30 m^2/kg). The lack of emphysema reflects the known inverse association of PD with smoking. There was a non-significant trend for PD patients to have a higher risk of self-reported osteoporosis, osteopaenia, or to be on bone protection than controls (2.33, p=0.06).

An intrinsic problem of clinical trials is the difficulty recruiting frailer patients with more co-morbidities, therefore artificially sampling a healthier population of both cases and controls. Using cardiovascular and cerebrovascular disease as an example, a comparison of the PD group with the expected UK prevalence of these conditions again showed only minor differences (Table 4.8). These results suggest that the Discovery sample reflects a real-world UK population rather than just a healthier subgroup.

Table 4.7 – Co-morbidities compared between PD and control group

	PD n=945	**Controls** n=295	**PD vs Controls**[1]
	n (%)	n (%)	OR, p-value
Vascular events and risks			
Cardiovascular or cerebrovascular disease	136 (14.4)	20 (6.8)	1.92, p=0.01
Angina	83 (8.8)	6 (2.0)	3.87, p=0.002
Stroke or TIA	43 (4.6)	9 (3.1)	1.30, p=0.48
Myocardial infarction	38 (4.0)	7 (2.4)	1.32, p=0.51
Heart failure	12 (1.3)	1 (0.3)	3.80, p=0.20
Hypertension	327 (34.6)	93 (31.5)	1.01, p=0.90
Hypercholesterolaemia	300 (31.8)	91 (30.9)	0.92, p=0.58
Diabetes	71 (7.5)	12 (4.1)	1.64, p=0.12
Atrial fibrillation	22 (2.3)	8 (2.7)	0.68, p=0.36
BMI (units m^2/kg)	27.3 (4.4)	27.4 (4.7)	1.00, p=0.97
Respiratory			
Asthma	100 (10.6)	26 (8.8)	1.28, p=0.29
Emphysema	6 (0.6)	5 (1.7)	0.32, p=0.07
Chronic bronchitis	14 (1.5)	4 (1.4)	1.03, p=0.96
Musculoskeletal			
Rheumatoid Arthritis	10 (1.1)	1 (0.3)	3.48, p=0.24
Gout	56 (6.1)	19 (6.4)	0.75, p=0.32
Fracture from a fall in last 3 years	11 (1.2)	7 (2.4)	0.50, p=0.19
Osteoporosis, osteopaenia or on bone protection	39 (4.1)	6 (2.0)	2.33, p=0.06
Cancer			
Bowel cancer	12 (1.3)	3 (1.0)	1.00, p=1.00
Breast cancer	15 (1.6)	7 (2.4)	0.95, p=0.91[2]
Lung cancer	5 (0.5)	0	∞
Prostate cancer	34 (3.6)	6 (2.0)	1.21, p=0.68[3]
Melanoma	16 (1.7)	7 (2.4)	0.57, p=0.22

[1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years) in each model; [2]Women only; [3]Men only

Table 4.8 – Comparison of vascular events in PD group to UK population estimates (Bhatnagar et al. 2015)

		Estimated UK prevalence (%)	PD group (%, 95% CI)	Estimated UK prevalence (%)	PD group (%, 95% CI)	Estimated UK prevalence (%)	PD group (%, 95% CI)
	Ages	55-64	55-64	65-74	65-74	75+	75+
Men	Myocardial infarction	3.6	4.0, 1.5-8.6	7.1	4.3, 2.2-7.6	12.1	9.7, 5.4-15.7
	Angina	3.6	7.4, 3.7-12.8*	8.8	11.3, 7.7-15.9	17.0	16.6, 10.9-23.6
	Stroke or TIA	2.7	2.0, 0.4-5.8	6.4	7.0, 4.2-10.9	14.9	6.3, 2.9-11.5*
Women	Myocardial infarction	0.9	0, 0-4.4	2.1	2.1, 0.4-6.0	5.5	3.3, 0.4-11.5
	Angina	1.7	3.7, 0.8-10.3	4.7	4.2, 1.5-8.8	4.4	4.6, 7.1-26.6
	Stroke or TIA	2.0	1.2, 0-6.6	4.4	4.2, 1.5-8.8	12.4	10.0, 3.8-20.5

*Denotes figure when population estimate lies outside of the 95% confidence interval for the PD group.

4.2.4.3 Vascular risk – Qrisk-2 score

The QRisk-2 score predicts the risk of a myocardial infarction or stroke within 10 years, given as a percentage, validated for individuals between the ages of 25 to 84 (Hippisley-Cox et al. 2008). This study used the published algorithms to determine this vascular risk but some of the risk factors (such as renal function, postcode and family history of early onset vascular disease) were unavailable so the results should be viewed with caution. Patients with a defined cardiovascular or cerebrovascular event were excluded. Cardiovascular risk was similar between cases and controls.

Table 4.9 – Q-Risk score

	PD n=783	**Controls** n=271	**PD vs Controls**[1]
	mean (sd)	mean (sd)	OR, 95% CI, p-value
Vascular risk (QRisk-2) score	17.1 (10.9)	14.2 (10.5)	1.18, 0.93-1.50, p=0.17

[1]Logistic regression model adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

4.2.4.4 Summary

The cohort has a typical profile of co-morbidities and suspected risk factors for PD, compared to both a control population and expected population prevalence. Angina was more common in the PD than control group, despite adjusting for age and gender, but there were no differences in other self-reported vascular events (stroke, TIA and myocardial infarction) and vascular risk factors (hypertension, hyperlipidaemia, diabetes). This implies that this individual result may be a chance finding, but could also relate to under recognition and under treatment of concomitant vascular risk in the PD population.

4.2.5 ADDICTIVE BEHAVIOURS AND PERSONALITY

Table 4.10 – Group comparison of addictive behaviours and personality profile

	PD n=945	**Controls** n=295	**PD vs Controls**[1] (OR, p-value)
Smoking			
Ever smoked (n(%))	383 (40.6)	128 (43.3)	0.72, 0.54-0.96, p=0.02
Current smokers (n(%))	23 (2.5)	14 (4.8)	0.44, 0.21-0.92, p=0.03
Smoking history in pack years (median (IQR))[a]	0 (0-7.5)	0 (0-7.5)	0.81, 0.68-0.97, p=0.02
Alcohol			
Ever consumed alcohol (n(%))	744 (80.6)	249 (84.7)	0.70, 0.47-1.04, p=0.07
Prior alcohol consumption in units per week (median (IQR))[b]	6 (1-14)	7 (2-18)	0.72, 0.57-0.90, p=0.004
Current alcohol consumption in units per week (median (IQR))[b]	4 (0-10)	6 (1-14)	0.62, 0.49-0.77, p<0.001
Caffeine			
Prior caffeine use in total beverages per day (median (IQR))[c]	5 (3-6)	5 (3-6)	1.01, 0.96-1.07, p=0.74
Current caffeine use in total beverages per day (median (IQR))[c]	4 (2-6)	4 (3-6)	0.97, 0.91-1.02, p=0.23
Big Five Inventory[d]:			
Extraversion (mean(SD))	24.3 (6.7)	27.1(6.7)	0.77, 0.70-0.85, p<0.001
Neuroticism (mean(SD))	22.2 (6.6)	19.8 (6.7)	1.34, 1.20-1.49, p<0.001
Agreeableness (mean(SD))	36.8 (5.1)	36.8 (4.9)	1.04, 0.94-1.15, p=0.48
Openness (mean(SD))	34.8 (7.1)	37.2 (6.9)	0.79, 0.71-0.89, p<0.001
Conscientiousness (mean(SD))	35.8 (5.8)	36.4 (5.8)	0.96, 0.87-1.06, p=0.48

[1]Logistic regression model adjusting for age, gender and socio-economic position, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years), wealth and education were added as proxies for socio-economic position; [a]Smoking pack years were analysed as a 3 level ordinal variable (never smoked, 0-10 pack years, >10 pack years); [b]Alcohol consumption was analysed as a 3 level ordinal variable based on UK guidelines (no alcohol, 1-14 units per week, >14 units per week); [c]Caffeine use was analysed as a 3 level ordinal variable based on cups of caffeine containing beverages (0-3 cups per day, 4-5 cups, >5 cups); [d]Each personality domain was analysed as quintiles of the total domain score

4.2.5.1 Addictive behaviours

There is evidence that PD patients engage in less addictive behaviours than the general population, such as smoking, alcohol and caffeine use (Hernan et al. 2002, Zhang et al. 2014). Conversely, a significant proportion of patients can develop impulsive-compulsive behaviours (ICB) when treated with dopaminergic replacement therapies, particularly dopamine agonists, increasing the frequency of these behaviours above that of the general population (Callesen et al. 2013). The rates of these behaviours are shown in Table 4.10 to compare the patient groups using pre-morbid or current consumption levels.

PD patients were less likely to have smoked regularly (at least 1 cigarette a day for 6 months) than controls (OR 0.72, p=0.02). Looking at total cigarette consumption (pack years smoked) in smokers only, this showed similar rates of consumption if they smoked. This fits with the majority of epidemiological evidence that suggests that PD is associated with lower rates of smoking (Nicoletti et al. 2010). The differences in smoking rates are explained by less PD patients ever smoking, rather than an intensity of smoking, as has been reported (Kenborg et al. 2015). Comparing past and current smoking rates, PD patients were more likely to have given up smoking than controls, potentially a result of disease progression affecting reward sensitivity in this group (OR went from 0.72 to 0.44), although there were only very few patients who remained current smokers. A similar finding was reported in early, untreated PD although we are unable to comment on the reasons for individuals quitting smoking (Moccia et al. 2015).

Alcohol consumption was less in the PD group, both currently and pre-morbidly as has been reported (Zhang et al. 2014). There may have been a trend that PD patients were less likely to have ever drunk alcohol than controls, but this was less pronounced (OR 0.70, p=0.07). Looking at past alcohol consumption using the same model but excluding those who do not drink, the PD group consume less (OR 0.68, p=0.02). While the

inverse relationship overall between PD and smoking and alcohol is similar, the dose relationship is only seen in the alcohol consumption. This may be explained by differences in the different behaviours of smoking and drinking, deliberate misreporting or recall bias. Caffeine beverage consumption was similar across groups, possibly because this does not assess the absolute caffeine value of different drinks.
The addition of personality to these models had only a modest effect on the differences between cases and controls.

4.2.5.2 Personality

The inverse relationship of addictive behaviours and PD has been widely investigated. Less is known about the effect of PD on personality traits. Assessment of personality varies depending on the model used, however some report that patients with established PD have a profile of less novelty seeking and more harm avoidance. In this cohort, patients with early PD were more neurotic ($p<0.001$), less extraverted ($p<0.001$) and less open than controls ($p<0.001$). This is largely consistent with previous studies looking at personality in PD. While still controversial, it has been argued that reduced striatal dopaminergic signalling may cause these personality traits. The addition of cognitive and mood assessments to the model made little difference to this result, with more detail previously published in a separate study (Baig et al. 2017).

4.2.5.3 Summary

The inverse relationship between addictive behaviours and Parkinson's, well reported in the literature, is replicated here. Also, published personality differences in this group are replicated. This provides some reassurance that the Parkinson's group is representative and not a selection of a certain 'personality type' who volunteers for clinical trials. This is potentially important as regards neuropsychological assessments which are a key feature of this study.

4.3 COMMON MUTATIONS

Table 4.11 – Common mutations

	PD n=945	**Controls** n=295
Common mutations (n(%)):		
• GBA	16 (1.7)	1 (0.3)
• LRRK2	7 (0.7)	0

As expected, the number of carriers were small, with GBA being the most common identified mutation in sporadic PD (see Table 4.11). The low numbers makes it difficult to draw meaningful inferences as regards phenotype. Previous large scale studies have found slightly higher rates of GBA mutations in their cohorts (3% to 4.8%) (Neumann et al. 2009, Sidransky et al. 2009, Lesage et al. 2011). Not all known variations were screened for in this study, although N370S and L444P account for 70% of known GBA mutations associated with PD (Lesage et al. 2011). An alternative explanation is that the populations sampled for these other studies were younger and thus more likely to be a carrier (each referenced study screened participants with a mean disease onset of <60 years old). This cohort identified 1.7% (95% CI 1.0%-2.7%) which is consistent with the findings of the Campaign cohort, which identified 2.3% (95% CI 0.9%-5.0%) patients with these mutations (Winder-Rhodes et al. 2013). The mean age at diagnosis of the PD carriers was 61.2 (sd 7.6) which was younger than the rest of the PD group. Only one case in the control group screening positive (aged 57, no parkinsonism and not hyposmic). Despite the higher number of Parkinson's carriers, the few numbers affected are the likely reason for the lack of a significant difference in the comparison.

The LRRK2 mutation G2019S is the most common cause of familial Parkinson's worldwide. In this cohort no R1441C mutations were identified, while 0.7% (95% CI 0.3%-1.5%) were G2019S carriers. This is consistent with the results of the large mutli-centre study by the GEO-PD consortium (48/6995 caucasian cases (0.7%)) as the most common LRRK2 mutation (Ross et al. 2011). The small number of cases precludes

phenotypic analysis. Of the PD cases which were carrying LRRK2 mutations, 2 had relatives with PD, each with 2 affected family members. The mean age at diagnosis was 67.1 (sd 11.0), similar to the rest of the cohort.

4.3.1.1 Summary

These genetic findings strengthen the contention that this cohort is representative of an unselected ‘real world’ PD population.

4.4 NON-MOTOR SYMPTOMS

Multiple studies have shown that non-motor symptoms (NMS) are common in established PD, but less is known about the early phases. This cohort demonstrates that they are very common even in early PD. A particular strength of this study was the use of validated measures of NMS domains, rather than just recording the presence or absence of each symptom, which allows for the severity of the symptoms to be assessed. The increased frequency of NMS in PD cases is well established, the comparison here with the control group is shown in Table 4.12:

Table 4.12 – Comparison of NMS between cases and controls.

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (OR, 95% CI, p-value)[1]
Total NMS Score (median (interquartile range))[a]	2 (3-4)	1 (0-2)	2.79, 2.38-3.28, p<0.001

[1]Logistic regression model adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [a]NMS score refers to the total number of non-motor symptoms screening positive (maximum 9) including assessments for cognition, anxiety, depression, impulsivity, orthostatic hypotension, pain, hyposmia, RBD and daytime sleepiness, this score was stratified into quintiles for the analysis. Participants with more than one missing assessment were excluded.

4.4.1.1 Effect of age and gender on NMS

The PD group underwent a greater number of assessments than the control group. The frequency of NMS using the full range of clinical assessments (maximum 18 symptoms, see Table 3.3), with group comparisons detailed in Table 4.13 and
Table 4.14. Using this composite measure, all patients were found to have at least one NMS. The distribution is similar when split by age, while the modest reduction in NMS experienced by women more obvious in the age groups under 70 (see Figure 4.5). This sum score is only a reference guide as the underlying symptoms are quite broad in aetiology, the differences are at best modest and analysis of each component would be useful. In a regression model adjusting for gender and disease duration, older age was associated with more NMS symptoms (OR 1.24, $p<0.001$). Gender and disease duration were not associated with the number of NMS symptoms experienced.

The UPDRS I (non-motor symptoms) was also performed in the PD group only. Only 9 of the included PD cases (1.6% of those with a complete assessment) did not experience any non-motor symptoms on the UPDRS I (mean 8.8, SD 5.1). The same association with older age was found with the UPDRS I total (OR 1.12, $p=0.007$) as with the NMS composite score above.

Figure 4.5 – Total number of NMS in PD group split by age and gender. Total number of NMS (maximum 18) refers to the sum of positive assessments for: cognition, hallucinations and psychosis, apathy, fatigue, anxiety, depression, impulsivity, orthostatic hypotension, urinary disturbance, hypersialorrhoea, pain, hyposmia, daytime sleepiness, RBD, disturbed sleep, sexual dysfunction, swallowing and constipation: the total NMS experienced were slightly less in younger women than the other groups.

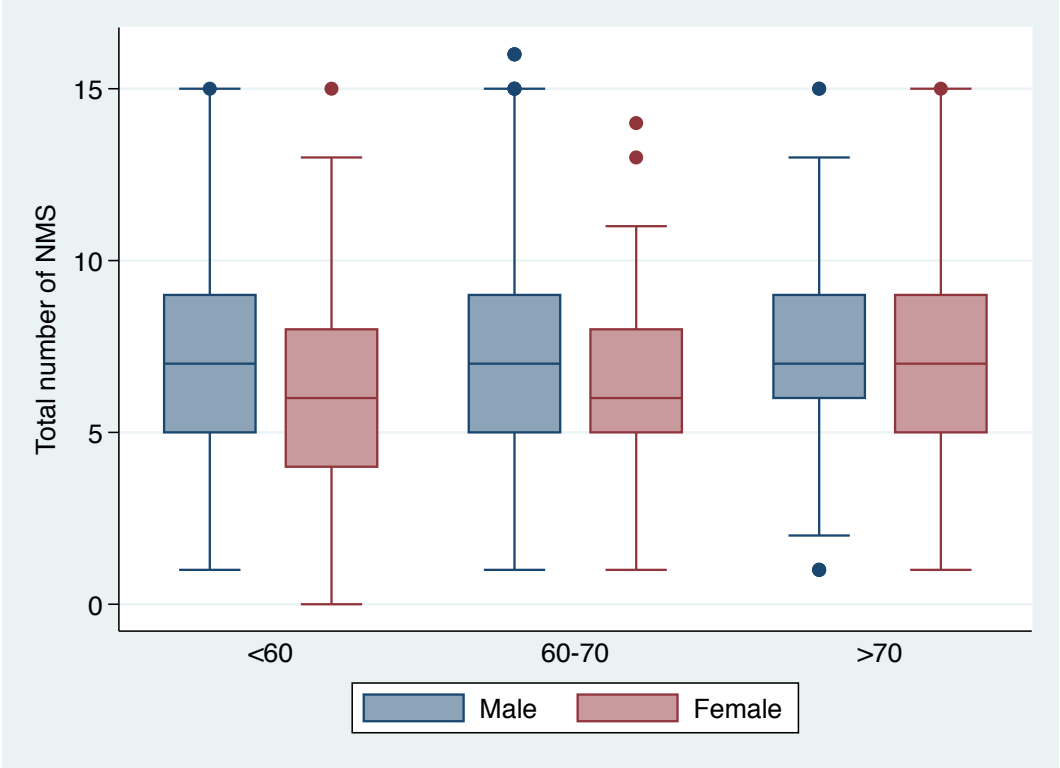


Table 4.13 – NMS stratified by age

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]
		<60	60-70	>70	
	n=945	n=204	n=356	n=385	
NMS total (median (IQR))	7 (5-9)	6 (4-8.5)	7 (5-9)	7 (5-9)	1.24, 1.11-1.38, p<0.001

[1]Ordinal logistic model adjusting for gender and disease duration from diagnosis, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years). Total number of NMS (maximum 18) refers to the sum of positive assessments for: cognition, hallucinations and psychosis, apathy, fatigue, anxiety, depression, impulsivity, orthostatic hypotension, urinary disturbance, hypersialorrhoea, pain, hyposmia, daytime sleepiness, RBD, disturbed sleep, sexual dysfunction, swallowing and constipation. This was stratified into quintiles for analysis.

Table 4.14 – NMS stratified by gender

	Total PD group	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[1]
		Male	Female	
	n=945	n=610	n=335	
NMS total (median (IQR))	7 (5-9)	7 (5-9)	6 (5-8)	1.21, 0.88-1.65, p=0.24

[1]Logistic model adjusting for age and disease duration from diagnosis, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years). Total number of NMS (maximum 18) refers to the sum of positive assessments for: cognition, hallucinations and psychosis, apathy, fatigue, anxiety, depression, impulsivity, orthostatic hypotension, urinary disturbance, hypersialorrhoea, pain, hyposmia, daytime sleepiness, RBD, disturbed sleep, sexual dysfunction, swallowing and constipation. This was stratified into quintiles for analysis.

4.4.2 NEUROPSYCHIATRIC SYMPTOMS

4.4.2.1 Cognition

Table 4.15 - Cognitive scores

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p-value)
Montreal Cognitive Assessment (median (IQR))	25 (23-27)	27 (25-29)	
Montreal Cognitive Assessment[a]: • Normal cognition (n(%)) • Possible mild cognitive impairment (n(%)) • Possible dementia (n(%))	663 (71.1) 142 (15.2) 128 (13.7)	258 (87.5) 26 (8.8) 11 (3.7)	2.58, 1.77-3.79, p<0.001[1]
Phonemic fluency (median (IQR))[b]	11 (8-14)	12 (11-15)	
Phonemic fluency – poor performer (<20th centile) (n (%))	161 (17.2)	22 (7.6)	2.40, 1.50-3.85, p<0.001[2]
Semantic fluency (median (IQR))	10 (8-12)	12 (10-14)	
Semantic fluency – poor performer (<20th centile) (n (%))[c]	195 (20.8)	20 (6.9)	3.28, 2.02-5.32, p<0.001[2]
Premorbid IQ estimate (median (IQR))[d]	120.5 (116.4-123.9)	122.2 (118.9-125.5)	
Premorbid IQ estimate – categories (n (%))[e]: • <110 or poor performer • 110-120 • >120	163 (27.7) 252 (42.8) 174 (29.5)	8 (10.5) 34 (44.7) 34 (44.7)	0.45, 0.28-0.70, p<0.001[1]

[1]Ordinal Logistic regression used to calculate odds ratios, adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [2]Logistic regression used to calculate odds ratios, adjusting for age and gender; [a]Cognition (MOCA) categories: normal cognition 24-30, mild cognitive impairment 22-23, dementia 0-21, [b]Letters beginning with F, A and S for 1 minute each, [c]Boys' and animal names for 1 minute each, [d]Premorbid IQ estimate based on the National Adult Reading test (Nelson et al. 1991, Bright et al. 2016), data from 589 PD patients and 76 controls only available, ranges shown only for those with complete test; [e]Poor performers were defined as those making 5 or more errors in the first half of the test, after which the test was discontinued, these categories were used in the regression model for analysis

The PD group performed worse on all three cognitive assessments (see Table 4.15 and Figure 4.6 for graph of MOCA comparison). These differences persisted, despite adjusting for age and gender, using the MOCA (OR 2.58, $p<0.001$), phonemic fluency (OR 2.40, $p<0.001$) and semantic fluency (OR 3.28, $p<0.001$). The MOCA score is adjusted for education years as part of the scoring (addition of one point for less than 12 years in full time education), thus the addition of education years to the model as a co-variate made little difference.

These findings are consistent with the literature, although reported rates vary due to differences in assessment and definition. Cognition is well known to be affected early in PD with multiple studies providing evidence that this is present at diagnosis at similar rates (Aarsland et al. 2009, Yarnall et al. 2014) and also in prodromal cohorts before the onset of motor symptoms (Rolinski et al. 2016). This includes the relatively poor performance on semantic compared to phonemic fluency, which is reported to be more impaired in PD (Henry et al. 2004). It is useful to note that 29% of the PD group screened positive for mild cognitive impairment or dementia even at this early stage in the disease which is a significant proportion. This is despite excluding patients who developed dementia within the first year or preceding their motor symptoms were excluded as having dementia with Lewy bodies. This early cognitive impairment is increasingly recognised.

Only a subset of patients, at the time of download, had an estimate of their pre-morbid IQ. The use of the National adult reading test (NART) for this estimate is based on the relative preservation of verbal ability over time in aging adults (Hedden et al. 2004). There is an apparent difference of a lower IQ in the PD group, despite adjustment for age and gender. The addition of socio-economic position to the model (using wealth and education years as proxies) made little difference to the model (OR 0.48, 95% CI 0.29-0.79, $p=0.004$). The pre-morbid IQ estimate is also associated positively with the MOCA

score (regression model adjusting for age, gender and socio-economic position: OR 2.11, 95% CI 1.61-2.77, p<0.001). It has been established that verbal ability is affected in PD, so it is possible that the cognitive effects of Parkinson's are responsible for this difference rather than a bias in recruitment. Despite previous reports, it may be less useful as a marker of pre-morbid IQ in PD studies. As discussed in chapter 4.2.1.4, the population is based in an affluent and educated part of the country, which may account for the relatively high mean IQ. The original validation study for this estimate reported a mean IQ of 109 with only 18% of their sample scoring >120 (Bright et al. 2016). This may also reflect improvement over time. It has been demonstrated that performance on this test improves over time, the Flynn effect (Norton et al. 2016).

In the PD group, there was considerable overlap between the results when split by gender or age group. However, male gender (p<0.001) and older age (p<0.001) were associated with a worse performance on the MOCA (see Table 4.16).

Older age was associated with a poorer performance on phonemic fluency (using letters F, A, and S) but not with semantic fluency (boys' and animal names). There was apparent effect of age on the NART performance as would be expected (Hedden et al. 2004). While gender had little effect on NART performance, it was slightly surprising to find that women performed better on semantic fluency, with a similar trend seen in phonemic fluency (Tombaugh et al. 1999, Zec et al. 1999, Henry et al. 2004). There is, however, good evidence to suggest that performance on verbal fluency tasks is affected differently by gender across categories (Gerlach et al. 2016).

Figure 4.6 – Group comparison of MOCA scores

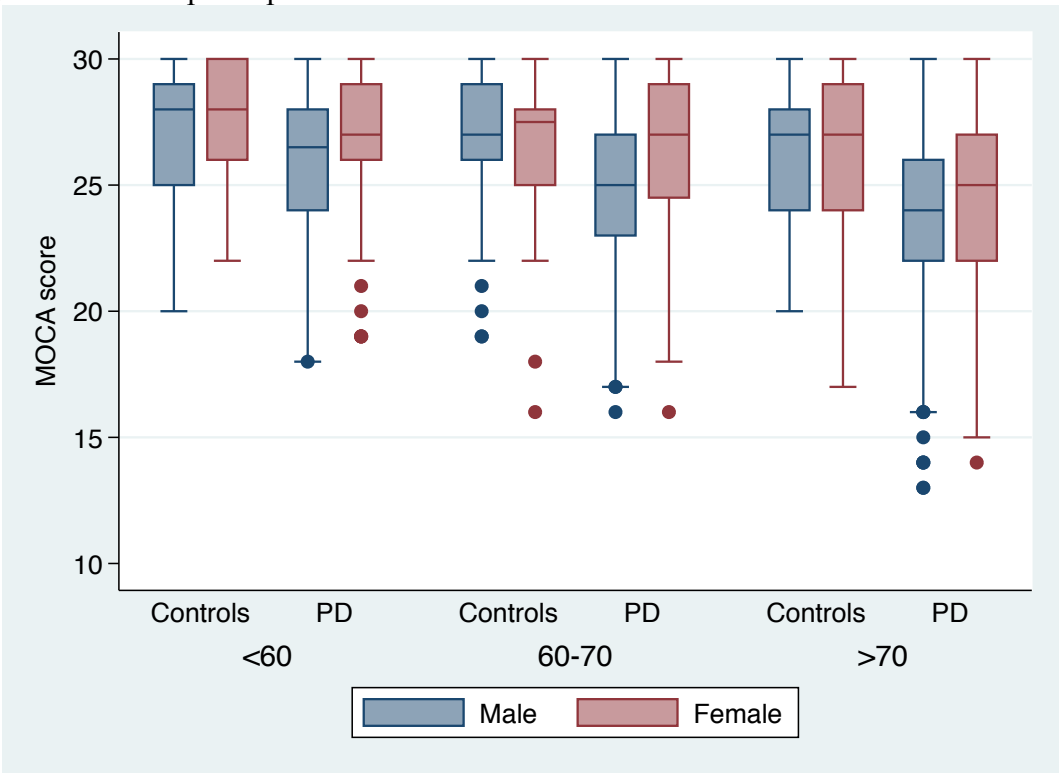


Table 4.16 – Cognitive scores of PD group stratified by age and gender

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[2]
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
MOCA score (median (IQR))	25 (23-27)	27 (25-28)	26 (24-28)	24 (22-26)	1.60, 1.43-1.78, p<0.001*	25 (23-27)	26 (24-28)	0.62, 0.45-0.85, p=0.003*
MOCA categories (n (%))[a]								
• Normal cognition	663 (71.1)	180 (88.2)	264 (75.4)	219 (57.8)		403 (67.1)	260 (78.3)	
• Possible MCI	142 (15.2)	14 (6.9)	50 (14.3)	78 (20.6)		105 (17.5)	37 (11.1)	
• Possible dementia	128 (13.7)	10 (4.9)	36 (10.3)	82 (21.6)		93 (15.5)	35 (10.4)	
Phonemic fluency (median (IQR))	11 (8-14)	11 (9-14)	12 (9-14)	10 (8-13)	1.17, 1.03-1.32, p=0.01	11 (8-13)	11 (9-14)	0.74, 0.51-1.07, p=0.11
Phonemic fluency – poor performer (n (%))	161 (17.2)	31 (15.2)	47 (13.4)	83 (21.7)		114 (18.8)	47 (14.2)	
Semantic fluency (median (IQR))	10 (8-12)	10 (8-12)	10.5 (8-13)	9 (8-12)	1.07, 0.94-1.22, p=0.32	10 (8-12)	11 (8-13)	0.27, 0.16-0.44, p<0.001[2]
Semantic fluency – poor performer (n (%))	195 (20.8)	26 (12.8)	47 (13.4)	64 (16.7)		117 (19.3)	20 (6.0)	
Pre-morbid IQ estimate (n (%)):								
• <110 or poor performer		30 (21.1)	33 (14.2)	44 (20.6)	1.01, 0.91-1.13, p=0.81*	67 (18.0)	40 (18.3)	1.20, 0.88-1.65, p=0.25*
• 110-120		51 (35.9)	89 (38.2)	70 (32.7)		142 (38.2)	68 (31.3)	
• >120		61 (43.0)	111 (47.6)	100 (46.7)		163 (43.8)	109 (50.2)	

[a]Cognition (Montreal cognitive assessment) screening thresholds: normal cognition 24-30, mild cognitive impairment 22-23, dementia 0-21; [b]Poor performers as defined by lowest 20th centile; [1]Logistic regression model adjusting for gender and disease duration, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [2]Logistic regression model adjusting for age and disease duration; *Ordinal logistic regression.

4.4.2.2 Mood disorders

Table 4.17 – Comparison of mood disorders across groups

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p-value)
Depression (Beck's Depression Inventory-II):			
BDI score (median (interquartile range))	8 (4-12)	4 (1-7)	
Depression category (n(%))[a]: • Mild • Moderate • Severe	 97 (11.0) 51 (5.8) 10 (1.1)	 9 (3.2) 9 (3.2) 0	3.46, 2.07-5.78, p<0.001[1]
Anxiety (Leeds Anxiety and Depression Scale):			
LADS score (median (interquartile range))	3 (1-5)	2 (0-3)	
Anxiety positive screen (n(%))[b]	159 (17.3)	17 (5.9)	4.04, 2.38-6.86, p<0.001[2]

[a]Beck Depression Inventory II: minimal depression 0-13, mild depression 14-19, moderate depression 20-28, severe depression 29-63; [b]Positive anxiety screen if LADS ≥ 7, [1]Ordinal logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); [2]Logistic regression adjusting for age and gender

Anxiety (17.3% in PD vs 5.9% in controls) and depression (17.9% in PD vs 6.4% in controls) were both more common in the PD group compared to controls (see Table 4.17). Depression scores were similar across gender and age groups. Anxiety symptoms were a little less common in men over 70 compared to women but there was considerable overlap. Additional sensitivity analysis was performed on the BDI (examining the affective and somatic symptoms of depression separately) with only a minor effect on the results.

Anxiety rates in the community population vary depending on definition and assessment tool, but in those over 50 estimates vary between 0.9 and 6.7% (Volkert et al. 2013), similar to this control group. The prevalence of depression in older people (older than 65) in the UK is estimated at 8.7% (95%CI 7.3-10.2)(McDougall et al. 2007) which is slightly higher than found in the control group (6.4%). This may be because those with

more depressive symptoms are less likely to participate in research, the inclusion of controls under 65 or because of different diagnostic criteria.

Regression models with anxiety and depression as the dependent variables with age, gender and disease duration were performed. Younger age (OR 0.97, p=0.001) and female gender (OR 1.68, p=0.004) were associated with anxiety. Younger age was also associated with depression (OR 0.98, p=0.02) but not gender. Disease duration was not associated with either mood disorder.

As mentioned above, heterogeneity in study design makes inferences in mood disorders across age and gender difficult to compare across studies, with conflicting reports on the effect of age and gender in depression (Djernes 2006). Using the same regression model in the control group, the same association with younger age and anxiety was seen (OR 0.94, p=0.02) but not in depression. More consistent was the finding of higher rates of anxiety in women, which has been reflected here (McLean et al. 2011).

4.4.2.3 Impulsivity, psychosis, apathy and fatigue

Table 4.18 – Neuropsychiatric symptoms compared across groups

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p-value)[1]
Impulse Control Behaviours (QUIP-S)[a] positive screen (n(%))	197 (21.9)	61 (21.4)	1.10 (0.79-1.55), p=0.56
Hallucinations & psychosis (UPDRS) (n (%))	105 (11.2)	n/a	n/a
Apathy (UPDRS) (n (%))	194 (20.6)	n/a	n/a
Fatigue (UPDRS) (n (%))	663 (70.8)	n/a	n/a

[a]ICB (Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease): scoring positive on any category, [1]Logistic regression adjusted for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Impulsive disorders screened positive at a similar rate across all groups (see Table 4.18). This is likely because the screening questionnaire was designed for PD and is not validated for non-cases. Even still, being a screening questionnaire, it is sensitive and less specific leaving it at risk for false positives. While it has become clear that ICD are more common in PD patients who are on dopaminergic treatments (Voon et al. 2011), a more in depth assessment would be required to determine true prevalence. A more in depth interview has been conducted on this cohort and found that 5.3% (95% CI 3.9%-6.9%) of PD patients had suffered ICD (data not yet published), compared to 1.4% (95% CI 0.4%-3.4%) of control participants. Accepting this caveat, younger age ($p<0.001$), disease duration ($p=0.005$), and LEDD ($p=0.004$) were associated with a positive QUIP, although gender was not.

Fatigue is very commonly reported in PD, but this symptom is associated with many other symptoms (such as mood, pain and motor disability) which can all contribute to its severity, making specific inferences difficult. Reports of fatigue in the literature vary widely, most likely due to the form of assessment as fatigue remains difficult to characterise. In this cohort, 71% of those with fatigue reported only 'slight' symptoms. Rates were similar across gender and age groups. The presence of depression (OR 3.16,

p<0.001) and anxiety (OR 3.98, p<0.001) were both associated with fatigue in a regression model also including age, gender and disease duration as co-variates. This association is well described, but in addition 64.5% of those without mood disorders had symptoms of fatigue.

Apathy has also shown an association with fatigue but is increasingly recognised as common in PD, distinct from other mood disorders, even at the early stages (Muhammed et al. 2016). Of those patients who did not report symptoms of depression and anxiety, 12.9% reported symptoms of apathy. There is an association though. The presence of depression (OR 3.47, p<0.001) and anxiety (OR 3.32, p<0.001) were both associated with apathy in a regression model also including age, gender and disease duration as co-variates. Rates were similar across gender and age groups. The prevalence of each of these symptoms are comparable with the ICICLE-PD study (Khoo et al. 2013, Pont-Sunyer et al. 2015).

Visual hallucinations or psychotic symptoms were perhaps more prominent at this early stage than previously thought (11.2%). The described symptoms were mostly 'slight' (88% of those who reported symptoms) with only a few describing them as 'mild' (12%) and none reporting moderate or severe. The key distinction is that all these patients had not lost insight that their illusions (misinterpretation of real stimuli) and hallucinations (spontaneous false sensations) were not real. Historically this was considered a late symptom, as an early feature it had been more commonly associated with dementia with Lewy bodies. It may be under reported as, if the patient has insight, they dismiss them. Visual hallucinations were associated with a higher LEDD (p=0.01) and a longer disease duration (p=0.002) in a regression model, but not age or gender.

4.4.3 GASTROINTESTINAL

Table 4.19 - Comparison of gastrointestinal symptoms

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p value)[1]
Constipation (Honolulu Ageing Study)[a] (n (%))	459 (49.1)	102 (34.8)	1.80, 1.36-2.38, p<0.001
Chewing and swallowing (UPDRS) (n (%))	195 (20.8)	n/a	

[a]Constipation (HAS): positive if <1 daily bowel movement or use of laxatives, [1]Ordinal logistic regression used to calculate odds ratios, adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Constipation was common with almost half of the PD group reporting symptoms or using laxatives. Both constipation ($p<0.001$) and laxative use ($p<0.001$) were higher in the PD group compared to controls (see Table 4.19). Constipation is also common in non-cases with a third of the control group symptomatic, as would be expected in this age group.

The replication of similar rates of chewing or swallowing difficulties (21%) in this larger cohort lends weight to previous smaller studies suggesting an earlier evolution of these symptoms than previously recognized (Muller et al. 2011, Khoo et al. 2013). While well established as a late complication of PD, swallowing difficulties has a significant impact on morbidity and quality of life.

Constipation was more common in older age (OR 1.26, $p<0.001$) and in women (OR 1.39, $p=0.007$), but was not affected by disease duration. These differences are well reported in the literature (McCrea et al. 2009).

4.4.4 SLEEP

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio (95% CI), p value) [1]
RBD[a] (RBD Sleep Questionnaire) (median(interquartile range))	4 (2-6)	2 (1-4)	
-Positive screen for RBD (n(%))	299 (33.3)	42 (14.9)	2.68 (1.87-3.84), p<0.001
Daytime somnolence (ESS)[b] (median(interquartile range))	7 (4-10)	5 (3-8)	
-Positive screen for daytime somnolence (n(%))	228 (24.5)	28 (9.7)	2.80 (1.84-4.26), p<0.001
Sleep disturbance (UPDRS) (n (%))	668 (71.2)	n/a	

[a]RBD (Rapid Eye Movement Sleep Behaviour Disorder): positive screen if ≥6, [b]Daytime somnolence (Epworth Sleepiness Scale): positive screen for daytime somnolence if ESS≥10, [1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

The links between PD and sleep disturbances are complex. The rates of self-reported sleep disturbances are high as expected (71.2%) in the PD group, with 24% describing moderate to severe symptoms. These are associated with a worse quality of life and can have a negative impact on other symptoms also. There are a broad range of sleep disturbances associated with PD causing these symptoms including RBD, excessive daytime somnolence (EDS), 'sleep attacks' (sudden onset of sleep without a prodrome), restless leg syndrome, periodic limb movements of sleep, disruption in sleep architecture and circadian rhythm dysfunction (Videnovic et al. 2013, French et al. 2016). Sleep disturbances can often precede the onset of the motor symptoms. Sleep can also be further disrupted by other NMS such as nocturia or mood disorders.

The number of cases reporting RBD in this cohort (33%) are similar to the results of a recent meta-analysis, with a similar comparison with non-cases (Zhang et al. 2017). The presence of RBD in PD patients has been associated with more severe symptoms in this cohort, its presence perhaps associated with a more aggressive subtype of PD (Rolinski et al. 2015). RBD symptoms may start before the onset of motor symptoms. Adult onset

RBD is commonly associated with conversion to a neurodegenerative disorder, most commonly a synucleinopathy, such as PD (more than 80% in long term follow up) (Postuma et al. 2015). An inherent problem of this, and other similar studies, is the use of screening questionnaires to define the presence of RBD rather than the more specific (but much more expensive and resource intense) use of overnight polysomnography. EDS is associated with PD itself, co-existent sleep disorders (such as obstructive sleep apnoea), disruption of circadian rhythms and sleep architecture, and as a side effect of dopamine replacement therapies (particularly dopamine agonists) (Videnovic et al. 2013). While 26% of the treated PD patients screened positive for EDS, fewer drug naïve PD patients were affected (17%) but still a significant proportion.

Both RBD and EDS were more common in men, despite adjusting for age and disease duration, but the rates were similar across age groups, as has been reported (Picillo et al. 2013). The addition of LEDD or use of dopamine agonists to the model made little difference. However conversely, sleep disturbance was reported more frequently in women compared to men (OR 1.43, p=0.02), adjusted for age, disease duration and dopamine agonist use.

4.4.5 AUTONOMIC

Table 4.20 - Comparison of autonomic symptoms across groups

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio (95% CI), p value)[1]
Postural drop in systolic BP (mmHg)(mean(sd))	6.3 (15.9)	0.1 (12.3)	
Orthostatic hypotension (n(%))[a]	187 (19.9)	19 (6.5)	3.17, 1.93-5.23, p<0.001
Urinary problems (UPDRS) (n (%))	590 (62.9)	n/a	
Saliva and drooling (UPDRS) (n (%))	450 (48.0)	n/a	

[a]Positive result if systolic drop of blood pressure (BP) of ≥20mmHg or a diastolic drop of ≥10mmHg, [1]Logistic regression used to calculate odds ratios, adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years).

Autonomic symptoms (see Table 4.20), as with sleep disorders, may precede the onset of the motor symptoms. Despite being increasingly common with age, they are more common in PD (Muller et al. 2013).

Orthostatic hypotension was more common in the PD group (3.17, p<0.001). 36% of the PD group and 26% of controls were taking anti-hypertensive medication. These differences persisted despite adjusting for the use of anti-hypertensive medication in the regression model or excluding those on treatment.

Urinary symptoms, in those who reported them, were mainly only slight (63%). They can be multifactorial, potentially neurogenic or structural in origin. Stress incontinence in women and prostatic symptoms in men are increasingly common with age, but the assessment tool was not sensitive to the cause of the symptoms

Excessive salivary dribbling was more common than previously thought, supporting the findings of previous smaller studies (Muller et al. 2011, Khoo et al. 2013). Sialorrhea may result from a combination of excessive salivary production, impairment of salivary clearance or inability to maintain saliva in the mouth (Srivanitchapoom et al. 2014).

Regression models were performed for each of these symptoms, including age, gender and disease duration as co-variates. Both orthostatic hypotension (OR 1.43, $p<0.001$) and urinary symptoms (OR 1.22, $p<0.001$) were more common with age, but hypersialorrhoea was not (OR 1.03, $p=0.55$). This is expected, as hypersialorrhoea, as opposed to the others, is not a common consequence of aging.

Women were less likely to experience postural hypotension (OR 0.60, $p=0.006$) and hypersialorrhoea (OR 0.49, $p<0.001$) than men, with both genders experiencing similar urinary symptoms. This gender bias of more autonomic symptoms in men has recently been reported but few studies have been performed (Picillo et al. 2013, Liu et al. 2015).

4.4.6 SENSORY

Table 4.21 – Sensory and pain symptoms in the PD and control groups

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p value)[1]
Sniffin total score (median (interquartile range)	7 (5-9)	12 (11-14)	
Hyposmia (Sniffin) (n(%))	748 (82.7)	43 (14.9)	26.1 (18.0-37.8), p<0.001
Pain and other sensations (UPDRS) (n (%))	747 (79.6)	n/a	
Moderate or extreme pain (EQ5D) [a] (n(%))	521 (55.4)	103 (35.4)	2.42 (1.83-3.21), p<0.001

[a]Pain (EQ5D): positive if rated moderate or extreme, [1]Logistic regression used to calculate odds ratios, adjusting for age and gender.

Hyposmia is well established as being very common in PD (see Table 4.21), usually starting before the onset of the motor symptoms (Muller et al. 2011, Noyce et al. 2012). Far less is known about pain and other sensations in PD. While common, the causes of pain are varied with patients experiencing pains associated with motor complications (i.e. dyskinesia and dystonia), centrally mediated burning pains, painful parasthesia, muscle cramps, and joint and muscle aches associated with stiffness. The assessment used in this cohort (EQ5D) does not differentiate these PD associated pains from any other causes (such as osteoarthritis). Despite this, there was clearly an increase in symptoms compared to controls (2.42, p<0.001). Assessing the pain and other sensations using the UPDRS scale (a 5 point scale rather than the 3 point scale of EQ5D), half of those reporting symptoms described them as slight, but there were still 13% describing moderate to severe symptoms.

Women were less likely to be hyposmic than men (OR 0.58, p=0.002), adjusting for age and disease duration, in-keeping with previous studies (Picillo et al. 2013, Liu et al. 2015). They experienced more pain and other sensations though (OR 1.60, p=0.001), and these symptoms were more common with age (OR 1.12, p=0.03) but not disease duration.

4.4.7 SEXUAL DYSFUNCTION

Sexual dysfunction can be considered part of the spectrum of autonomic disorders, but considered here separately. Erectile dysfunction was reported by 43% (n=254) of the PD group, which is apparently similar to some published prevalence studies, but estimates vary considerably (Hatzimouratidis 2007). Previous work has suggested that this is associated and may even be a prodromal marker for PD, but the absence of the assessment from non-cases prevents further conclusions.

Sexual dysfunction, more broadly, has been reported more commonly in men compared to women, findings consistent with this cohort (26% in men vs 5% in women; OR 0.16, $p<0.001$) (Mollenhauer et al. 2013). In total, 18.5% (n=170) of the PD group described sexual dysfuction. Age and disease duration had little effect.

4.4.8 NON-MOTOR SYMPTOMS SUMMARY

In order of frequency, the most common NMS experienced in PD were *hyposmia, pain, sleep disturbance, urinary symptoms, and fatigue,* each affecting over half of subjects. The PD group experienced more or performed worse on each measure compared to controls, aside from the impulsivity screening questionnaire. These differences persisted despite adjusting for age and gender in regression models. This is unsurprising, given that these symptoms are known to be associated with PD. The largest difference between the two groups was hyposmia (OR 26.1, $p<0.001$) with anxiety (OR 4.0, $p<0.001$) the next largest. The relationship between these symptoms, made worse by PD, and the underlying pathophysiological processes may predict the rate of progression.

Cross-comparison of NMS between different cohorts is often limited by the types of screening tool used. This cohort exhibited a similar spectrum of NMS in our PD cohort to that reported in the ICICLE-PD study (Khoo et al. 2013), a similar cohort, and others as referenced through the chapter. This study also reported that urinary disturbance, saliva and drooling, hyposmia, and constipation were very common. Anxiety, low mood and forgetfulness were more prevalent in their cohort. This may reflect the means of assessment (the NMSQuest used in their study dichotomously rates each symptom as present/absent), while severity-rating scales were mainly used in this cohort. The similar rates of NMS in early and incident PD cohorts robustly confirms the typical nature of this large cohort.

The effects of age and gender in PD are shown to be important in a number of symptoms, with their effects depicted graphically in Figure 4.7 and Figure 4.8. The association of older age with cognitive and autonomic symptoms fits with what is known about aging and thus may progress at a faster rate in the context of PD. The association of younger age with mood disorders was only small, and may reflect an underlying trend in the population. Although women experienced more anxiety and pain, they performed

better on the cognitive tasks and experienced less autonomic and sleep disturbances. Based on the non-motor symptoms alone, this in part supports the hypothesis that women develop a less aggressive phenotype than men, potentially due to neuroprotective effects of hormones.

Figure 4.7 – Age: graph depicting odds ratios (grey bars) with 95% confidence intervals (black error bars) for the effect of age on individual non-motor symptoms in a logistic regression model adjusting for gender and disease duration where each dependent variable was dichotomised into present or absent, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years).

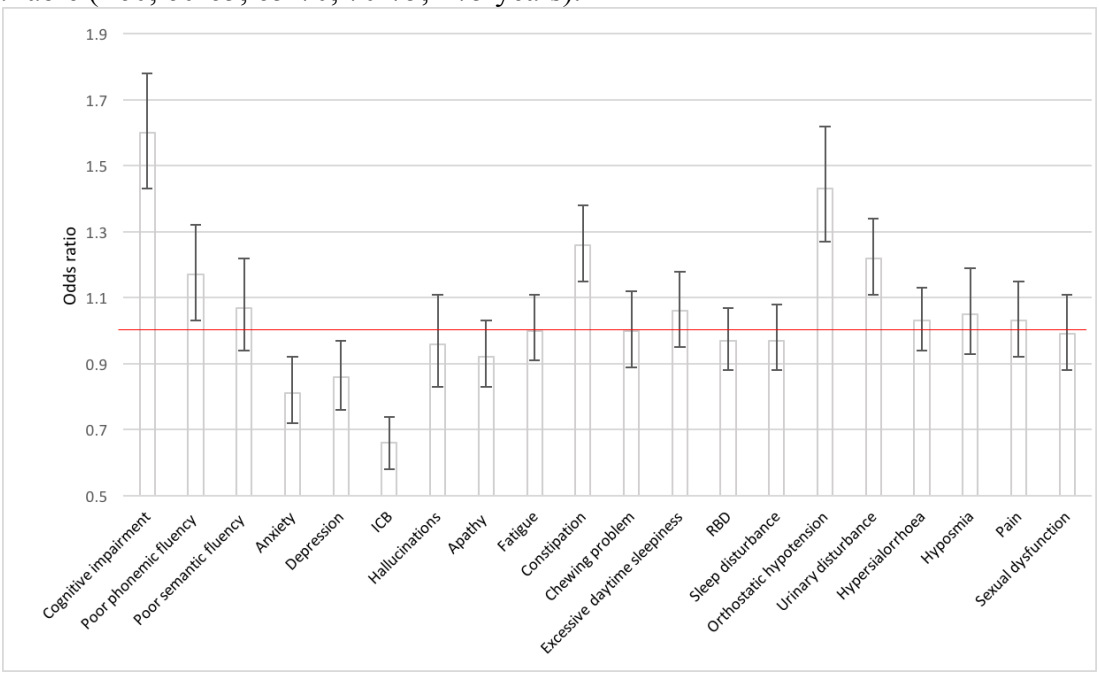
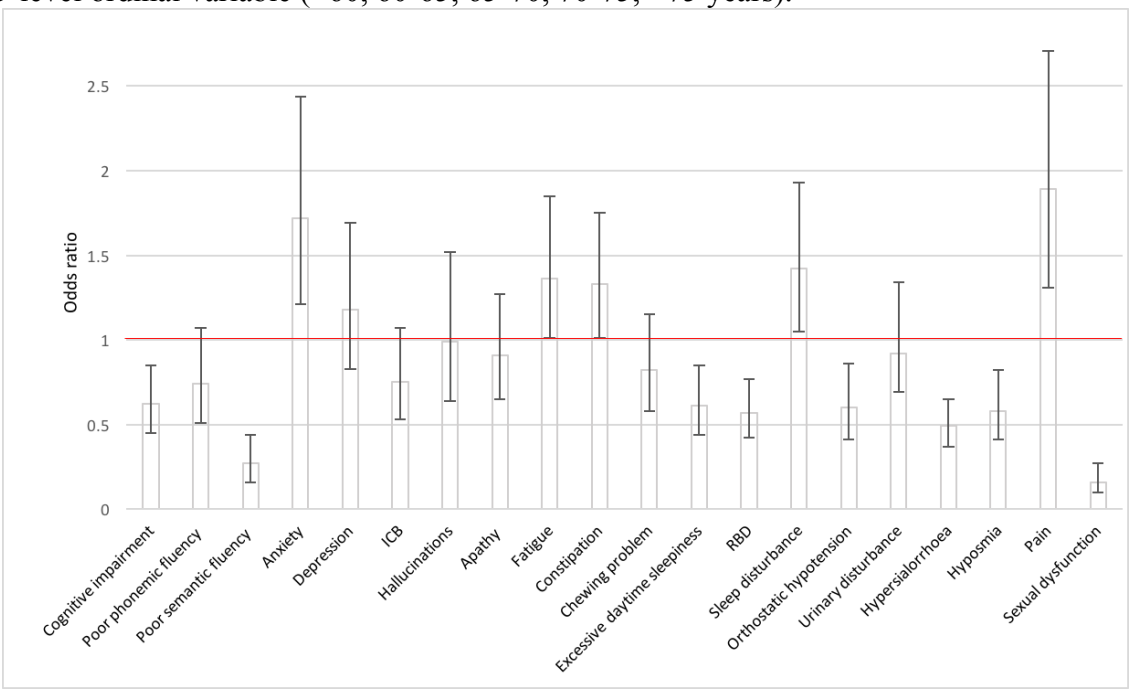


Figure 4.8 - Gender: graph depicting odds ratios (grey bars) with 95% confidence intervals (black error bars) for the effect of female gender on individual non-motor symptoms in a logistic regression model adjusting for age and disease duration where each dependent variable was dichotomised into present or absent, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years).



4.5 MOTOR FEATURES

Selected using the UK brain bank criteria, included patients with Parkinson's had bradykinesia with at least one of tremor, rigidity and postural instability. These individual features progress at different rates and respond differently to dopaminergic medications. Participants also may perform better or worse depending when tested in relation to their last medication dose. All patients in this study were examined on their usual medication or in the 'on' phase if they had motor fluctuations. Unrelated co-morbidities in patients of this age, such as arthritis, may also impact performance. Factors potentially related to PD, such as cognition, will also affect performance. All these factors make motor assessment complex. However, despite these caveats, unsurprisingly the PD group did worse in comparison to the control group in all motor assessments (see Table 4.23). There may be an element of bias in this selection, where more physically disabled volunteers are less likely to participate in research, but these results are as expected.

4.5.1 UPDRS III MOTOR SCORE, HOEHN AND YAHR STAGE AND MOTOR COMPLICATIONS

Table 4.22 – Motor scores

MOTOR SCORES	**PD** (n=945)	**Controls** (n=295)
MDS-UPDRS III (mean (SD))	26.5 (10.8)	1.7 (2.7)
MDS-UPDRS IV (mean (SD))	0.3 (1.1)	n/a

The mean UPDRS III score in this cohort (26.5, see Table 4.22) is comparable to the ICICLE-PD study (and other similar cohorts) which reported a mean UPDRS III of 27.9 (Khoo et al. 2013). The majority were Hoehn and Yahr stage 2 (70%) as would be expected in early PD with the majority consistent with the tremor dominant (55%) phenotype.

Motor impairment increased with age (1.35, $p<0.001$) but not gender (see Table 4.23). Some parkinsonian signs were elicited from control subjects and, using gender as a co-variate, and were also strongly associated with increasing age (OR 1.88, 95% CI 1.58-2.23, $p<0.001$). Up to 15% of controls were affected by at least one of tremor, rigidity and bradykinesia although the vast majority were only slight. This is in-keeping with a recent population based study which reported a similar prevalence of motor signs in an unaffected population, also describing a significant association with increasing age (Keezer et al. 2016). Whether this represents a subclinical neurodegenerative process, such as a synucleinopathy or cerebrovascular disease, or the effect of co-morbidities is not clear. The effect of age was also reflected in the Hoehn and Yahr staging with 78% of the PD participants in H&Y stage 3 being older than 70. Older age was associated with a greater chance of displaying the PIGD phenotype compared to tremor dominant. Women were also more likely to develop the PIGD than men ($p=0.01$). Despite these differences, there is significant overlap between the groups. These results should be viewed with a little caution though, as while disease duration had little effect, higher

LEDD were associated with the PIGD phenotype. Dopaminergic medications differ in their efficacy between individuals and against individual motor symptoms (for example some may experience more effect on their rigidity than their tremor), which may change the phenotype despite the addition of LEDD to the model. However, at this early stage of PD and with a large sample size this is probably less of an issue. Only including those who were drug naïve in the same model, did not show the same effects of age and gender, but the sample was small (n=127).

The lateralising motor signs examined (i.e. tremor, rigidity and bradykinesia) seemed to affect each side evenly across the cohort. Parkinsonism scores were higher on the left in 37% of cases, on the right in 40% of cases and similar bilaterally in 23% of cases (a difference in UPDRS III score between each side of less than 4).

Only 8% of the cohort experienced motor complications at baseline, but most were slight with no functional impact. Of the PD group, 5% reported motor fluctuations (89% of which were slight) and 4% (36) had dyskinesia (of which 92% had no functional impact). Although difficult to make conclusions on these relatively small numbers, unsurprisingly longer disease duration and higher LEDD were associated with the risk of early motor complications in a regression model, while H&Y stage, age and gender made little difference as co-variates. At H&Y stage 3, 14% of participants reported motor complications, with relatively fewer reporting problems at stage 2 (8%) and stage 1 (6%), but the higher stages correlate strongly with disease duration and LEDD.

Table 4.23 – Motor scores stratified by age and gender

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[1]
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
UPDRS III (mean (SD))	26.5 (10.8)	22.7 (9.6)	25.6 (10.3)	29.2 (11.1)	1.35, 1.24-1.46, p<0.001	27.0 (10.8)	25.5 (10.6)	0.81, 0.64-1.03, p=0.09
Hoehn and Yahr Stage (n (%)):					1.57, 1.41-1.74, p<0.001			1.33, 0.98-1.80, p=0.06
• 1	216 (23.0)	78 (38.4)	88 (24.9)	50 (13.0)		139 (22.9)	77 (23.1)	
• 2	661 (70.2)	121 (59.6)	256 (72.3)	284 (74.0)		438 (72.0)	223 (67.0)	
• 3	64 (6.8)	4 (2.0)	10 (2.8)	50 (13.0)		31 (5.1)	33 (9.9)	
Motor subtype (n (%)):					1.12, 1.02-1.22, p=0.02			1.50, 1.15-1.96, p=0.003
• Tremor dominant	509 (54.9)	124 (61.7)	188 (54.5)	197 (51.7)		347 (57.9)	162 (49.4)	
• Indeterminate	114 (12.3)	27 (13.4)	46 (13.3)	41 (10.8)		69 (11.5)	45 (13.7)	
• Postural instability and gait disorder	304 (32.8)	50 (24.9)	111 (32.2)	143 (37.5)		183 (30.6)	121 (36.9)	
Laterality[a] (n (%)):					0.98, 0.90-1.07, p=0.69			0.82, 0.64-1.05, p=0.12
• Right	373 (39.9)	85 (41.9)	155 (44.0)	133 (34.9)		243 (40.2)	130 (39.2)	
• Left	348 (37.2)	94 (46.3)	121 (34.4)	133 (34.9)		208 (34.4)	140 (42.2)	
• Symmetrical	215 (23.0)	24 (11.8)	76 (21.6)	115 (30.2)		153 (25.3)	62 (18.7)	

[a]Laterality was determined by a UPDRS III score of 4 or greater on one side or symmetry if the difference was less, for the regression model right sided laterality was a higher order than left, [1]Ordinal logistic regression used to calculate odds ratios, adjusting for age, gender, disease duration and levodopa equivalent dose, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

4.5.2 TIMED MOTOR ASSESSMENTS

Table 4.24 - Functional motor assessments

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p value)
Purdue board peg test (mean(sd)):			
Total pin insertion	28.7 (6.8)	37.5 (6.8)	10.2, 5.6-18.7, p<0.001[1]
Total assembly	17.6 (5.9)	25.0 (6.7)	11.2, 6.0-20.9, p<0.001[1]
Flamingo Balance Test (n(%)):			
• Abandoned or <10 seconds	207 (22.6)	32 (10.9)	2.68, 1.99-3.60, p<0.001[2]
• 10 to 29 Seconds	268 (29.3)	52 (17.8)	
• 30 seconds	440 (49.1)	209 (71.3)	
Get up and go, poor performance (n(%))	271 (29.5)	19 (6.4)	8.1 (4.3-15.3), p<0.001[1]
Falls within last 6 months (n (%))[a]	68 (16.5)	10 (8.7)	2.11 (1.04-4.29), p=0.05[1]

[1]Logistic regression used to calculate odds ratios, adjusting for age and gender, [2]Ordinal logistic regression used to calculate odds ratios, adjusting for age and gender, [a]The falls questionnaire was added after the study started, information from 410 PD cases and 115 controls only was available

Despite being on usual treatment and, if suffering motor fluctuations, being in the 'on' phase, PD patients performed worse at each of the functional tasks (see Table 4.24). Older age was associated with worse performance, as would be expected, on each functional motor assessment, the association with falls probably less pronounced due to the smaller sample. Men performed worse at the Purdue peg board test (total pin insertion) with similar results for the assembly test (see Figure 4.9 and Figure 4.10). This result was very similar when using the full score rather than just poor performers. This trend was the same seen when including only the control group and so is unlikely to be specific to PD. Conversely, women performed worse on the flamingo balance task (OR 1.48, p=0.004) and the get up and go test (OR 1.62, p=0.006), again this same trend seen in the control group.

These tests differ in that the Purdue peg board task is a measure of dexterity while the flamingo and get up and go tasks also include a measure of balance. In chapters 4.2.2.3 and 4.5.1, women reported more balance problems at diagnosis and also demonstrated more PIGD symptoms than men. These findings may reveal an underlying pre-disposition in women which are made relatively worse more rapidly in PD than in men. There was less data available about falls as this was added after the start of data collection. However, even at this early stage, PD patients were at greater risk of falls than controls ($p=0.05$). Similar to the functional assessments which include balance, age (OR 1.38, $p=0.009$) and female gender (OR 2.30, $p=0.003$) were associated with worse performance.

Table 4.25 - Functional motor assessments stratified by age and gender

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[1]
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
Purdue board peg test (mean(sd))[a]:								
• Total pin insertion	28.7 (6.8)	32.9 (6.3)	29.1 (6.3)	26.1 (6.3)	1.56, 1.40-1.74, p<0.001	27.4 (6.5)	31.0 (6.9)	0.44, 0.31-0.60, p<0.001[1]
• Total assembly	17.6 (5.9)	21.6 (6.2)	17.9 (5.3)	15.2 (5.1)	1.64, 1.47-1.83, p<0.001	16.9 (5.7)	18.9 (6.2)	0.70, 0.51-0.96, p=0.03
Flamingo Balance Test (n(%)):								
• Abandoned or <10 seconds	207 (22.6)	14 (7.1)	60 (17.4)	133 (35.7)	1.90, 1.72-2.09, p<0.001	125 (21.2)	82 (25.2)	1.51, 1.15-1.98, p=0.003
• 10 to 29 Seconds	268 (29.3)	26 (13.1)	108 (31.5)	134 (35.9)		172 (29.2)	96 (29.5)	
• 30 seconds	440 (49.1)	158 (79.8)	176 (51.2)	106 (28.4)		293 (49.7)	147 (45.2)	
Get up and go, poor performance (n(%))	271 (29.5)	15 (7.5)	64 (18.6)	138 (36.8)	1.74, 1.53-1.97, p<0.001	128 (21.6)	89 (27.3)	1.66, 1.19-2.32, p=0.003
Falls within last 6 months (n (%))[b]	68 (16.5)	15 (14.3)	17 (10.9)	36 (24.0)	1.32, 1.09-1.61, p=0.004	35 (13.0)	33 (23.4)	2.28, 1.31-3.96, p=0.003

[a]Subjects in lowest 20th centile considered poor performers used for analysis, [b]The falls questionnaire was added after the study started, information from 410 PD cases and 115 controls only was available, [1]Ordinal logistic regression model adjusting for age, gender, disease duration and LEDD, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Figure 4.9 - Purdue total score split by age, gender and group

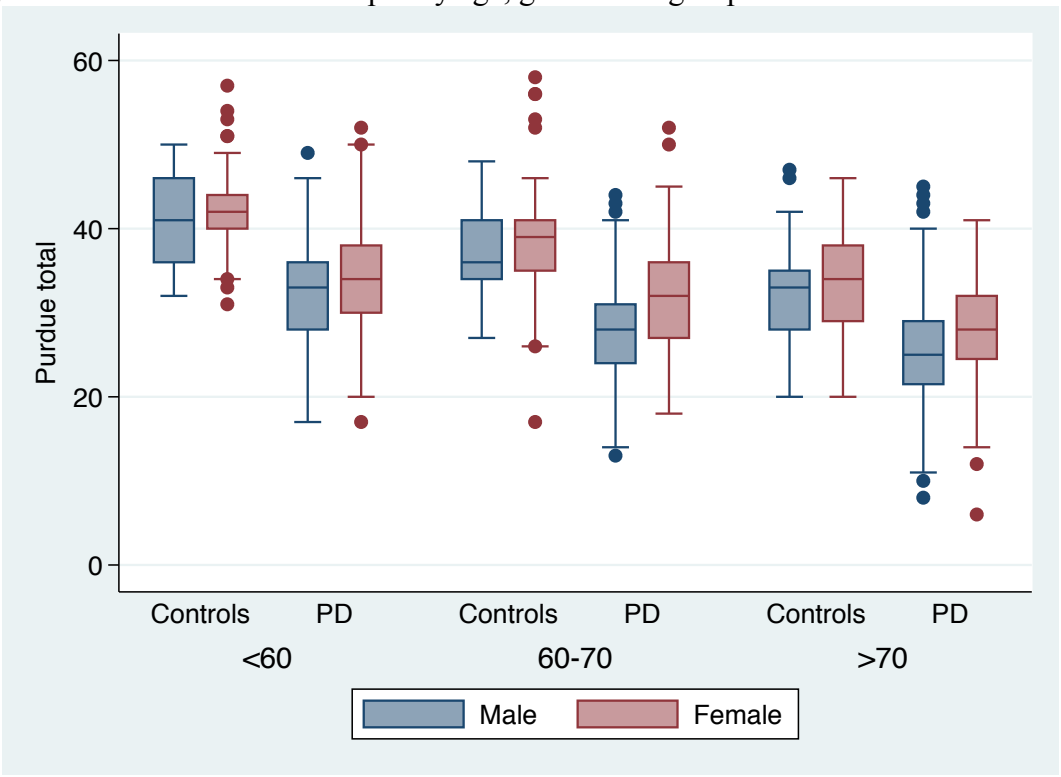
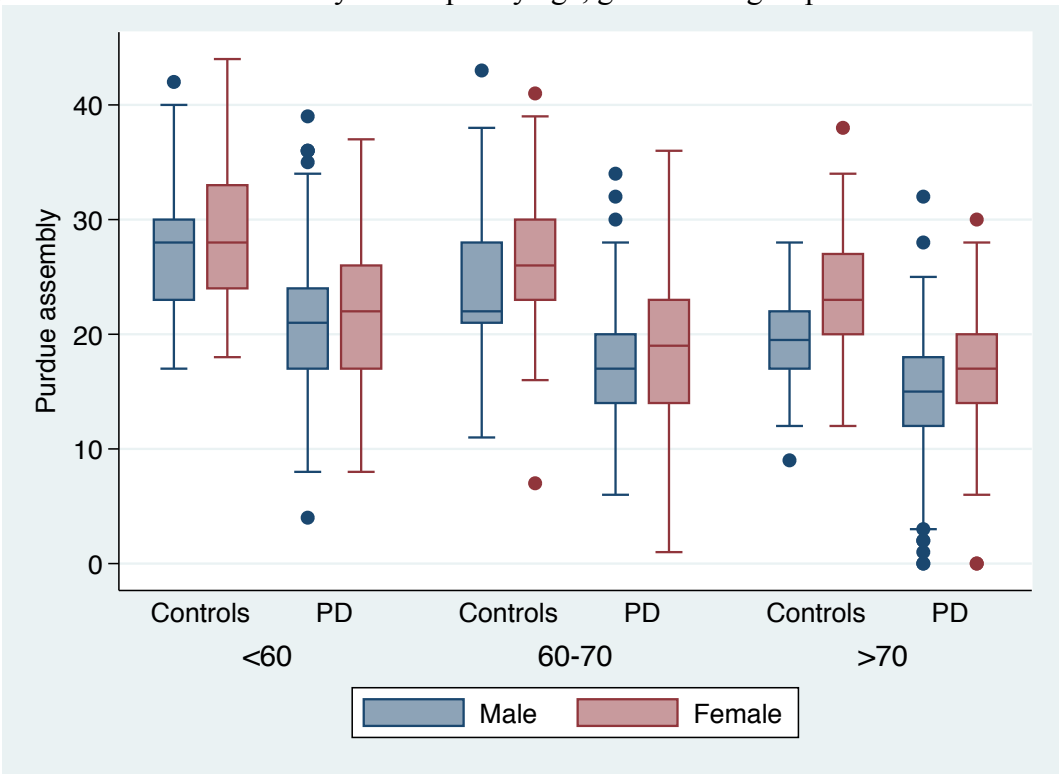


Figure 4.10 - Purdue assembly score split by age, gender and group



4.6 ACTIVITIES OF DAILY LIVING AND QUALITY OF LIFE

Table 4.26 – Group comparison of activities of daily living and quality of life

	PD (n=945)	**Controls** (n=295)	**PD vs Controls** (Odds Ratio, 95% CI, p value)
Schwab and England ADL:			
• Raw score (median (IQR))	90 (90-90)	100 (100-100)	
• No or minimal difficulty with ADL but independent (n (%))	729 (77.3)	287 (97.3)	10.5 (5.1-21.6), p<0.001
• Needs some assistance with ADL (n (%))	154 (16.3)	5 (1.7)	
• Not completely independent (n (%))	60 (6.4)	3 (1.0)	
UPDRS II (motor activities of daily living) (median (IQR))	4 (8-12)		
Quality of life (EQ-5D-3L)[a]:			
• Raw sum score (median (IQR))	7 (6-8)	5 (5-6)	
• Summary index (median (IQR))	0.80 (0.68-0.88)	1 (0.80-1)	3.89, 3.01-5.04, p<0.001

[a]The 5 domains of mobility, self-care, usual activities, pain and mood each on a 3 point scale which was then converted into a summary index score for comparison (range maximum 1 for highest quality of life, minimum -0.28 for lowest)(EuroQol 1990), the summary index was then stratified into quintiles for analysis and inverted (i.e. highest quintile associated with worse quality of life); [1]Ordinal logistic regression used to calculate odds ratios, adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Parkinson's patients had more difficulty with their ADL than controls, and also had a lower quality of life (Table 4.26). As would be expected, older age was associated with more difficulty with ADL and a worse quality of life, but the results for gender were more complicated. There was a trend toward more difficulty with both their ADL as well as their motor ADL. As shown earlier in this chapter, women performed better on cognitive tasks and were more dextrous, but had more difficulties with mood and balance. The Schwab and England questionnaire encompasses ADL broadly and could be affected by both cognition and motor impairment, while the UPDRS II is more

focussed on the motor difficulties. While these scales are broad, it is possible it is more weighted to tasks which affect women more. The alternative is an effect of the method of data collection, as mood may influence how disability is reported. The addition of anxiety or depression in the model reduces the association of gender with disability (OR reduces from 1.37 to 1.25 (95% CI 0.88-1.77, p=0.21)).

The motor activities of daily living score revealed that most (98%) described at least slight motor impairment (median 8, IQR 4-12). The most commonly described symptoms were tremor (80%), trouble getting out (bed, car or deep chair) (64%) walking (57%), dressing (53%) and handwriting (61%). Most of these symptoms were described as slight, with little or no functional impairment. Age and gender made little difference to these scores.

The EQ-5D-3L assesses 5 domains from which its summary score is calculated, one of which is mood (anxiety and depression combined) and so this may account for the worse quality of life outcome in women.

Table 4.27 – Activities of daily living and quality of life measures stratified by age and gender

	Total PD group	**Stratification by age at baseline**			Effect of age (OR, 95% CI, p-value)[1]	**Stratification by gender**		Effect of gender (OR, 95% CI, p-value)[1]
		<60	60-70	>70		Male	Female	
	n=945	n=204	n=356	n=385		n=610	n=335	
Schwab and England ADL:								
• Raw score (median (IQR))	90 (90-90)	90 (90-100)	90 (90-90)	90 (80-90)		90 (90-90)	90 (80-90)	
• No or minimal difficulty with ADL (n (%))	729 (77.3)	175 (86.2)	288 (80.9)	266 (69.3)	1.38, 1.23-1.54, p<0.001	478 (78.6)	251 (74.9)	1.37, 1.00-1.88, p=0.05
• Needs assistance with ADL (n (%))	154 (16.3)	22 (10.8)	50 (14.0)	82 (21.4)		97 (16.0)	57 (17.0)	
• Not completely independent (n (%))	60 (6.4)	6 (3.0)	18 (5.1)	36 (9.4)		33 (5.4)	27 (8.1)	
UPDRS II (mean (SD))	26.5 (10.8)	22.7 (9.6)	25.6 (10.3)	29.2 (11.1)	1.08, 1.00-1.17, p=0.06	27.0 (10.8)	25.5 (10.6)	0.83, 0.66-1.06, p=0.13
Quality of life (EQ-5D-3L)[a]:								
• Raw sum score (mean (SD))		7.0 (1.8)	6.9 (1.7)	7.2 (1.7)	1.07, 1.00-1.17, p=0.08	6.9 (1.7)	7.2 (1.7)	1.46, 1.15-1.86, p=0.002
• Summary index (mean (SD))		0.76 (0.21)	0.76 (0.23)	0.74 (0.21)		0.76 (0.22)	0.73 (0.21)	

[a]Laterality was determined by a UPDRS III score of 4 or greater on one side or symmetry if the difference was less, [1]Ordinal logistic regression used to calculate odds ratios, adjusting for age, gender, disease duration and levodopa equivalent daily dose, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

4.7 BIOCHEMICAL FEATURES AT BASELINE

Participants who were recruited between September 2010-September 2014 were eligible for inclusion. Unfortunately, some serum samples were not available for the biochemical analysis for a variety of reasons (for example difficulty in sample collection or use in another study).

The original grant proposal to fund this project planned to test EGF as a biomarker for cognition in the whole cohort. This was based on initial biomarker discovery work by Chen-Plotkin et al. who had identified EGF as the top analyte from a discovery cohort testing 102 plasma proteins with lower levels predicting a worse performance on cognitive testing.(Chen-Plotkin et al. 2011) This was then replicated in an independent cohort of early PD who found that lower levels were related to cognitive function and performance at 2 year follow up.(Pellecchia et al. 2013) Since then, the author of the original assay has shared unpublished data with our research team which demonstrated that in a larger cohort, the variability between centres were larger than the differences seen between individuals, despite the use of a rigorous protocol. Their preliminary analysis was based on 154 PD cases and 100 control cases from 19 clinical sites. Given this additional information, the decision was made to test for EGF as a pilot in a subset of the cohort before deciding whether to test the whole population, given the limited availability of the serum and the expense of the assay.

Figure 4.11 is a flow chart that outlines the included participants to the study (634 PD cases and 144 controls) and reasons for exclusions. Of these, EGF was tested in 215 PD cases and 95 controls (2 of which were included in the EGF study only).

Figure 4.11 – Flowchart of participants included in the biomarker study

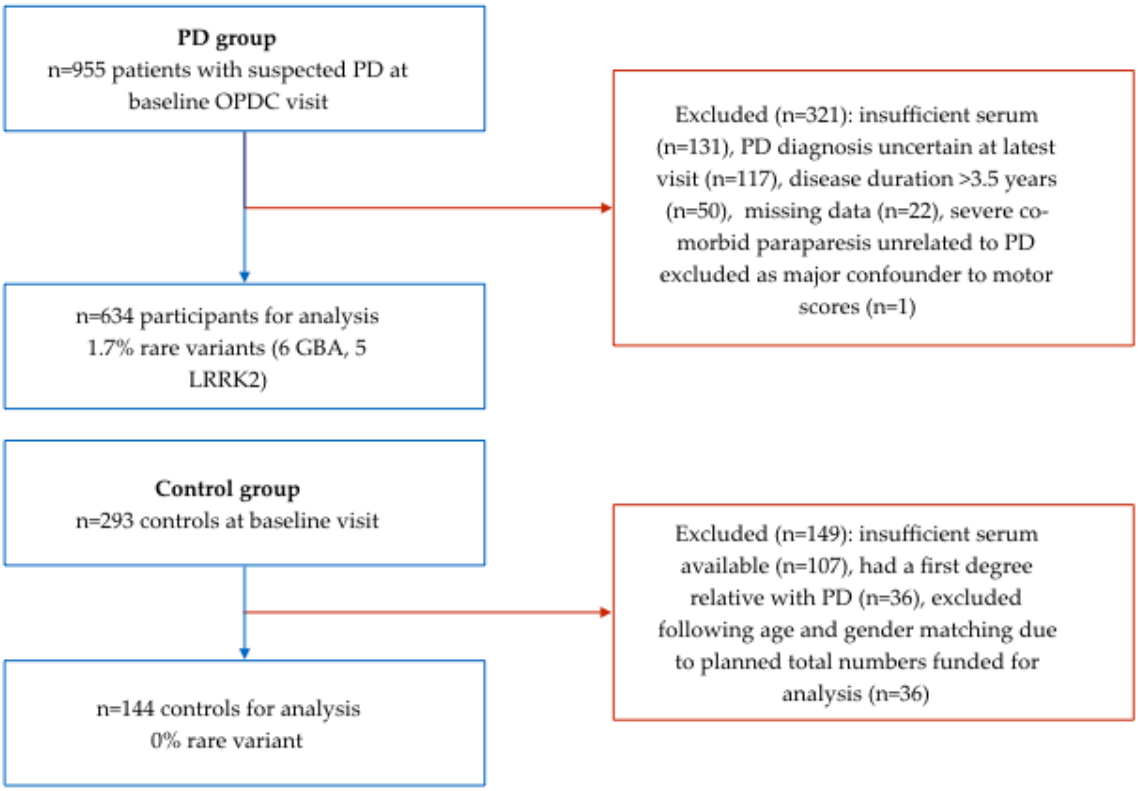


Table 4.28 – Demographics of participants included in the biochemical study

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)**
Age (mean, range (SD))	67.5, 32-89 (10.0)	66.0, 32-88 (11.3)	1.13, 0.80-1.58, p=0.49[1]
Gender (female n (%))	224 (35)	61 (42)	0.75, 0.52-1.08, p=0.12[2]
Ethnicity (non-white n (%))	14 (2)	2 (1)	
First-degree relatives with PD (n (%))	94 (15)	0	
Age of motoric symptom onset (mean, range (SD))	64.6, 28-87 (10.2)	n/a	
Disease duration from PD symptom onset in years (mean, range (SD))	2.9, 0.04-13.9 (1.9)	n/a	
Disease duration from diagnosis in years (mean, (SD))	1.3, 0.01-3.5 (1.0)	n/a	
MDS-UPDRS III (mean (SD))	26.3 (11.2)	2.4 (3.2)	
Hoehn and Yahr Stage (n(%)) 0 1 2 3	0 152 (24) 437 (69) 45 (7)		
Untreated PD (n (%))	75 (12)	n/a	
Levodopa equivalent daily dosage (treated patients only) (mean (SD))	334 (198)	n/a	
Treated participants were on the following medications (n (%))[a]: Levodopa Dopamine agonist MAOB-I	 355 (64) 190 (34) 166 (30)	n/a	
Cognition (Montreal Cognitive Assessment) (median(interquartile range))	25 (23-27)	27 (25-28.5)	

[a]Percentages relate to the number on each drug, some patients are on more than one class of drug. [1]Ordinal logistic regression model adjusting for gender, [2]Logistic regression model adjusting for age

4.7.1 COMPARISONS BETWEEN CASES AND CONTROLS

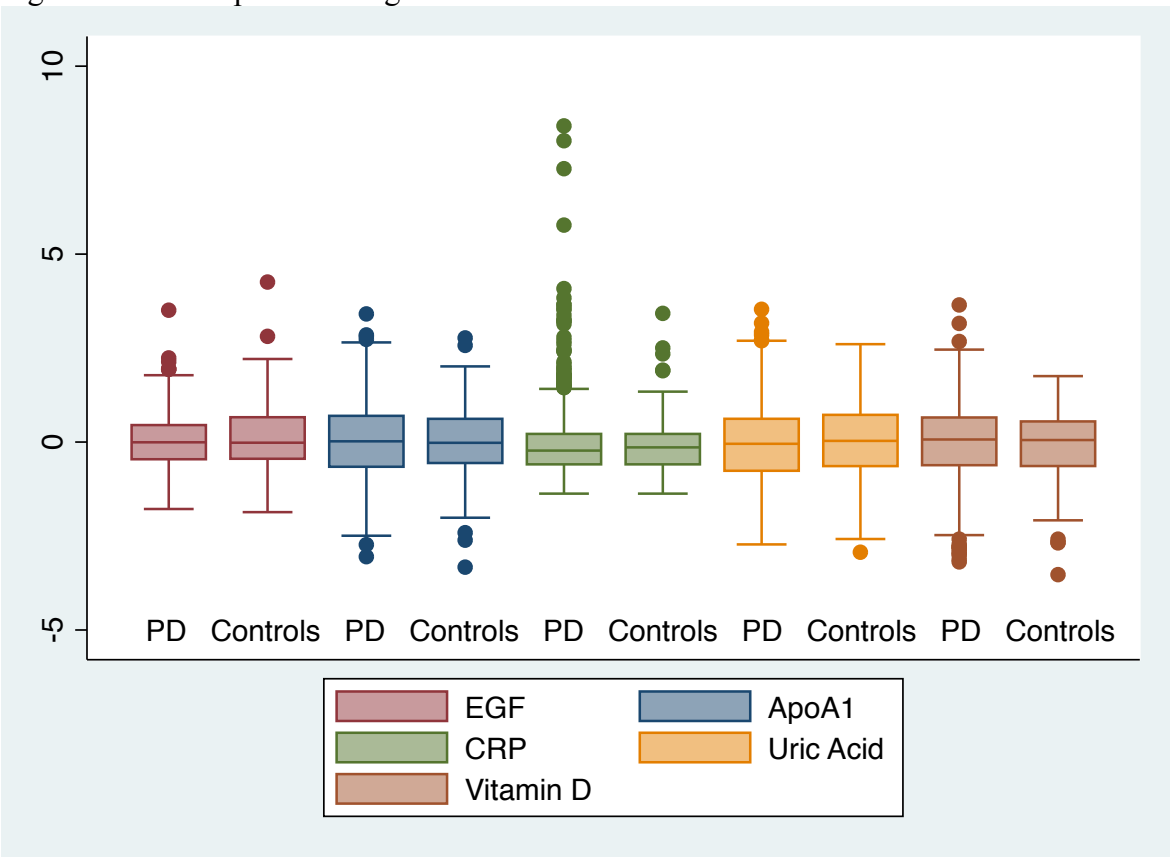
Table 4.29 – Baseline biochemistry results

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
ApoA1 (g/L) (median, IQR)	1.6 (1.4-1.8)	1.6 (1.5-1.8)	1.05, 0.92-1.21, p=0.45
CRP (mg/L) (median, IQR)	1.3 (0.6-2.5)	1.5 (0.6-2.5)	1.00, 0.88-1.13, p=0.98
Uric Acid (umol/L) (median, IQR)	307 (252-358)	313 (262-366)	0.84, 0.73-0.97, p=0.02
Vitamin D (nmol/L)* (median, IQR)	46.1 (31.7-62.4)	46.9 (30.8-59)	1.01, 0.89-1.15, p=0.91
EGF (ng/ml) (median, IQR)[a]	21.8 (12.5-33.8)	21.5 (12.7-40.2)	0.99, 0.84-1.18, p=0.94

[a]Total 341 cases were analysed for EGF, [1]Each biomarker was checked for normality and transformed if necessary (EGF and CRP by square root transformation; *Vitamin D was transformed by log transformation and trigonometric function for season) and then standardised to a mean of 0 and standard deviation of 1. Each biomarker was then stratified into quintiles and added to a logistic regression model to compare cases and controls, adjusting for age and gender.

The control population was similar in age and gender to the PD population. The clinical and demographics of the PD group were similar to the larger cohort (see Table 4.28). There was little difference between groups in each analyte (see Table 4.29 and Figure 4.12). Using regression models adjusting for age and gender, uric acid was lower in the PD group compared to controls (p=0.02). The other analytes were similar between cases and controls. However, the significant overlap precludes this from being useful diagnostically. Vitamin D, CRP and uric acid can also be categorised by established normal ranges into normal, high or low. Comparing these categories between groups in a regression model adjusting for age and gender did not show any differences. In the PD group, age did not make a significant difference to the result of each analyte. As has been reported, both uric acid (higher in men) and ApoA1 (higher in women) showed significant gender differences.

Figure 4.12 – Boxplot showing standardised scores of each biomarker



For each biomarker, the results (y-axis) were normalised (EGF and CRP by square root transformation; Vitamin D by log transformation and trigonometric function) and then standardised to a mean of 0 and standard deviation of 1.

4.7.2 APOLIPOPROTEIN A1

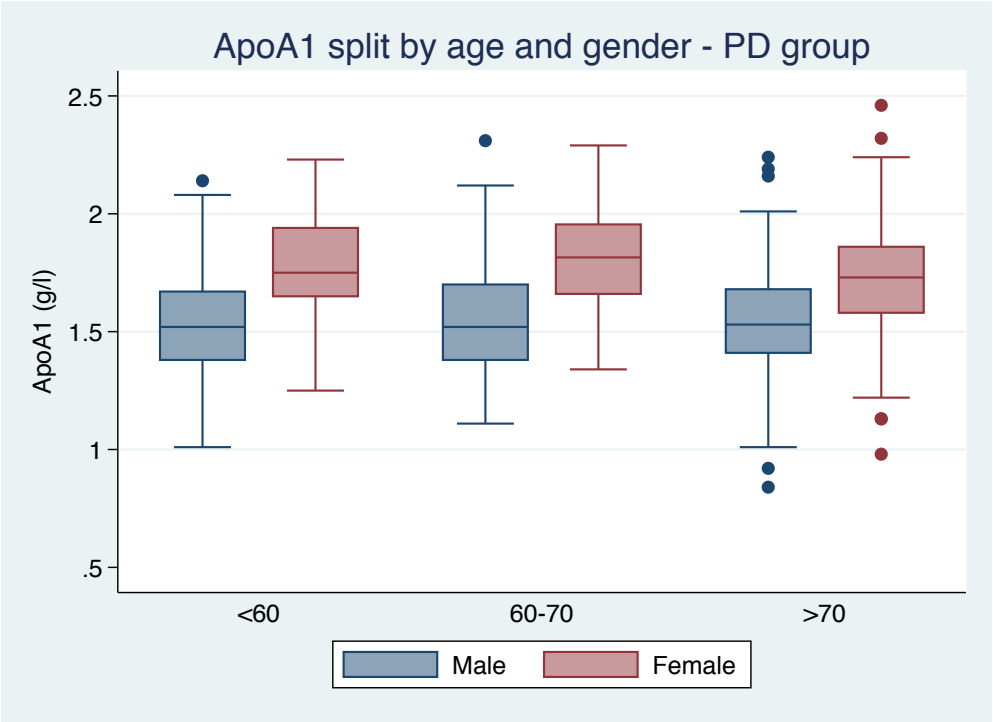
Table 4.30 – Apolipoprotein A1 group comparison

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
ApoA1 (g/L) (median, IQR)	1.6 (1.4-1.8)	1.6 (1.5-1.8)	1.05, 0.92-1.21, p=0.45
ApoA1 level (n (%))[a]: • Normal • Elevated	 452 (92) 38 (8)	 95 (89) 12 (11)	0.62, 0.31-1.24, p=0.18
On lipid lowering statin treatment (n (%))	190 (30)	33 (23)	

[a]Manufacturer guidance states normal ranges, although these are not clinically validated, for men and women older than 60: 0.73-1.83 g/L in men; and 0.91-2.24 in women,
[1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Although there was no apparent association between ApoA1 levels and controls (see Table 4.30) considering the whole group together, there was a difference when comparing the women only (see Figure 4.13). Women had higher circulating levels of ApoA1 which is as expected, thought due to the effects of oestrogen (Jungner et al. 1998). Comparing men only, adjusting for age, between cases and controls did not reveal a significant difference (OR 0.85; 95% CI 0.71,1.02; p=0.08). In women, those with PD had comparatively higher ApoA1 levels (OR 1.43; 95% CI 1.15, 1.77; p=0.001). This result is unexpected as it is contrary to reports in the literature which have associated higher levels of ApoA1 with a reduction in risk of PD or a better prognosis. The addition of the use of statins, which are known to modulate ApoA1 levels, made little difference to the results. Circulating levels of hormones may have affected the results, but most of the cohort were likely post-menopausal given their age. It is possible this is a chance finding or affected by an unknown variable, but a biological basis for this result is unlikely given the findings of similar studies (Jungner et al. 1998). An important limitation is that the ELISA used to measure ApoA1 is reliable for relative quantitation, rather than absolute quantitation, permitting the comparison of sample values within a run, but allowing less confidence between runs.

Figure 4.13 – ApoA1 results split by age and gender: women consistently had higher ApoA1 levels than men across all age groups.



4.7.3 C-REACTIVE PROTEIN

Table 4.31 – CRP group comparison

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
CRP (mg/L) (median, IQR)	1.3 (0.6-2.5)	1.5 (0.6-2.5)	1.00, 0.88-1.13, p=0.98
Elevated CRP (n (%))[a]	67 (11)	13 (9)	1.18, 0.63-2.21, p=0.60

[a]Locally approved normal range for CRP is 0-5mg/L, based on national guidelines and international consensus, [1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Unsurprisingly, this non-specific marker of inflammation was similar across groups, age and gender (see Table 4.31). While inflammatory change has been described in imaging studies in vivo as well as at post-mortem, the markers are not specific for diagnosis. Age and gender were not associated with variations in CRP.

4.7.4 URIC ACID

Table 4.32 – Uric acid group comparison

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
Uric Acid (umol/L) (median, IQR)	307 (252-358)	313 (262-366)	0.84, 0.73-0.97, p=0.02
Uric acid level (n (%))[a]: Low Normal Elevated	 17 (3) 547 (87) 68 (11)	 4 (3) 121 (84) 19 (13)	0.86, 0.53-1.41, p=0.56
Diagnosis of gout (n (%))	37 (6)	10 (7)	
Taking medication to reduce uric acid levels (n (%))	6 (1)	5 (3)	
Taking thiazide medication (n (%))	63 (10)	10 (7)	

[a]Locally approved normal ranges are gender specific: males 210-420 mmol/l; females 150-350 mmolL, [1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Although (comparing across the whole group) lower uric acid levels were associated with PD (see Table 4.32), this association is primarily due to differences in the women. Uric acid levels are influenced by oestrogen, causing the differences between genders (Mumford et al. 2013).

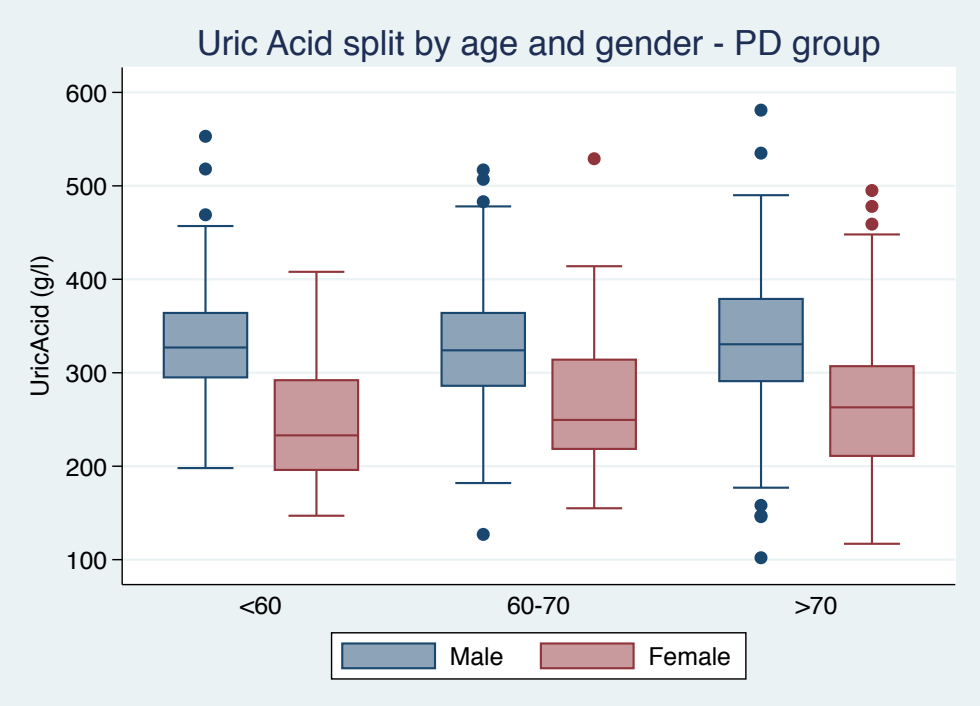
Comparing men only, adjusting for age, between cases and controls did not reveal a significant difference (OR 0.89; 95% CI 0.73,1.07; p=0.21). Comparing only the women showed a potentially greater difference with lower uric acid levels associated with PD (OR 0.80; 95% CI 0.65, 0.98; p=0.03). There was still considerable overlap though (see Figure 4.14), therefore the whole group analysis should be viewed with caution. When analysing a gender specific response, one recent meta-analysis found the association between low uric acid and PD was greater in men (Shen et al. 2013). This has been replicated in a recent well designed prospective study (Gao et al. 2016), while another more inclusive study (with less stringent criteria) found the association greater in women (Wen et al. 2017). Sensitivity analysis by adjusting for medication which lowers uric

acid levels (eg allopurinol) and thiazide medications (known to increase levels) made little difference to the results.

Measuring uric acid is problematic as many factors can alter levels. Exercise, diet, alcohol, body mass index, alcohol consumption, hypertension, and use of diuretics can alter levels. Adjusting for these individually did not alter the results significantly however (Weisskopf et al.).

Higher levels of uric acid are suspected to be protective against neurodegeneration, with the most common complication of hyperuricaemia being gout. We did not, however, find a difference in the rates of gout between the cases and controls in this cohort (regression model adjusting for age and gender: OR 0.78; 95% CI 0.37,1.62; p=0.50).

Figure 4.14 - Uric acid results split by age and gender



4.7.5 VITAMIN D

Table 4.33 – Vitamin D group comparison

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
Vitamin D (nmol/L) (median, IQR)	46.1 (31.7-62.4)	46.9 (30.8-59)	1.01, 0.89-1.15, p=0.91
Vitamin D level (n (%))[a]:			
• Severe deficiency	139 (22)	30 (21)	0.96, 0.76-1.21, p=0.74
• Deficiency	214 (34)	50 (35)	
• Sufficient	279 (44)	64 (44)	
Vitamin D supplementation (n (%))	21 (3)	2 (1)	

[a]Locally approved ranges for Vitamin D are: >50 nmol/L sufficient; 30-50 nmol/L deficiency; <30 nmol/L severe deficiency based on national guidelines and international consensus, [1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

Over half the cohort, either PD or controls, were considered vitamin D deficient by local guidelines (see Table 4.33). In this cohort 56% of participants, whether PD or controls, would be considered deficient which is in line with population studies although these do vary widely (Holick 2009). Comparison between cases and controls revealed no significant differences despite adjusting for age and gender. Further sensitivity analysis by including vitamin D treatment to the model did not affect this result. Age and gender did not influence the results.

Comparing our rates of vitamin D insufficiency with the similarly designed ICICLE-PD study, our control group (46.9 nmol/L; IQR 30.8-59)) was very similar to their results (47.2 nmol/L; IQR 30.6–64.7), but our PD participants had higher levels (46.1 nmol/L; IQR 31.7-62.4) than their study (35.9 nmol/L IQR 25.8–57.0). Despite this, the IQR had considerable overlap. The demographics are comparable between these cohorts and the locations are similar (therefore a comparable number of sunlight hours). The numbers in this other study are smaller (PD, n=129; controls, n=72) so this may have been a chance finding (see chapter 2.5.4.2).

Larger studies in Miami (Wang et al. 2015) (PD, n=478; controls, n=431) and Harvard (Ding et al. 2013) (PD, n=388; controls, n=283) had also showed significant differences. A direct comparison is more difficult as these studies used alternative ranges to define deficiency, <50nmol/L was considered deficient and <75nmol/L insufficient. Also, unlike Europe, milk and orange juice among other foods are fortified with vitamin D in the USA. This is a much larger proportion of deficiency than reported in both Harvard (PD, 17.6%; controls, 9.3%) and Miami (PD, 36%; controls 17%).

This evidence would suggest that vitamin D levels are similar in PD patients and controls. Strengths of this study are the large cohort, representative sample and early stage of the disease. Although we did not account for these potential confounding variables, other studies have suggested that potential biases such as impaired mobility limiting sunlight exposure, intestinal dysmotility inhibiting absorption and the potential interaction with dopaminergic medication have limited if any effect. While multiple smaller studies have suggested an association, this larger, representative cohort provides robust evidence that vitamin D is not associated with an increased risk of a diagnosis of PD.

4.7.6 EPIDERMAL GROWTH FACTOR

Table 4.34 – Epidermal growth factor group comparison

	PD (n=634)	**Controls (n=144)**	**PD vs Control group, OR (95% CI, p-value)[1]**
EGF (ng/ml) (median, IQR)[a]	21.8 (12.5-33.8)	21.5 (12.7-40.2)	0.99, 0.84-1.18, p=0.94

[a]Total 341 cases were analysed for EGF, [1]Ordinal logistic regression of quintiles adjusting for age and gender; [1]Logistic regression adjusting for age and gender, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years)

EGF levels were similar across groups, age and gender. This was only tested on a smaller subgroup of patients for the reasons listed above. As part of this study, we performed a comparison of serum and plasma EGF levels. Although the study by Pellecchia and colleagues (Pellecchia et al. 2013) used serum, the first publication identifying this as a potential biomarker was on plasma. There was no significant correlation between the plasma and serum EGF levels although there was a trend to an inverse relationship (Spearman's rank coefficient -0.44, p=0.08), which has previously been described (Lev-Ran et al. 1990). A potential confounding variable in this study was the lack of a platelet count, only serum was sampled from cohort participants. Higher platelet levels have been positively correlated with higher levels of serum EGF, a finding which we replicated in this study (Spearman's rank coefficient 0.50, p=0.03). These findings, and also the difficulty of replicability of results reported by the Chen-Plotkin group, make EGF unlikely to be a robust biomarker.

5 CLINICAL PREDICTORS OF PROGRESSION IN PARKINSON'S DISEASE

This chapter investigates whether individual or composite clinical markers can predict progression in Parkinson's disease. The primary outcomes are cognition, motor impairment and disability as measured by the MOCA, UPDRS III and the Schwab and England activities of daily living questionnaire, respectively.

The systematic review of clinical predictors (see 2.4.4) describes good evidence that MCI predicts PDD. Older age may predict motor and cognitive decline, male gender may predict more rapid motor progression in the early phases, and cognitive decline may be predicted by RBD symptoms, verbal fluency and memory dysfunction at baseline. Older age at onset and more severe non-tremor motor symptoms (such as PIGD and bradykinesia) at baseline may predict disability. These predictors are tested on the cohort in addition to other potential candidates.

5.1 MEASURES OF PROGRESSION

A summary of the demographics of those attending follow-up is detailed in Table 5.1. Recruitment for new PD participants has closed with follow-up assessments still ongoing. The data included for analysis was downloaded on 16/06/2016. Data from withdrawn participants was not available, although permission has been sought to access patient records for this in the future.

At the time the dataset was closed for this analysis, participants had been followed up for a mean of 2.0 years since study entry, on average 2.2 appointments per individual. This was an average follow-up of 3.3 years after diagnosis. Twelve participants missed their visit 2 appointment and were seen again at their third visit, all other follow-up appointments were consecutive.

The participants, as one might expect, with the longest follow-up tended to be younger but the differences across the groups did not quite reach significance (χ^2, p=0.06). The gender balance remained the same throughout follow-up.

Table 5.1 – Summary of demographics at follow-up assessment

	Visit 1 0 months	Visit 2 18 months	Visit 3 36 months	Visit 4 54 months
Numbers attended	945	682	382	120
Age (mean (SD))	67.2 (9.5)	69.0 (9.2)	70.2 (8.9)	71.0 (9.7)
Gender (female n (%))	335 (35.4)	238 (34.9)	133 (34.8)	44 (36.7)
Disease duration since diagnosis (median (IQR))	1.0 (0.4-2.0)	2.8 (2.1-3.7)	4.5 (3.7-5.5)	6.5 (5.4-7.3)

5.1.1 COGNITIVE IMPAIRMENT

Table 5.2 – Summary of Montreal cognitive assessment (MOCA) results over time

	Visit 1 0 months	Visit 2 18 months	Visit 3 36 months	Visit 4 54 months
Numbers attended	945	682	382	120
MOCA (median (IQR))	25 (23-27)	25 (22-27)	25 (23-27)	25 (23-28)
• Change in MOCA score from Visit 1 (mean (sd))		-0.7 (3.0)	-0.6 (3.0)	-1.1 (3.4)
• Change in MOCA per year from Visit 1 (mean (% change from baseline))		-0.4 (1.6%)	-0.2 (0.8%)	-0.2 (0.8%)
MOCA category[a]: • Normal • MCI • Dementia	 663 (71.1) 142 (15.2) 128 (13.7)	 433 (65.2) 105 (15.8) 126 (19.0)	 253 (68.6) 57 (15.5) 59 (16.0)	 78 (68.4) 14 (12.3) 22 (19.3)

[a]Cognition (MOCA) categories were also used in the regression model for analysis: normal cognition 24-30, mild cognitive impairment 22-23, dementia 0-21

The Montreal cognitive assessment was used to assess cognition, with MCI and dementia categorised using validated ranges (Litvan et al. 2012, Hu et al. 2014). This stability the MOCA over time (see Figure 5.1), with only small changes between each visit, are very similar to published results of similar studies, notably the PPMI (Fereshtehnejad et al. 2017) and DeNoPa (Mollenhauer et al. 2016) cohorts. As this data is only available for those who attended follow-up appointments, it is likely that this is an underestimate of the decline as those who had developed dementia, with associated functional impairment, are more likely not to re-attend. While the overall trend was a decline in MOCA in score over time, some patients did improve on repeat testing between visits (see Figure 5.2).

Figure 5.1 – Distribution of MOCA scores at each visit. The frequency of each score is displayed by the width of the bar. The orange bar represents the median score and the green and red bars represent the upper and lower quartile ranges respectively.

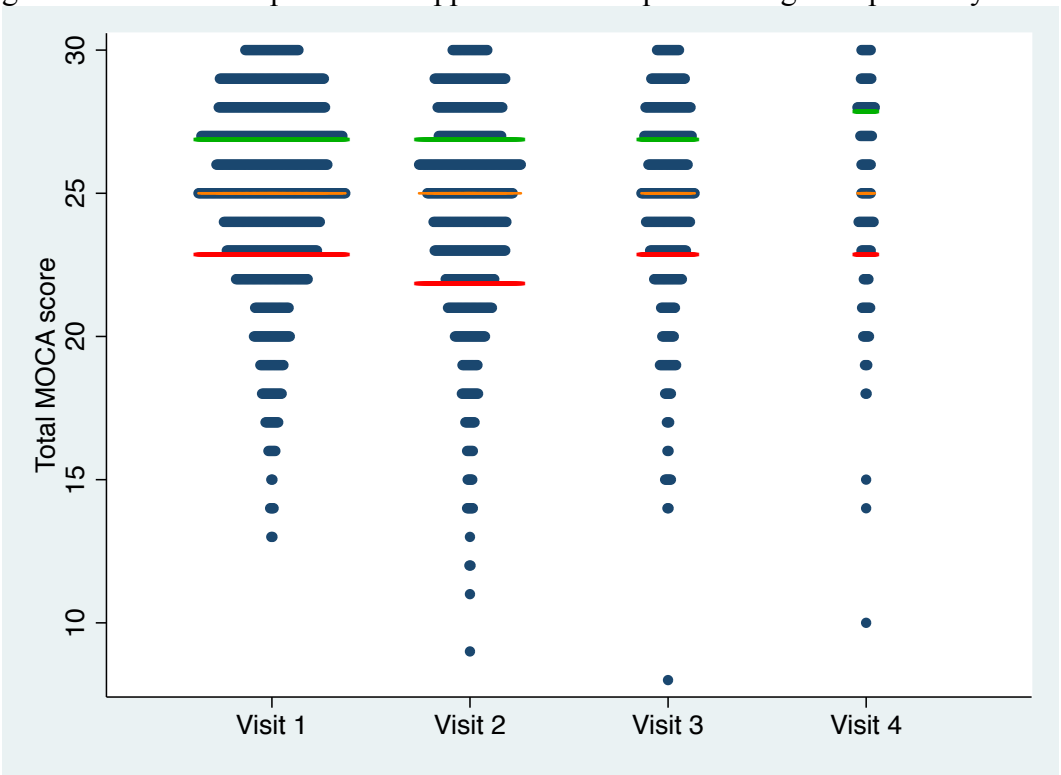
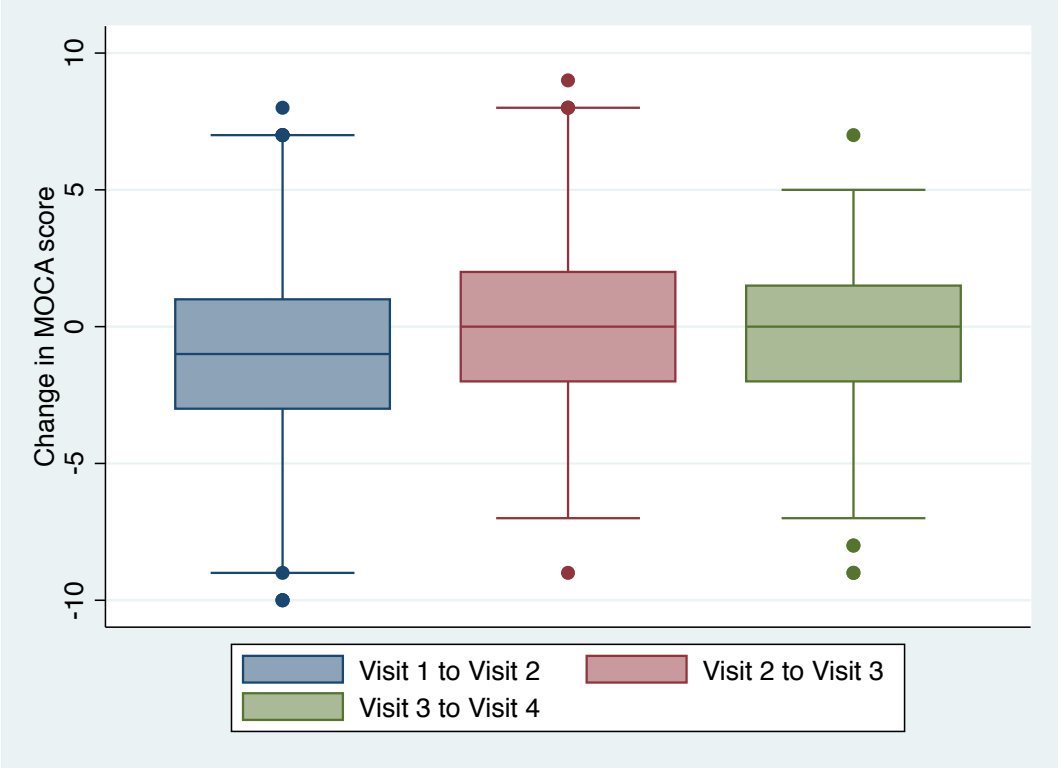


Figure 5.2 – Change in MOCA score between each visit



Using a mixed effect model to analyse the unadjusted effects of disease duration on MOCA (see Table 5.3), one would expect that at time of diagnosis an average patient with PD would score 25 on the MOCA. One would then expect that this would then decline by 0.2 points per year (i.e. 1 point every 5 years).

Table 5.3 – Effect of disease duration on MOCA

Disease Duration	**Association at intercept with disease duration from diagnosis of zero. Estimate (95% CI)**	**Outcome change per year for an increase of disease duration of 1 year. Estimate (95% CI), p-value**
MOCA (total score)	25.2 (24.9, 25.4)	-0.21 (-0.28, -0.14), p<0.001

This mixed effect model was unadjusted.

5.1.1.1 Influence of age and gender

Age and gender may affect progression of PD over time, with older age and male gender thought to predict a more rapid progression of symptoms. The caveat being that ICB and motor fluctuations are reported to be more common in younger patients and mood disorders are more common in women (Picillo et al. 2017).

As would be expected, older age and male gender were associated with a poorer baseline performance on the MOCA. However, while older age predicted a more rapid progression, gender did not seem to affect the rate of progression.

The interpretation here is that for every 5 years older that a patient with PD was diagnosed, one would expect that their MOCA score at diagnosis would be 0.5 point worse. Their performance on the MOCA would then decline by an average of 0.05 points faster per year. Despite its significance level, it remains clinically a small difference. Similarly the effect of gender is small, with a woman of the same age at diagnosis expected to gain a score of 0.7 higher than a man at diagnosis, but then continue to deteriorate at the same rate.

Table 5.4 – The influence of age and gender on cognitive decline

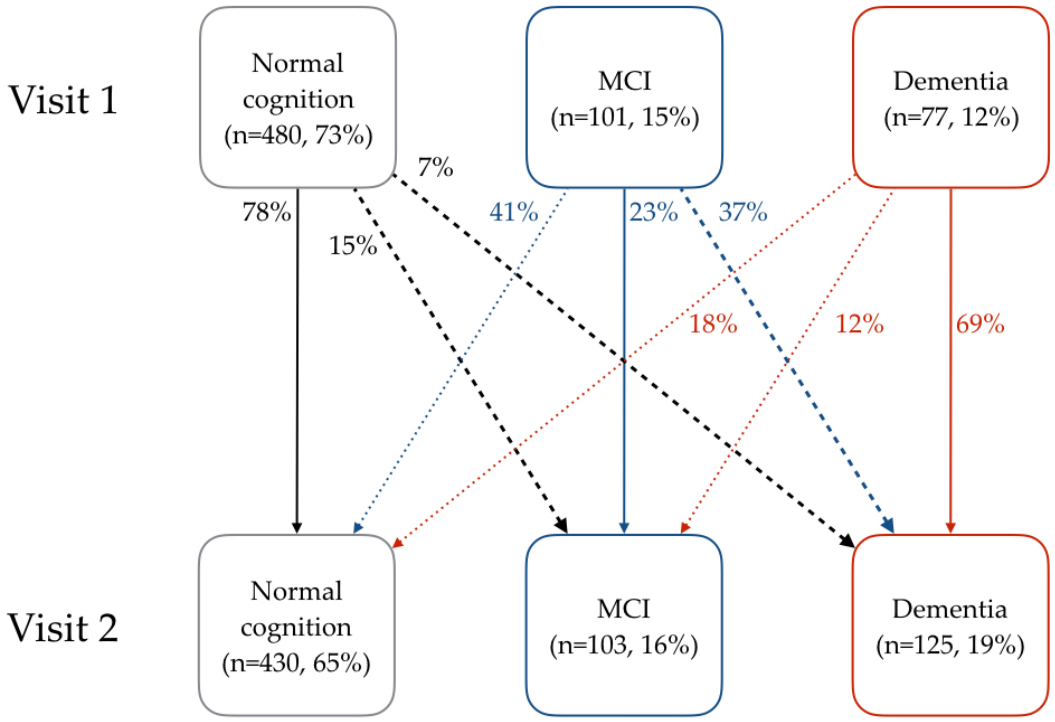
	Association with intercept for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value[1]	**Outcome change per year for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value[1]**	**Association with intercept for female compared males. Estimate (95% CI), p-value[2]**	**Outcome change per year for female gender. Estimate (95% CI), p-value[2]**
MOCA (total score)	-0.51 (-0.63, -0.39), p<0.001	-0.05 (-0.09, -0.01), p=0.02	0.74 (0.25, 1.23), p=0.003	0.04 (-0.11, 0.18), p=0.64

[1]Mixed effect model adjusting for gender and its interaction with time, age was centred on mean age at diagnosis; [2]Mixed effect model adjusting for age and its interaction with time

5.1.1.2 Milestones of cognitive impairment

Due to the modest changes of the MOCA over time, it may be that this may be insensitive to delineating poor performers. An alternative method would be to use a milestone outcome of 'new cognitive impairment' (i.e. new onset MCI or dementia). While this could provide a more clinically relevant measure of outcome, in the absence of a functional measure of cognitive impairment, it would still be limited by fluctuations of performance on the cognitive test. These fluctuations are shown in Figure 5.3, with only patients with data available for both visit 1 and visit 2 included.

Figure 5.3 – Figure displays transition of patients between cognitive categories between the first (baseline) and second visit, only including those with data available for both. Percentages in boxes relate to the percentage of the total (n=658). Percentages with arrows refers to the percentages of participants transitioning between their cognitive category at visit 1 to their cognitive category at visit 2. The solid line means no change between groups, the broken line means deterioration and the dotted line means improvement. Cognition categories: normal cognition 24-30, mild cognitive impairment 22-23, dementia 0-21. Due to the narrow categories, several scores shifted between the two visits.



These published category cut-points for MCI (Litvan et al. 2012) can be considered in the context of the normative data generated by the control group. The mean MOCA for the controls was 26.7 with a standard deviation (SD) of 2.7, making 24 equivalent to 1 SD below the mean and 22 equivalent to 1.5 SD below the mean.

While there is stability between the cognitively normal category from baseline to their first follow-up visit, the narrow category of MCI (MOCA score 22-23) means there is little room for fluctuation within each category. Therefore, many of the patients shifted category in their follow-up visit. Despite 69% of patients scoring in the demented category for both visit, the fact that 14 of these patients (18%) were then categorised as 'normal cognition' at follow-up (i.e. a minimum improvement of 3 points on the MOCA)

highlights the limitations of using cognitive scales in the absence of a functional measure.

Sixty-one (10.5%) of patients who, as per these criteria, were not demented at baseline then screened positive for dementia at their first follow-up visit (visit 2) and remained cognitively impaired (according to the same criteria) on further follow-up (if they attended). These categories could be used to test potential cognitive predictors in regression models, with the outcome of new-onset dementia.

The fluctuations between cognitive categories in this cohort are more evident than the results published by the ICICLE study (Lawson et al. 2017), primarily because of their larger bounds for classifying MCI and additional criteria for a diagnosis of PDD.

5.1.2 MOTOR IMPAIRMENT

Table 5.5 – Summary of UPDRS III scores over time

	Visit 1 0 months	Visit 2 18 months	Visit 3 36 months	Visit 4 54 months
Numbers attended	945	682	382	120
UPDRS III (median (IQR))	25 (18-34)	28 (20-36)	32 (24-42)	27.5 (19-41)
• Change in UPDRS III from Visit 1 (mean (SD))		3.1 (9.9)	8.8 (13.1)	6.2 (14.6)
• Change in UPDRS III per year (mean (% change from baseline))		1.9 (7.2)	2.7 (10.2)	1.3 (4.9)

The UPDRS III motor score is the most commonly used measure of motor progression over time. The change each year is modest, consistent with published studies (Evans et al. 2011, Velseboer et al. 2013, Mollenhauer et al. 2016, Fereshtehnejad et al. 2017). While the overall trend was a progression of symptoms, there was a small improvement at visit 4 compared to visit 3. As with the MOCA scores, this is likely explained by more impaired participants being less likely to attend (see Figure 5.4).

At each visit, the participants were examined in their ‘on’ state. Therefore, one could argue that variability in treatment response is being assessed rather than true disease progression. However, this study is designed to be applicable in a real world setting for applicability in both clinical practice and for drug trials. Artificially withdrawing treatment rather than assessing patients on their conventionally treated state would also be misleading. This information would not be applicable in the clinic and novel therapeutics need to improve on conventional treatment. In addition, from an ethical perspective, withdrawal of treatment could potentially cause harm to the patient. After 5 years from diagnosis, ‘on’ and ‘off’ scores may deteriorate at the same rate (Clissold et

al. 2006), thus, as long as the assessments are consistent this should make little difference.

Figure 5.4 - Distribution of UPDRS III scores at each visit. The frequency of each score is displayed by the width of the bar. The orange bar represents the median score and the green and red bars represent the upper and lower quartile ranges respectively. This graph shows that motor impairment increases modestly between visits.

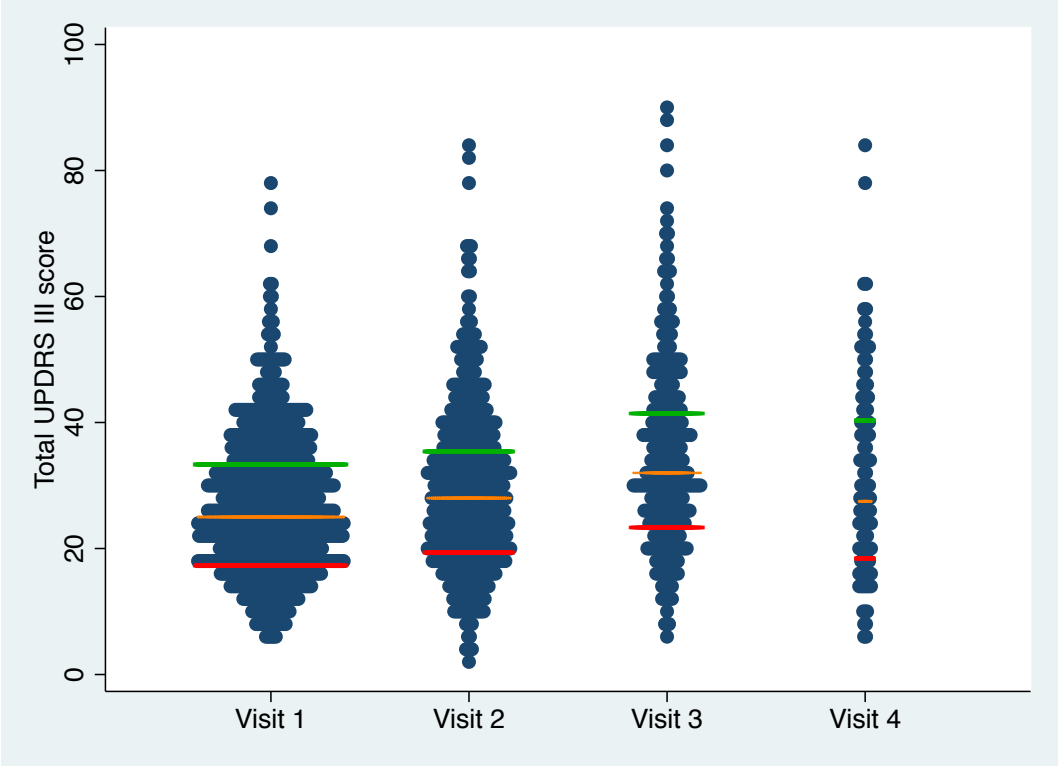


Table 5.6 - Effect of disease duration on UPDRS III

Disease Duration	**Association at intercept with disease duration from diagnosis of zero. Estimate (95% CI)[1]**	**Outcome change per year for an increase of disease duration of 1 year. Estimate (95% CI), p-value[2]**
UPDRS III (total score)	23.9 (23.0, 24.8)	2.14 (1.84, 2.45)

This mixed effect model was unadjusted.

Using a mixed effect model to analyse the unadjusted effects of disease duration on UPDRS III (see Table 5.6), one would expect that at time of diagnosis an average patient with PD would score 24 on the UPDRS III. One would then expect that this would then increase by around 2 points per year.

5.1.2.1 Influence of age and gender

Table 5.7 - The influence of age and gender on motor impairment over time

	Association with intercept for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value	**Outcome change per year for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value**	**Association with intercept for female gender. Estimate (95% CI), p-value**	**Outcome change per year for female gender. Estimate (95% CI), p-value**
UPDRS III (total score)	1.17 (0.72, 1.61), p<0.001	0.15 (-0.01, 0.31), p=0.07	0.37 (-1.39, 2.14), p=0.68	-1.09 (-1.71, -0.47), p=0.001

[1]Mixed effect model adjusting for gender and its interaction with time, age was centred on mean age at diagnosis; [2]Mixed effect model adjusting for age and its interaction with time

Older age was associated with a worse performance at baseline, but the absolute value was small (1 point worse for an increase in age of 5 years). There was also a trend that progression over time was more rapid but again this was a very small absolute difference and consistent with chance.

While gender had no apparent effect on the expected baseline motor impairment, female gender was associated with a less rapid progression (see Figure 5.5 and Figure 5.6). Women were almost entirely stable over the follow-up period, while men worsened over time. The literature in general reports that women may have a more benign phenotype earlier, but subsequently have more problems with motor fluctuations and dyskinesias (Picillo et al. 2017). These data support the former observation.

Figure 5.5 – UPDRS III scores displayed by visit and split by gender

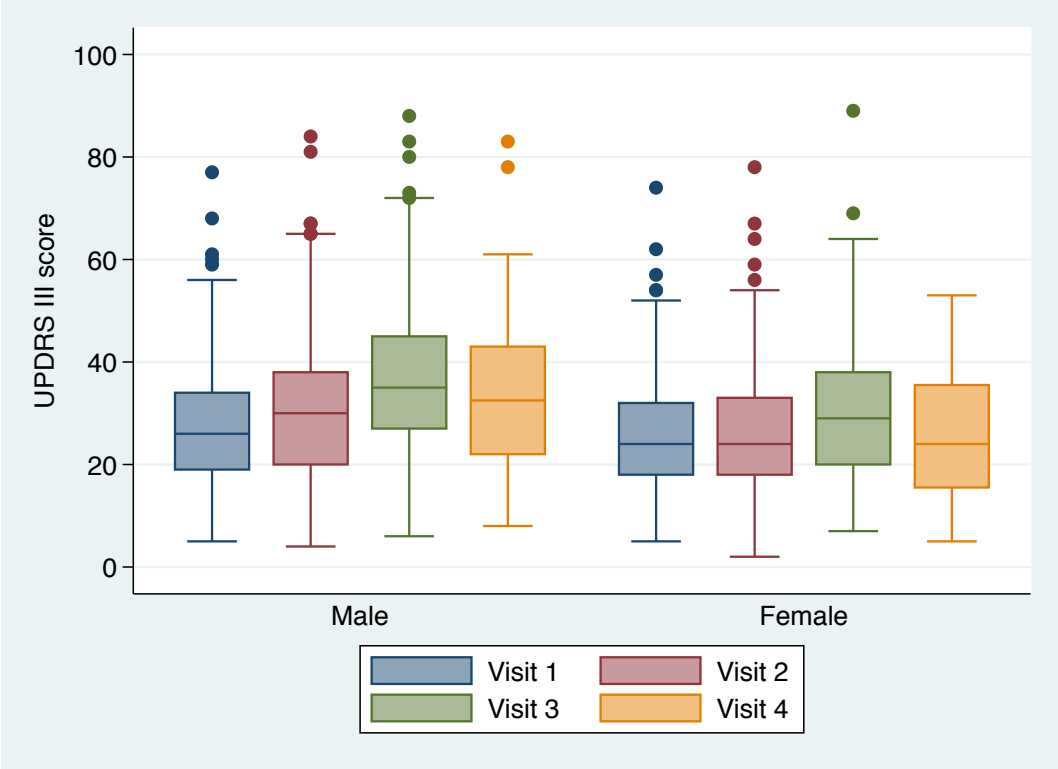
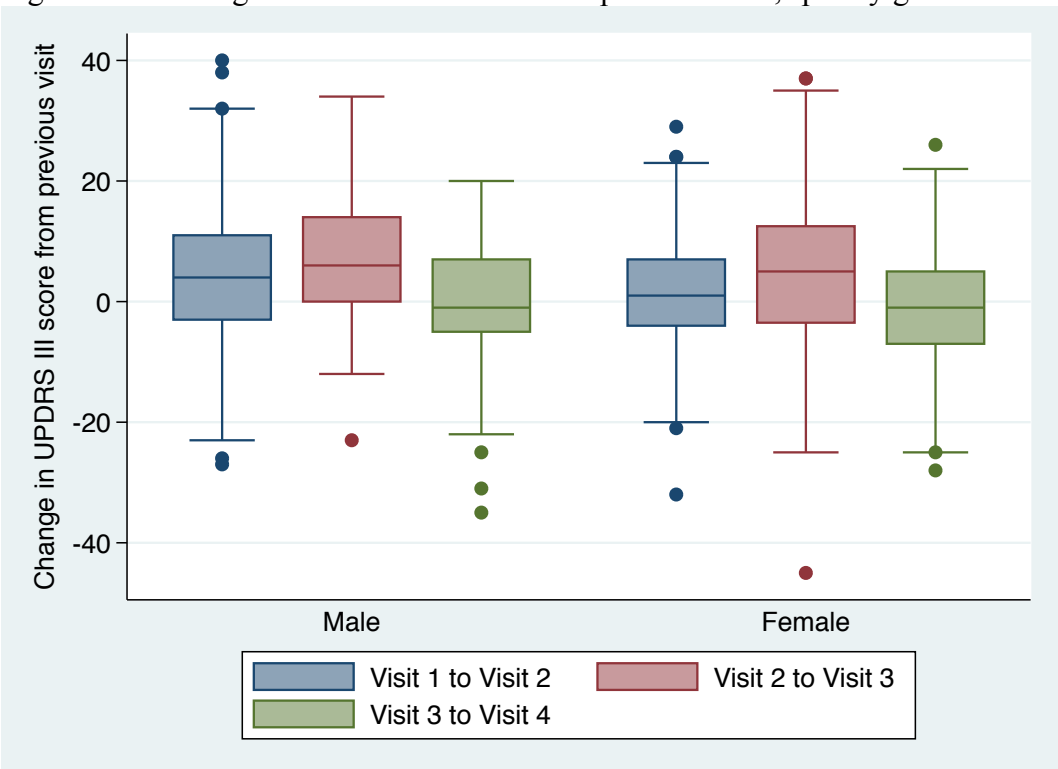


Figure 5.6 – Change in UPDRS III score from previous visit, split by gender

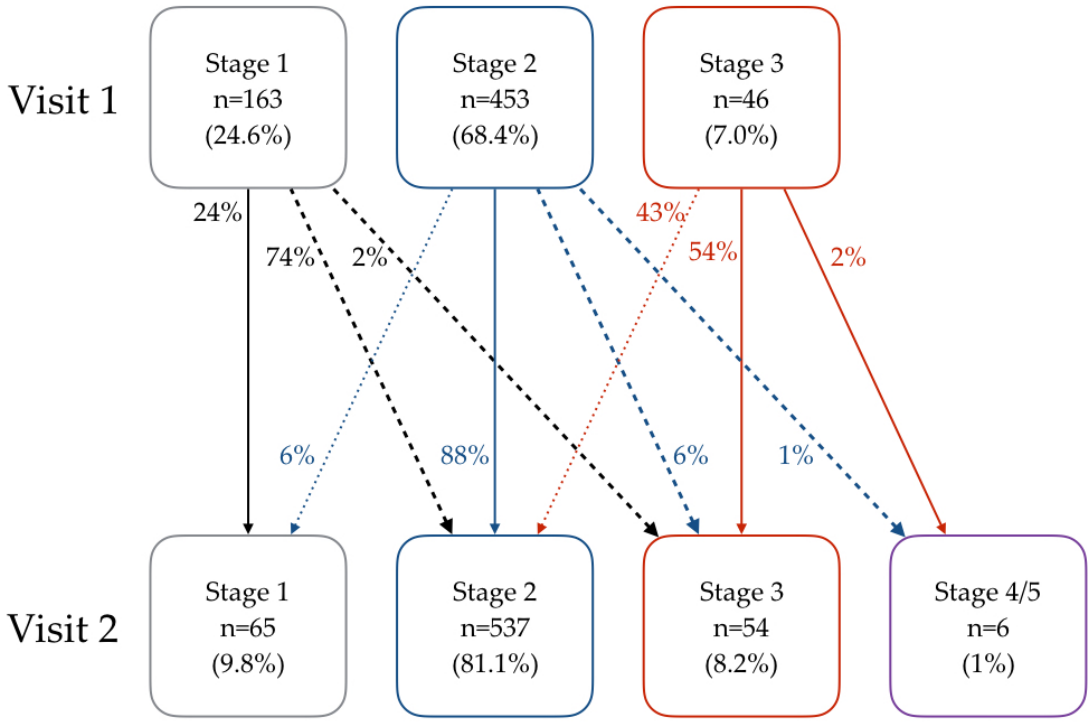


5.1.2.2 Milestones of motor impairment

Hoehn and Yahr staging is the most common staging system for PD. Stage 3, when an individual develops postural instability, is considered a key milestone as this is associated with a functional and cognitive decline. Postural stability is considered to be refractory to conventional treatments, as the impairment is not driven by dopamine deficiency (Albin et al. 2017).

The majority of patients are at stage 2 as expected with this length of follow-up (Zhao et al. 2010), with only patients with data available for both visit 1 and visit 2 included (see Figure 5.7). While it is possible that response to conventional therapies may shift some individuals between stages 1 and 2, it is perhaps surprising that 20 participants improved from stage 3 to stage 2. This may be due to an element of measurement error with some of these cases, day-to-day variability in motor performance, the influence of co-morbidities or potentially inconsistent medical optimisation.

Figure 5.7 – Figure displays transition of patients between Hoehn and Yahr stages between the first (baseline) and second visit, only including those with data available for both. Percentages in boxes relate to the percentage of the total (n=665). Percentages with arrows refers to the percentages of participants transitioning between stages at visit 1 to visit 2. The solid line means no change between groups, the broken line means deterioration and the dotted line means improvement. This graph shows how most participants were stage 2 by visit 2 with a surprising number of patients improving stages between visits (the reasons for which are discussed in the text).



5.1.3 DISABILITY

Table 5.8 – Summary of Schwab and England ADL scores over time

	Visit 1 0 months	Visit 2 18 months	Visit 3 36 months	Visit 4 54 months
Numbers attended	945	682	382	120
Schwab and England ADL (median (IQR))	90 (90-90)	90 (80-90)	90 (80-90)	80 (80-90)
• Change in S&E score from Visit 1 (mean (SD))		-3.3 (11.3)	-6.2 (11.8)	-8.0 (11.5)
• Change in S&E score per year (mean (% change from baseline))		-2.0 (2.3%)	-1.9 (2.1%)	-1.7 (1.9%)
Functional dependency based on S&E scale:				
• No or minimal difficulty with ADL but independent (n (%))	729 (77.3)	429 (63.6)	197 (51.8)	56 (47.1)
• Needs some assistance with ADL (n (%))	154 (16.3)	150 (22.2)	114 (30.0)	43 (36.1)
• Not completely independent (n (%))	60 (6.4)	96 (14.2)	69 (18.2)	20 (16.8)

Even at baseline, while 77% of this early PD cohort were independent, most had at least some difficulty with their simple ADL (see Table 5.8). By Visit 4, only 3 participants described themselves as completely independent with no difficulties. The overall trend of the scale was increasing disability over time, with the rate of progression slowing over time (see Figure 5.8). This apparent slowing of decline may be a reflection of more rapidly progressing participants being unable to attend follow up appointments. This rate of change is consistent with published data (Velseboer et al. 2013, Fereshtehnejad et al. 2017).

Figure 5.8 - Distribution of Schwab and England ADL scores at each visit. The frequency of each score is displayed by the width of the bar. The green and red bars represent the upper and lower quartile ranges respectively, the median value was the same as the upper quartile range at each visit.

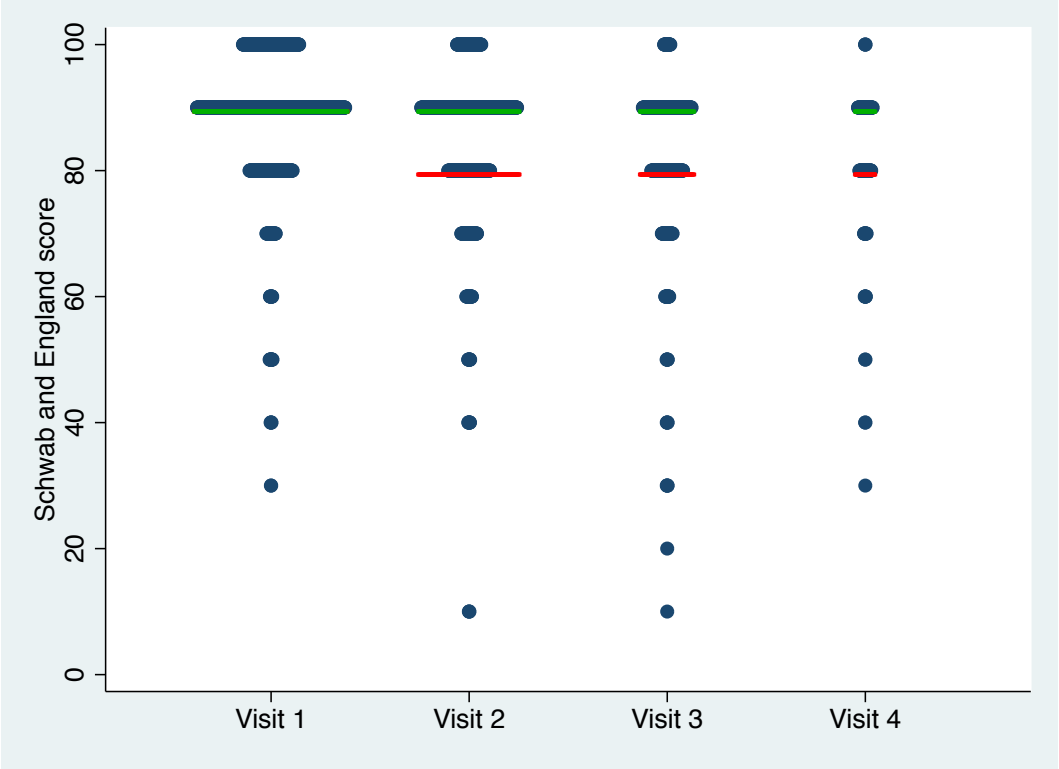


Table 5.9 - Effect of disease duration on disability

Disease Duration	**Association at intercept with disease duration from diagnosis of zero. Estimate (95% CI), p-value**	**Outcome change per year for an increase of disease duration of 1 year. Estimate (95% CI), p-value**
Schwab and England (total score)	90.9 (90.2, 91.7)	-1.92 (-2.22, -1.63)

This mixed effect model was unadjusted.

Using a mixed effect model to analyse the unadjusted effects of disease duration on disability (see Table 5.9), one would expect that at time of diagnosis an average patient with PD would score 90 points on the Schwab and England scale. One would then expect that this would then decline by an additional 2 points per year (i.e. a decline by one unit on the 11 point scale every 5 years).

5.1.3.1 Influence of age and gender

	Association with intercept for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value[1]	**Outcome change per year for each increase of age of diagnosis of 5 years. Estimate (95% CI), p-value[1]**	**Association with intercept for female gender. Estimate (95% CI), p-value[2]**	**Outcome change per year for female gender. Estimate (95% CI), p-value[2]**
Schwab and England (total score)	-0.80 (-1.19, -0.41), p<0.001	-0.31 (-0.46, -0.16), p<0.001	-1.85 (-3.40, -0.31), p=0.02	0.32 (-0.27, 0.91), p=0.29

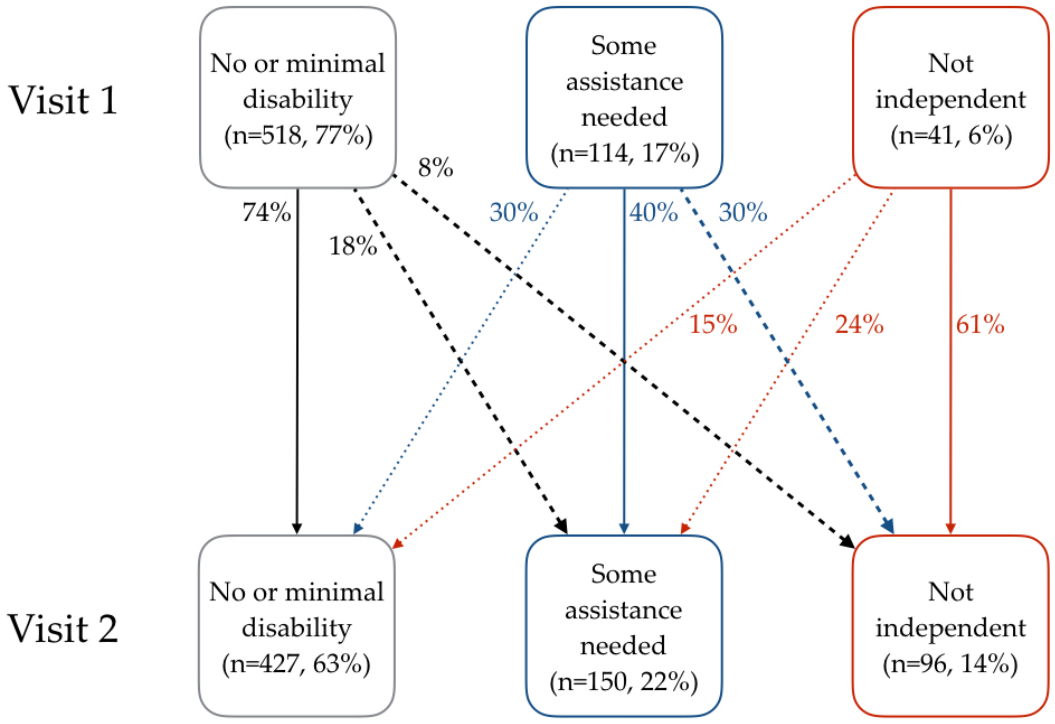
[1]Mixed effect model adjusting for gender and its interaction with time, age was centred on mean age at diagnosis; [2]Mixed effect model adjusting for age and its interaction with time

Age is associated with greater disability at baseline and faster progression. However, the actual difference is very small in clinical terms. Similarly, there was a small difference in baseline disability attributed to gender, but progression over time was similar.

5.1.3.2 Milestones of disability

Dependency is a key functional milestone. Figure 5.9 shows an overall trend of increasing dependency even this early in the disease course. As with the other measures of progression, there were some who improved as visit 2. While the actual number of those who improved from being dependent to minimal disability is small (n=6), it may suggest either improvement in a co-morbidity or medical optimisation of motor symptoms.

Figure 5.9 - Figure displays transition of patients between functional categories between the first (baseline) and second visit, only including those with data available for both. Percentages in boxes relate to the percentage of the total (n=673). Percentages with arrows refers to the percentages of participants transitioning between categories from visit 1 to visit 2. The solid line means no change between groups, the broken line means deterioration and the dotted line means improvement. This flowchart shows how dependency increases over time.



5.2 NON-MOTOR PREDICTORS OF BASELINE AND PROGRESSION OF COGNITIVE, MOTOR AND DISABILITY OUTCOMES

5.2.1 COGNITIVE TASKS

There is good evidence that poor visuo-spatial, verbal fluency and MCI predict earlier cognitive decline or dementia. Early hallucinations may also predict worse outcome. In this analysis the diagnosis of MCI was based on the MOCA, which was also used as the outcome, and thus was omitted as it would be a circular analysis. Cognitive tasks and their association with progression is shown in Table 5.10. Poor performance on each of the tasks, as would be expected, predicted a poorer cognitive outcome at diagnosis, but their sensitivity to rate of progression varied.

Verbal fluencies strongly predicted a worse performance on baseline cognition but did not predict a more rapid decline over time. The average difference in MOCA score at baseline was about 3 points worse for poor performers, which is a clinically relevant difference. This would imply that those with a poor performance on verbal fluency tasks at diagnosis would, assuming a linear decline, continue to perform at a worse level throughout their disease course and thus reach MCI and dementia milestones sooner. Poor fluency ability was not strongly predictive of motor or functional impairment. Sensitivity analysis by categorising fluencies into a categorical variable based on worst 20^{th} and 40^{th} centiles made little difference to the sensitivity of rate of change.

Poor performance on each of the visuo-spatial tasks also predicted poor performance at baseline. Pentagon copying and cube copying were also analysed as binary variables (simply a perfect copy or not) with similar results, but not as sensitive to rate of change. The pentagon and cube copying tasks were similar in their sensitivity to rate of change, although cube copying resulted in narrower confidence intervals improving its

sensitivity. This is probably a benefit of being a more complex task. The interpretation for cube copying predicts that at diagnosis the worst performers (i.e. 2 or more mistakes) would achieve 2 points less on the MOCA and lose an additional point every 5 years compared those who copied the cube correctly.

Cube and pentagon copying also predicted a more rapid motor decline. While cube copying was associated with less motor impairment at baseline and then with a more rapid progression, incorrect pentagon copying predicted worse motor impairment at diagnosis and then more rapid progression. Pentagon copying is the easier task with only 13% of PD participants making mistakes, while 37% failed to copy the cube correctly. Therefore, pentagon copying is only picking out those most affected. These worst performers are presumably the most rapidly progressive subtype across both motor and NMS which are described across all subtyping publications (Lawton et al. 2015, Fereshtehnejad et al. 2017). This is further evidenced by the inability to copy a pentagon predicting more rapid disability.

Errors on the trail task, as with the other cognitive tasks, predicted a worse cognitive performance at diagnosis but not over time. This is potentially in keeping with the hypothesis that while executive dysfunction may cause early cognitive impairment, deterioration into dementia is less rapid than in those with posterior cortical dysfunction (Williams-Gray et al. 2013).

It is interesting to reflect that while cube copying was associated with worse cognitive and motor outcomes, pentagon copying was associated with worse motor and functional ability outcomes. As they are likely underpinned by similar mechanisms, these differences may be due to the method of assessment. As the change over time of each outcome measure is small, a limitation of this model in these circumstances may be more reflective of the fixed effect and less sensitive to the effect over time.

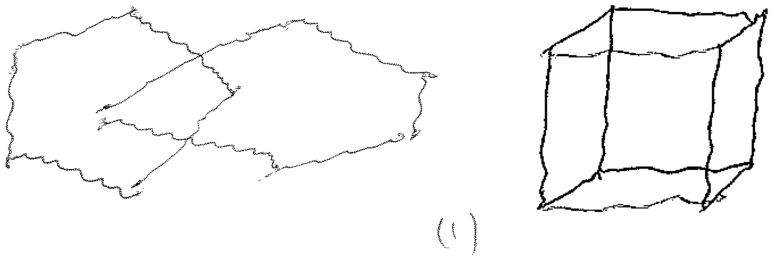
Table 5.10 – The association of baseline cognitive tasks with disease progression

	MOCA (total score)[1]		UPDRS III (total score)[1]		Schwab and England (total score) [1]	
	Intercept coefficient: estimate (95% CI), p-value	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
Semantic fluency	-2.87 (-3.54, -2.21), p<0.001	-0.03 (-0.24, 0.17), p=0.75	2.17 (-0.31, 4.65), p=0.09	0.41 (-0.45, 1.27), p=0.35	-1.97 (-4.16, 0.21), p=0.08	-0.55 (-1.37, 0.28), p=0.19
Phonemic fluency	-3.33 (-3.90, -2.76), p<0.001	0.09, (-0.10, 0.29), p=0.34	1.65 (-0.60, 3.90), p=0.15	0.68 (-0.13, 1.50), p=0.10	-0.40 (-2.36, 1.57), p=0.69	-0.47 (-1.25, 0.30), p=0.23
Pentagon copying*[b]	-1.31 (-1.88, -0.76), p<0.001	-0.16 (-0.33, -0.33), p=0.07	2.28 (0.25, 4.31), p=0.03	0.73 (0.01, 1.45), p=0.05	-2.21 (-3.92, -0.51), p=0.01	-0.81 (-1.43, -0.19) p=0.01
Cube copying*[c]	-1.02 (-1.33, -0.71), p<0.001	-0.11 (-0.20, -0.02), p=0.02	-0.43 (-1.58, 0.72), p=0.47	0.75 (0.35, 1.15), p<0.001	-0.19 (-1.14, 0.77), p=0.70	-0.19 (-0.54, 0.15), p=0.27
Connection errors (cube copying)*[d]	-0.86 (-1.18, -0.54), p<0.001	-0.13 (-0.23, -0.04), p=0.006	-0.54 (-1.71, 0.62), p=0.36	0.65, (0.24, 1.06), p=0.002	-0.03 (-1.01, 0.94), p=0.94	-0.10 (-0.46, 0.26), p=0.41
Clock copying[e]	-0.83 (-1.10, -0.56), p<0.001	0.01 (-0.07, 0.09), p=0.79	1.36 (0.39, 2.33), p=0.006	0.05 (-0.29, 0.39), p=0.77	0.16 (-0.69, 1.01), p=0.71	-0.28 (-0.59, 0.30) p=0.08
Trail task errors[f]	-1.92 (-2.27, -1.56) p<0.001	0.09 (-0.02, 0.20), p=0.10	0.28 (-1.07, 1.63), p=0.69	0.40 (-0.08, 0.87), p=0.10	-0.91 (-2.05, 0.22), p=0.11	-0.19 (-0.60, 0.23), p=0.38

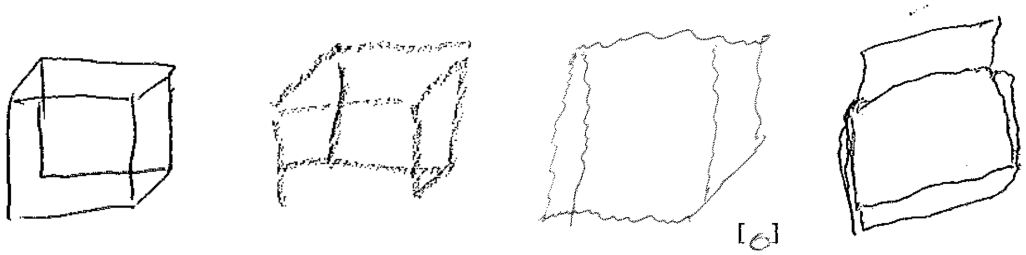
*Data was available for 781 participants; [a]Verbal fluencies were analysed as a binary variable with poor performers <20th centile; [b]Pentagon copying was scored from 0-2 (Ala et al. 2001); [c]Cube copying task was scored from 0-2 (one mark lost for each of 3D construction, all lines present, no extra lines, parallel lines or orientation) (Maeshima et al. 1997); [d]The number of incomplete connection points on the cube copying task were categorised into 0 errors, 1-3 errors, >3 errors; [e]Clock copying was marked 0-3, with 1 point each for clock outline, numbers and hands; [f]The number of errors on the trail task were categorised into 0 errors, 0-4 errors and >4 errors for analysis; [1]Linear mixed effects model using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

Figure 5.10 – Examples of cube and pentagon copying by PD participants:

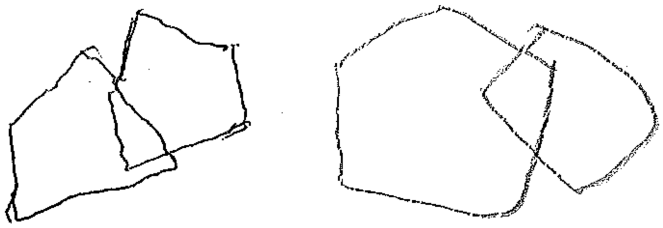
Examples of a good performance:



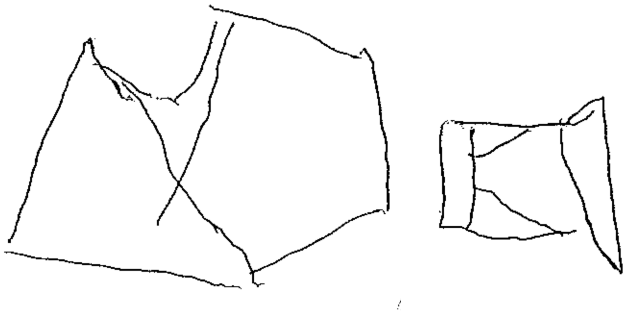
Examples of poor cube copying:



Examples of poor pentagon copying:



Example of participant who scored 0 on both pentagon and cube copying:



5.2.2 HALLUCINATIONS, MOOD AND APATHY AS PREDICTORS OF PROGRESSION

Both anxiety and depression were associated with worse cognitive performance, although depression appeared to have a fixed effect while anxiety had a stronger association with change over time. This result should be viewed with caution as the differences are small and the mood disorders themselves can affect performance on cognitive testing. Hallucinations did not predict a worse cognitive outcome at this stage, but this may be due its relative rarity early in PD. Only11% experienced hallucinations, of which most were considered slight or fleeting with the others only considered mild. Depression, anxiety and apathy were all associated with a worse motor score with an estimated 2.6 to 3.6 points higher on UPDRS III at diagnosis. Again, it is unclear how much this related to the underlying pathophysiology or the symptoms themselves affecting effort during the assessment. There have been reports that dopamine replacement therapies can alleviate mood symptoms in PD, which would fit with an underlying common neurobiological basis, but this is not established (Chaudhuri et al. 2006).

Anxiety and depression were predictors of a significant increase in functional disability. On the Schwab and England scale, an individual with either mood disorder would be expected to be one category lower on this 11 point scale after 5 years, which represents a significant decline in function. Fatigue and apathy were also associated with a functional decline, but not as strongly. These symptoms are disabling in their own right and while, there may be an element of a negative outlook affecting an individual's response, the direct effect is plausible. This particularly highlights the need for recognition and treatment of these disabling symptoms.

Table 5.11 - The association of baseline neuropsychiatric symptoms with disease progression

	MOCA (total score)[1]		**UPDRS III (total score)[1]**		**Schwab and England (total score) [1]**	
	Intercept coefficient: estimate (95% CI), p-value	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
Depression[a]	-0.89 (-1.51, -0.27), p=0.005	-0.05 (-0.23, 0.13), p=0.57	2.60 (0.35, 4.86), p=0.02	0.10 (-0.65, 0.85), p=0.80	-3.98 (-5.94, -2.02), p<0.001	-1.02 (-1.71, -0.3), p=0.004
Anxiety[b]	-0.55 (-1.19, 0.09), p=0.09	-0.22 (-0.41, -0.03), p=0.02	3.89 (1.58, 6.20), p=0.001	0.18 (-0.62, 0.98), p=0.66	-3.84 (-5.86, -1.81), p<0.001	-1.11 (-1.87, -0.35), p=0.004
Hallucinations[c]	-0.03 (-0.79, 0.74), p=0.95	-0.06 (-0.26, 0.14), p=0.57	0.88 (-1.88, 3.64), p=0.53	0.01 (-0.86, 0.87), p=0.99	-2.45 (-4.91, 0.002), p=0.05	0.11 (-0.72, 0.93), p=0.80
Apathy[c]	-0.11 (-0.70, 0.47), p=0.71	-0.05 (-0.23, 0.14), p=0.63	3.55 (1.45, 5.64), p=0.001	-0.57 (-1.34, 0.20), p=0.15	-1.80 (-3.66, 0.54), p=0.06	-0.75 (-1.49, -0.01), p=0.05
Fatigue[c]	-0.13 (-0.64, 0.38), p=0.62	-0.06 (-0.22, 0.09), p=0.41	0.88 (-0.96, 2.72), p=0.35	0.35 (-0.29, 0.99), p=0.29	-2.29 (-3.88, -0.69), p=0.005	-0.70, (-1.30, -0.09), p=0.03

[a]Beck Depression Inventory II: minimal depression 0-13, mild depression 14-19, moderate depression 20-28, severe depression 29-63; [b]Positive anxiety screen if LADS ≥7, [c]Present or absent, based on UPDRS I; [1]Linear mixed effects model using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

5.2.3 GASTROINTESTINAL AND AUTONOMIC SYMPTOMS AS PREDICTORS OF PROGRESSION

Hypersialorrhoea predicted a modest decline in cognitive function. However, almost half the cohort described at least slight symptoms. Considering only those with at least mild symptoms (a UPDRS score of >1), the estimate of the slope coefficient is similar but reduces slightly from -0.23 to -0.19 (p=0.02). The mechanisms underlying this are unclear but while previously it was thought to be an over-production of saliva, current opinion is that it is probably multi-factorial with clearance being a major driver (Karakoc et al. 2016). The symptoms are not thought to be dopamine responsive with autonomic dysfunction thought to be the underlying pathophysiology. The lack of association of either orthostatic hypotension or urinary disturbance with cognitive impairment makes it unlikely to be an association with autonomic dysfunction more broadly. This association has not been described in the literature and thus should be viewed with caution.

Chewing and swallowing difficulty, hypersialorrhoea and urinary disturbance were each associated with a worse motor and functional impairment as a fixed effect, but not more rapid progression over time. Historically these features were thought to occur in more advanced disease, but are now described more commonly earlier.

Table 5.12 - The association of baseline gastrointestinal and autonomic symptoms with disease progression

	MOCA (total score)[1]		**UPDRS III (total score)[1]**		**Schwab and England (total score) [1]**	
	Intercept coefficient: estimate (95% CI), p-value	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
Constipation[a]	-0.56 (-1.04, -0.09), p=0.02	0.03 (-0.11, 0.18), p=0.64	0.41 (-1.30, 2.13), p=0.64	0.10 (-0.50, 0.71), p=0.73	-0.55 (-2.05, 0.95), p=0.48	-0.12 (-0.70, 0.46), p=0.68
Chewing and swallowing difficulty[b]	-0.42 (-1.01, 0.17), p=0.17	-0.10 (-0.27, 0.08), p=0.17	3.14 (1.01, 5.28), p=0.004	0.43 (-0.29, 1.15), p=0.24	-5.49 (-7.33, -3.64), p<0.001	-0.22 (-0.90, 0.46), p=0.52
Hypersialorrhoea[b]	0.17 (-0.30, 0.65), p=0.47	-0.23 (-0.37, -0.08), p=0.002	3.96 (2.27, 5.65), p<0.001	-0.09 (-0.69, 0.51), p=0.77	-2.69 (-4.17, -1.20), p<0.001	-0.31 (-0.88, 0.27), p=0.29
Orthostatic hypotension[c]	-0.20 (-0.81, 0.42), p=0.53	-0.08 (-0.26, 0.10), p=0.38	0.56 (-1.63, 2.78), p=0.61	0.18 (-0.57, 0.93), p=0.63	-1.58 (-3.49, 0.34), p=0.11	-0.04 (-0.75, 0.68), p=0.92
Urinary disturbance[b]	-0.22 (-0.70, 0.27), p=0.39	0.15 (-0.003, 0.30), p=0.05	2.97 (1.21, 4.73), p=0.001	-0.13 (-0.75, 0.49), p=0.68	-2.34 (-3.88, -0.81), p=0.003	0.002 (-0.59, 0.60), p=0.99

[a]Constipation: positive if <1 daily bowel movement or use of laxatives; [b]Present or absent, based on UPDRS I; [c]Positive result if systolic drop of blood pressure (BP) of ≥20mmHg or a diastolic drop of ≥10mmHg; [1]Linear mixed effects model using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

5.2.4 SLEEP, SENSORY AND TOTAL NUMBER OF NON-MOTOR SYMPTOMS AS PREDICTORS OF PROGRESSION

RBD in PD has been associated with worse cognition, whether assessed by polysomnography or by screening questionnaire (Rolinski et al. 2013). Again, the presence of RBD was associated with a poor cognitive performance at diagnosis, but not with a subsequent more rapid progression. Patients with RBD without PD already have cognitive deficits similar to early PD (Rolinski et al. 2016) which fits with this analysis. Thus, while it appears to be a fixed effect, it would likely predict an earlier dementia. The effects of daytime somnolence has been associated with cognitive impairment before, but not as commonly as RBD (Zhu et al. 2014), but appear to have a stronger inverse association with cognition and functional ability than RBD. It's possible that being somnolent may cause individuals to underperform on cognitive testing which leads to this association. In addition, daytime somnolence is more disabling than RBD in performing ADL. A potential confounder is that it is a side effect of dopaminergic medication and the apparent association is in fact just a reflection of a higher LEDD. Understandably, pain limited ADL at diagnosis and predicted subsequent progressive disability. However, it is important to note that this measure did not take into account the nature of the pain symptoms.

More NMS strongly predicted worse cognitive, motor and functional impairment at diagnosis. This reflects the multi-system nature of PD and the broad spectrum of NMS which are not only interlinked with cognition and motor dysfunction but are responsible for disability in itself. Vascular risk and individual personality traits did not predict more rapid progression in early PD, while diabetes had a small effect on disability over time only (data not shown).

Table 5.13 - The association of baseline sleep and sensory symptoms with disease progression

	MOCA (total score)[1]		UPDRS III (total score)[1]		Schwab and England (total score)[1]	
	Intercept coefficient: estimate (95% CI), p-value	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
Sleep						
RBD (RBD Sleep Questionnaire)[a]	-0.71 (-1.21, -0.21), p=0.006	0.06 (-0.09, 0.21), p=0.43	-0.66 (-2.49, 1.18), p=0.48	0.32 (-0.31, 0.95), p=0.32	-1.39 (-2.93, 0.15), p=0.08	0.19 (-0.41, 0.78), p=0.54
Daytime somnolence (ESS)[b]	-0.76 (-1.32, -0.20), p=0.007	-0.16 (-0.32, 0.01), p=0.06	1.60 (-0.39, 3.59), p=0.12	0.45 (-0.23, 1.14), p=0.19	-1.98 (-3.72, -0.23), p=0.03	-0.62 (-1.27, 0.03), p=0.06
Sensory						
Hyposmia	-0.61 (-1.24, 0.02), p=0.06	-0.10 (-0.29, 0.09), p=0.30	-0.43 (-2.73, 1.86), p=0.71	0.80 (0.01, 1.60), p=0.05	-1.12 (-3.10, 0.87), p=0.27	0.40 (-0.35, 1.14), p=0.30
Pain	-0.63 (-1.10, -0.16), p=0.009	0.06 (-0.09, 0.20), p=0.43	2.01 (0.32, 3.71), p=0.02	-0.01 (-0.61, 0.59), p=0.97	-3.98 (-5.44, -2.53), p<0.001	-0.62 (-1.17, -0.05), p=0.03
Total NMS	-0.80 (-1.08, -0.51), p<0.001	0.05 (-0.04, 0.13), p=0.27	1.56 (0.52, 2.61), p=0.003	0.24 (-0.12, 0.60), p=0.20	-2.51 (-3.42, -1.60), p<0.001	-0.35 (-0.69, 0.01), p=0.05

[a]RBD (Rapid Eye Movement Sleep Behaviour Disorder): positive screen if ≥6; [b]Daytime somnolence (Epworth Sleepiness Scale): positive screen for daytime somnolence if ESS≥10; [c]Present or absent, based on UPDRS I; [d]Pain (EQ5D): positive if rated moderate or extreme; [e] Total number of NMS (maximum 18) refers to the sum of positive assessments for: cognition, hallucinations and psychosis, apathy, fatigue, anxiety, depression, impulsivity, orthostatic hypotension, urinary disturbance, hypersialorrhoea, pain, hyposmia, daytime sleepiness, RBD, disturbed sleep, sexual dysfunction, swallowing and constipation. This was stratified into categories of 0-6, 7-8 or >8 for analysis; [1]Linear mixed effects model using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

5.3 MOTOR PREDICTORS

Postural instability, PIGD phenotype and the get up and go test are intrinsically similar and each predicts a worse cognitive function at baseline, consistent with multiple studies. The association of motor complications with worse cognition is not clear, because at diagnosis it predicts a better score followed by a slightly more rapid progression. This is likely not significant as the overall effect would be approximately neutral after 4 years from diagnosis, with further extrapolation for several years after that beyond the validity of the current data. The Purdue assembly task predicted both a worse outcome at baseline and a more rapid progression. This association has been reported before and may imply a more global impairment or be a sign of poor attention signalling cognitive dysfunction (Anang et al. 2014).

The timed motor assessments and postural instability each predicted greater motor impairment at diagnosis, with the Purdue assembly task having the largest fixed effect. Postural instability showed a trend toward predicting a more rapid progression. Postural instability, PIGD phenotype and the get up and go test each had a large fixed effect, but only the get up and go test was associated with more rapid progression following this. Each of the timed motor tests predicted a more rapid decline, but the get up and go test showed the strongest association.

Falls did not predict outcomes, but data was only available for 411 participants (data not shown). Motor symmetry, bradykinesia, tremor and postural signs, based on UPDRS III scores, did not predict cognitive decline, but were associated with a greater disability at diagnosis.

Table 5.14 - The association of baseline motor symptoms with disease progression

	MOCA (total score)[1]		UPDRS III (total score)[1]		Schwab and England (total score)[1]	
	Intercept coefficient: estimate (95% CI), p-value	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
Motor complications[a]	1.24 (0.26, 2.22), p=0.01	-0.32 (-0.59, -0.05), p=0.02	0.51 (-3.04, 4.06), p=0.78	-0.66 (-1.79, 0.46), p=0.25	-2.76 (-5.99, 0.48), p=0.10	-0.49 (-1.56, 0.57), p=0.36
PIGD phenotype	-0.78 (-1.28, -0.27), p=0.003	-0.03 (-0.19, 0.12), p=0.68	-0.37 (-2.26, 1.51), p=0.70	0.25 (-0.41, 0.91), p=0.46	-6.35 (-7.97, -4.73), p<0.001	-0.03 (-0.66, 0.60), p=0.93
Postural instability[b]	-0.54 (-1.06, -0.02), p=0.04	-0.10 (-0.25, 0.05), p=0.18	4.49 (2.63, 6.34), p<0.001	-0.61 (-1.24, 0.02), p=0.06	-4.69 (-6.31, -3.08), p<0.001	-0.49 (-1.07, -0.10), p=0.10
Timed motor tests						
Purdue assembly	-0.74 (-1.27, -0.21), p=0.006	-0.25 (-0.41, -0.09), p=0.002	5.61 (3.73, 7.49), p<0.001	0.01 (-0.66, 0.67), p=0.98	-1.81 (-3.49, -0.14), p=0.03	-1.05 (-1.67, -0.42), p=0.001
Flamingo	-0.47 (-1.09, 0.15), p=0.14	-0.13 (-0.33, 0.07), p=0.20	3.87 (1.67-6.08), p=0.001	0.31 (-0.49, 1.11), p=0.45	-1.54 (-3.49, 0.42), p=0.12	-0.94 (-1.70, -0.17), p=0.02
Get up and go test	-0.98 (-1.57, -0.38), p=0.001	-0.17 (-0.35, 0.01), p=0.06	5.11 (3.00, 7.23), p<0.001	0.59 (-0.15, 1.32), p=0.12	-4.92 (-6.67, -3.07), p<0.001	-0.97 (-1.66, -0.28), p=0.006

[a]The presence of any motor complication including on/off fluctuations and dyskinesias as rated by the UPDRS IV; [b]Present or absent, based on UPDRS III; [1]Linear mixed effects model using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

5.4 SUMMARY OF CLINICAL PREDICTORS

Poor performance on visuo-spatial tasks and the Purdue assembly task robustly predicted worse cognitive outcomes both at baseline and over time (see Figure 5.11 and Figure 5.12).

This work reproduces the findings of the Campaign cohort, in which early visuo-spatial dysfunction predicted an earlier dementia (Williams-Gray et al. 2009). This is an important result, as the previous study, while well designed, was much smaller (n=147) thus reproducibility in a much larger cohort adds considerable weight to the evidence.

Fluencies predicted a significantly worse cognitive outcome at diagnosis and thus likely an earlier dementia, but not over time, which may be due to limitations of this model.

The association between performance on the Purdue assembly task and cognition is perhaps surprising, given that it is a measure of dexterity. This association has been shown before, albeit in more advanced disease (Anang et al. 2014). Given its association with disability also, it may indicate a more rapid global progression.

Anxiety was associated with worse cognitive and functional ability scores, but this should be viewed with a little caution as the symptoms themselves may affect the rating of each of these measures. Similarly, this may be true for the association of depression with disability. However, each of these symptoms is disabling in their own right.

Get up and go was a good predictor of disability, which makes intuitive sense as it is a sensitive measure of mobility. Postural instability was associated with disability over time and all 3 outcomes at diagnosis. Taken in isolation, it appeared a more sensitive predictor of motor impairment and disability than the PIGD phenotype, but not cognition.

A number of other tasks predicted worse baseline outcomes, but the actual effect in many of these was small, and may not be clinically relevant.

Figure 5.11 – This diagram shows which clinical assessments were associated with a worse performance on each of the 3 tested outcomes at diagnosis. All those with a significance level of $p<0.05$ are included, the tasks with a significance level of $p<0.02$ are in capitals. *Cognitive tasks include: semantic fluency, phonemic fluency, cube copying, connection errors on cube copying, trail task errors

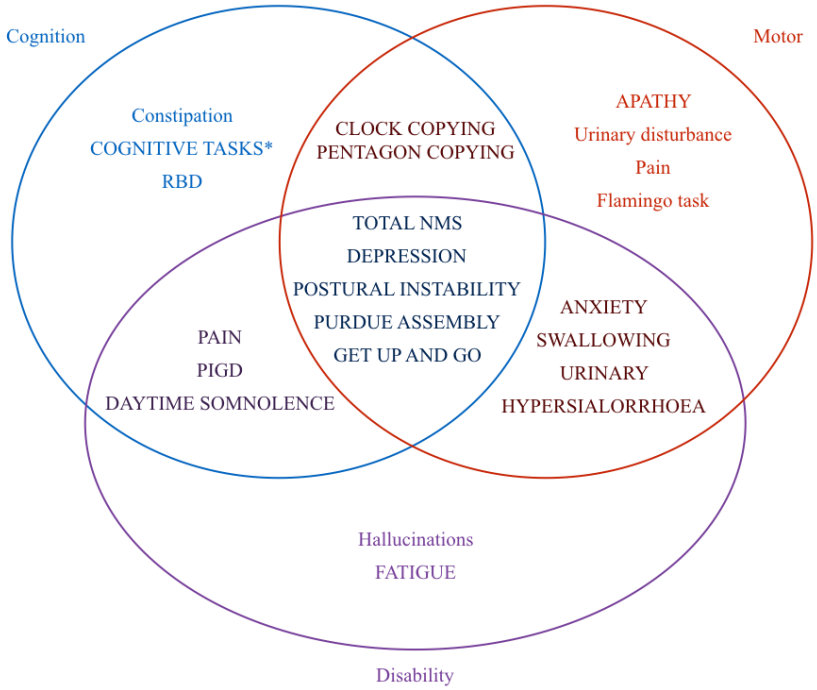
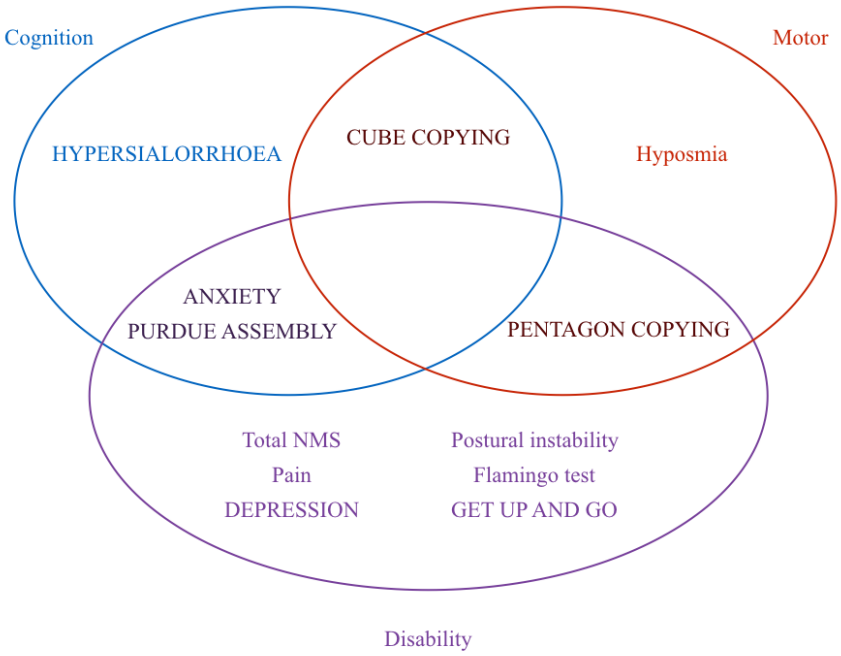


Figure 5.12 - This diagram shows which clinical assessments were associated with a more rapid deterioration on each of the 3 tested outcomes at diagnosis. All those with a significance level of $p<0.05$ are included, the tasks with a significance level of $p<0.02$ are in capitals.



5.5 MOTOR AND NON-MOTOR PREDICTORS OF EARLY CLINICALLY RELEVANT MILESTONES

To test whether the potential clinical predictors are robust, they can be modelled against the onset of milestones. Dementia and loss of independence are both important stages in progression. Using the onset of Hoehn and Yahr stage 3 as a motor milestone was considered, but as this implies postural instability which is not clearly a marker of global motor progression and thus thought to be less useful.

New onset dementia and dependence were defined using the MOCA (dementia score <23, n=61) and S&E scale (dependence <80%, n=68). At the first visit, to be included, they must not have fulfilled these criteria. To account for the variation seen between follow-ups (see Figure 5.3 and Figure 5.9), only those who had a sustained deficit (i.e. had not improved on future visits) were considered to have achieved the milestone. For each category, only patients with data available from both visits 1 and 2 were included due to the lack of information on withdrawn participants.

Individual regression models with new dementia or dependency as the dependent variable were used to test each potential candidate. Each regression model was adjusted for age at diagnosis, gender and disease duration at baseline. All potential candidate variables which predicted the milestone with a significance level of $p<0.05$ were eligible for the stepwise selection model. A backward stepwise selection model was then used with a significance threshold of $p<0.02$. Receiver operator characteristic analysis was the performed on the selected model.

5.5.1 PREDICTING DEMENTIA

Each potential clinical predictor was analysed in a logistic regression model to ascertain its association with dementia, adjusting for age and gender. Models with a significance level of $p<0.05$ are included in Table 5.15. Of note, both gender and disease duration from diagnosis did not meet the inclusion criteria once adjusted for age and either disease duration or gender respectively.

In the model, the number of connection errors was included as the visuo-spatial task as it had a greater odds ratio (OR 1.95) than both cube copying (OR 1.37) or pentagon copying (OR 1.18). The others were excluded due to co-linearity. Similarly, phonemic fluency had a greater association (OR 5.45) than semantic fluency (OR 3.75).

Age at diagnosis, cube copying, phonemic fluency and the Purdue assembly test were all included in the final model as independent predictors of dementia. This is reassuring as this matches the results from the mixed effect models using a different method which one could argue is more clinically relevant. Figure 5.13 graphically displays the odds ratios. Visuo-spatial dysfunction and poor fluency have both been reported as predictors of cognitive impairment which fits with these results. Again, the Purdue assembly task is strongly associated with more rapid cognitive impairment which is supportive against a chance finding, although this is the same data set.

The area under the ROC curve for this model is estimated at 0.81 (95% CI 0.74-0.89) Figure 5.14. Using a probability cut-off of 0.2 (see Figure 5.15), the specificity was 89.5% with a negative predictive value of 95.4%. However, the sensitivity was modest at 61.2% with a positive predictive value of only 39.5%. This probability cut-off was chosen after examining tabulating the ROC analysis results, as a compromise between sensitivity and specificity. Thus, while this would not be useful to screen for dementia, the relatively high specificity would be useful delineate at risk groups for clinical trials and mechanistic studies.

In the PPMI cohort, hyposmia, RBD, depression and UPDRS III at 2 years follow-up gave an AUC of 0.76 (95% CI 0.68-0.83). They also reported a high negative predictive value and poor specificity, although their predictive clinical variables were notably quite different to this study. The inclusion of DAT imaging and CSF $A\beta_{42}$ improved the AUC to 0.80 (95% CI 0.74-0.87) which is more similar to the results of this study. However, their number of cases was smaller as they only included 2 years of follow-up since diagnosis while this study was a bit longer at about 3 years.

A collaboration using both the CARPA and Campaign cohorts to create and then validate the model, identified a combination of age, a higher UPDRS axial score and semantic fluency predicting a worse outcome. However, their outcome was a combination of both motor and cognitive markers (Velseboer et al. 2016). Despite this, the findings of age and fluency being predictive is again robustly reproduced.

By far the largest study to date included a broad range of disease durations, rather than just early PD. This combination of cohorts (n=3,200) including more advanced PD, found age at onset, baseline MMSE, years of education, motor exam score, sex, depression, and GBA status predicted dementia at 10 years (Liu et al. 2017). These differences are discussed in more detail in chapter 7.2.

Other published models with more advanced PD cohorts have identified EDS (Zhu et al. 2014), cardiovascular autonomic dysfunction, RBD, color discrimination ability, gait dysfunction and loss of dexterity as predictive of dementia (Anang et al. 2014).

Between all these studies, age remains the most consistent risk factor but almost all the other factors vary between published studies. Reassuringly for this model, there is good evidence for the predictive value of the visuo-spatial and verbal fluency tasks identified. Further validation of this model on an independent cohort would be beneficial.

Table 5.15 – Potential clinical predictors of dementia at the first follow-up visit (visit 2)

	New dementia at visit 2, OR (95% CI, p-value)[1]	**Odds ratios of selected variables for final model OR (95% CI, p-value)[2]**
	n=488	**n=488**
Age at diagnosis	1.93 (1.54 (2.41), p<0.001*	1.65 (1.26-2.17), p<0.001*
Phonemic fluency	5.57, 2.86-10.8, p<0.001	4.03 (1.87-8.70), p<0.001
Connection errors on cube copying	1.94 (1.31-2.86), p=0.001	1.64 (1.09-2.45), p=0.02
Purdue assembly poor performer	2.59, 1.46-4.60, p=0.001	3.66 (1.86-7.21), p<0.001
Excessive daytime somnolence	2.04 (1.12-3.71), p=0.02	n/a
Postural instability	2.28, 1.29-4.02, p=0.005	n/a
Total NMS score 10 or more	2.02 (1.00-1.30), p=0.05	n/a

[1]Unless specified otherwise, logistic regression models adjusting for age, gender and disease duration, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); *Logistic regression models adjusting for gender and disease duration; [2]Odds ratio if included in final regression model with new dementia as the dependent variable; Phonemic fluency and purdue assembly were analysed as a dichotomous variables (poor performers <20th centile); Connection errors on cube copying was analysed as a 3 level ordinal variable of 0-2 mistakes in constructing corners.

Figure 5.13 – Odds ratios of model for factors predicting dementia, with their 95% confidence intervals, plotted for comparison (Table 5.15). Age at diagnosis was analysed as a 5 level ordinal variable, cube copying errors was analysed as a 3 level ordinal variable, and both phonemic fluency and purdue assembly were analysed as a dichotomous variables (poor performers <20th centile).

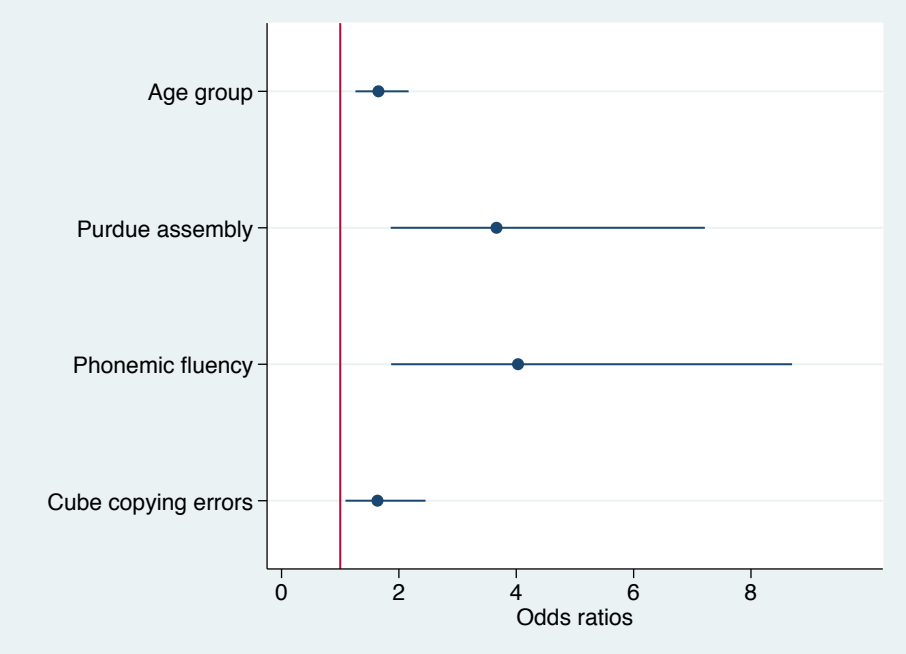


Figure 5.14 – ROC curve depicting model of factors predicting dementia including age at diagnosis, Purdue assembly task, phonemic fluency and cube copying connection errors

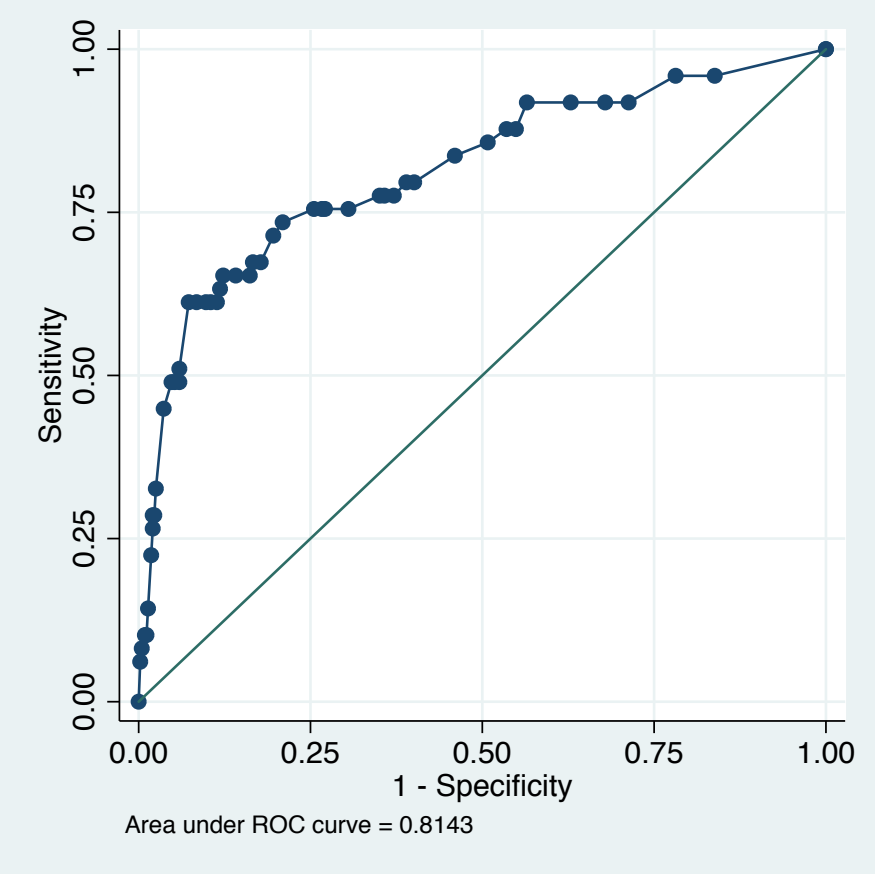
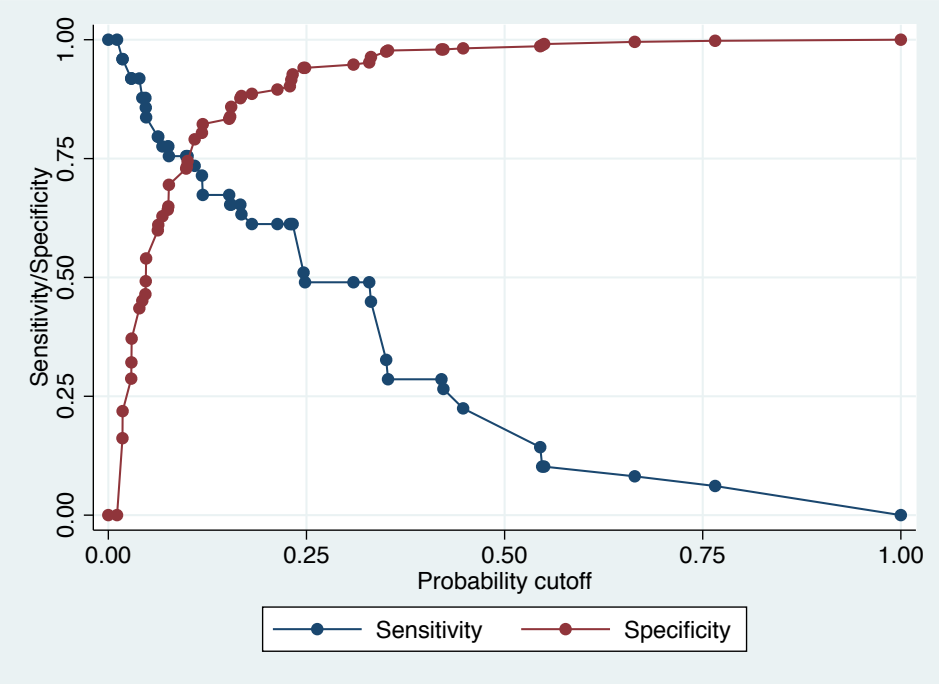


Figure 5.15 – Sensitivity and specificity graph plotted against probability as per regression model including age at diagnosis, Purdue assembly task, phonemic fluency and cube copying connection errors



5.5.2 PREDICTING DEPENDENCY

The same method was used to predict dependency as for the dementia model. Each potential clinical predictor was analysed in a logistic regression model, adjusting for age and gender, to select variables associated with a significance level of $p<0.05$. These results are shown in Table 5.16. In the regression model, each of the visuo-spatial tasks failed to meet the significance level.

Age at diagnosis, Purdue assembly task, anxiety, pain and postural instability predicted new dependency at the first follow-up visit. This is again reassuring as these were the factors which came out most strongly in the mixed effect model. The absence of a cognitive test is notable, as while pentagon copying predicted more rapid disability over time in the previous model, this was not reproduced. Baseline MCI and dementia in the model were not independent predictors of new dependency this early in the disease course. Intuitively, poor dexterity and loss of postural stability (thus worse mobility) would lead to difficulty performing household tasks. Also, anxiety and pain are disabling in themselves, thus their predictive value for dependency in a group of patients would make sense. Both of these symptoms are more common in PD compared to controls (see chapters 4.4.2.2 and 4.4.6) and so may affect the disease course.

The area under the ROC curve for this model is estimated at 0.79 (95% CI 0.73-0.84) (see Figure 5.17), which is a similar result to the dementia model. Using a probability cut-off of 0.2 (see Figure 5.18), the specificity was 85.9% with a negative predictive value of 94.2%. The sensitivity was modest at 55.4% with a positive predictive value of only 31.9%. This probability cut-off was chosen after examining tabulating the ROC analysis results, as a compromise between sensitivity and specificity. This model is therefore useful to select at risk cases, but not as useful as a screening tool. Potentially these could be used to select high risk patients for clinical trials and help inform individuals about future planning.

Older age and axial impairment has been shown to predict disability in the PINE study up to 12 years following diagnosis, although we did not reproduce their findings on smoking and cognitive impairment (Macleod et al. 2016).

Age remains an important predictive factor for disability, with postural instability a key factor also. The Purdue assembly task, also shown to predict cognitive impairment, is again shown to be an important predictive factor for progression.

Table 5.16 - Potential clinical predictors of dependency at the first follow-up visit (visit 2)

	New dependency at visit 2, OR (95% CI, p-value)[2]	**Odds ratios of selected variables for final model OR (95% CI, p-value)[2]**
	n=612	**n=612**
Age at diagnosis	1.57 (1.29-1.91), p<0.001*	1.42 (1.14-1.78), p=0.002*
Purdue assembly poor performer	3.05, 1.91-4.89, p<0.001	3.12 (1.78-5.50), p<0.001
Anxiety	3.14 (1.61-6.11), p=0.001	2.34 (1.56-5.51), p=0.02
Pain	3.14 (1.73-5.70), p<0.001	2.94 (1.56-5.51), p=0.001
Postural instability	2.82 (1.66-4.76), p<0.001	2.11 (1.21-3.67), p=0.009
Total NMS score 10 or more	2.46 (1.35-4.50), p=0.003	n/a
Chewing and swallowing difficulty	2.25 (1.24-4.08), p=0.007	n/a
Fatigue	2.80 (1.38-5.65), p=0.004	n/a
Depression	2.56 (1.34-4.89), p=0.005	n/a
Semantic fluency	1.94 (1.01-3.74), p=0.05	n/a
Hypersialorrhoea	2.24 (1.30-3.85), p=0.004	n/a
PIGD phenotype	3.21 (1.79-5.75), p<0.001	n/a
Get up and go	2.50 (1.41-4.43), p<0.001	n/a

[1]Unless specified otherwise, logistic regression models adjusting for age, gender and disease duration, age was analysed as a 5-level ordinal variable (<60, 60-65, 65-70, 70-75, >75 years); *Logistic regression models adjusting for gender and disease duration;
[2]Odds ratio if included in final regression model with new dependency as the dependent variable

Figure 5.16 - Odds ratios of model for factors predicting dependency plotted for comparison (Table 5.16). Age at diagnosis was analysed as a 5 level ordinal variable, anxiety, pain, postural instability and Purdue assembly were analysed as a dichotomous variables (poor performers <20th centile).

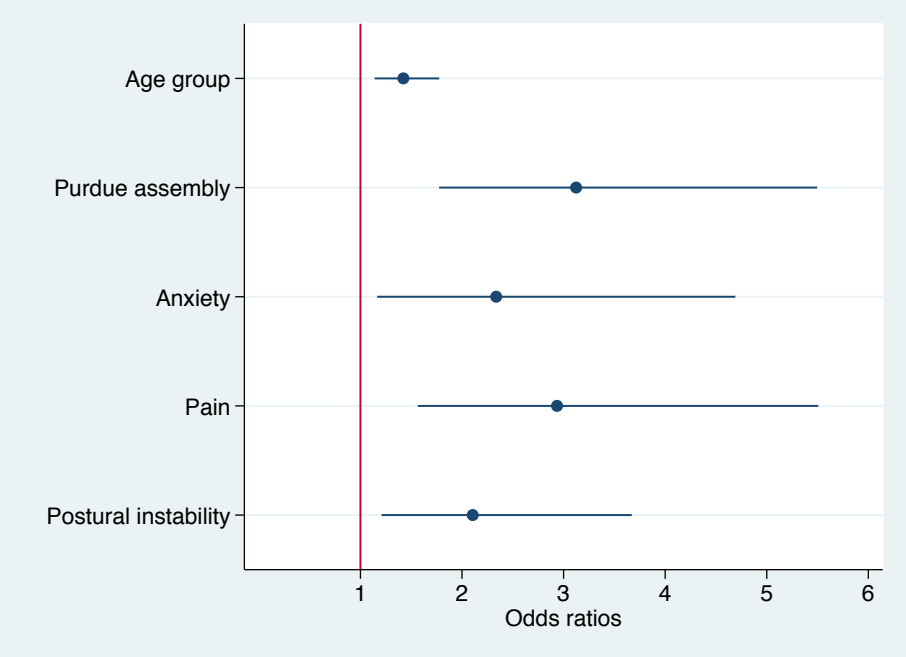


Figure 5.17 - ROC curve depicting model of factors predicting dependency including age at diagnosis, Purdue assembly task, anxiety, pain and postural instability

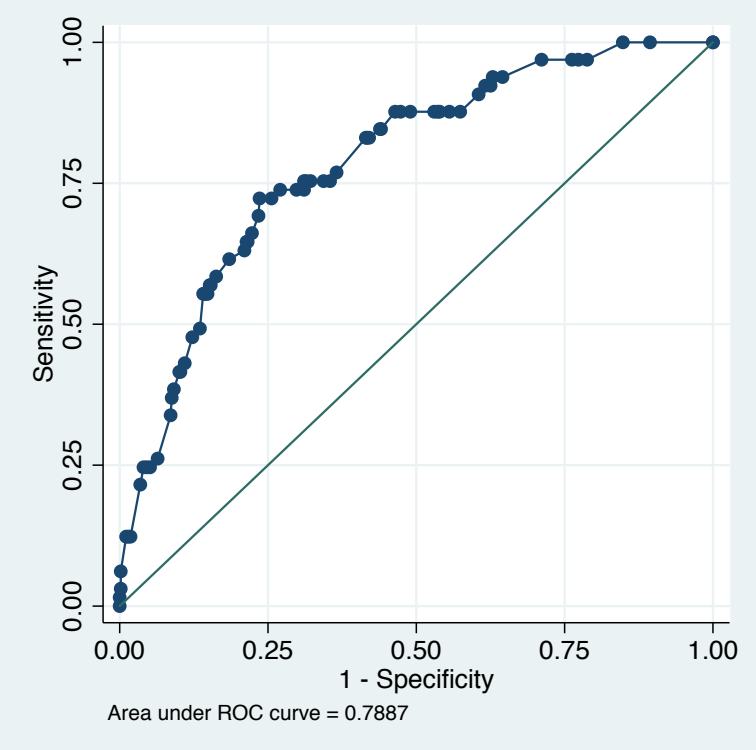
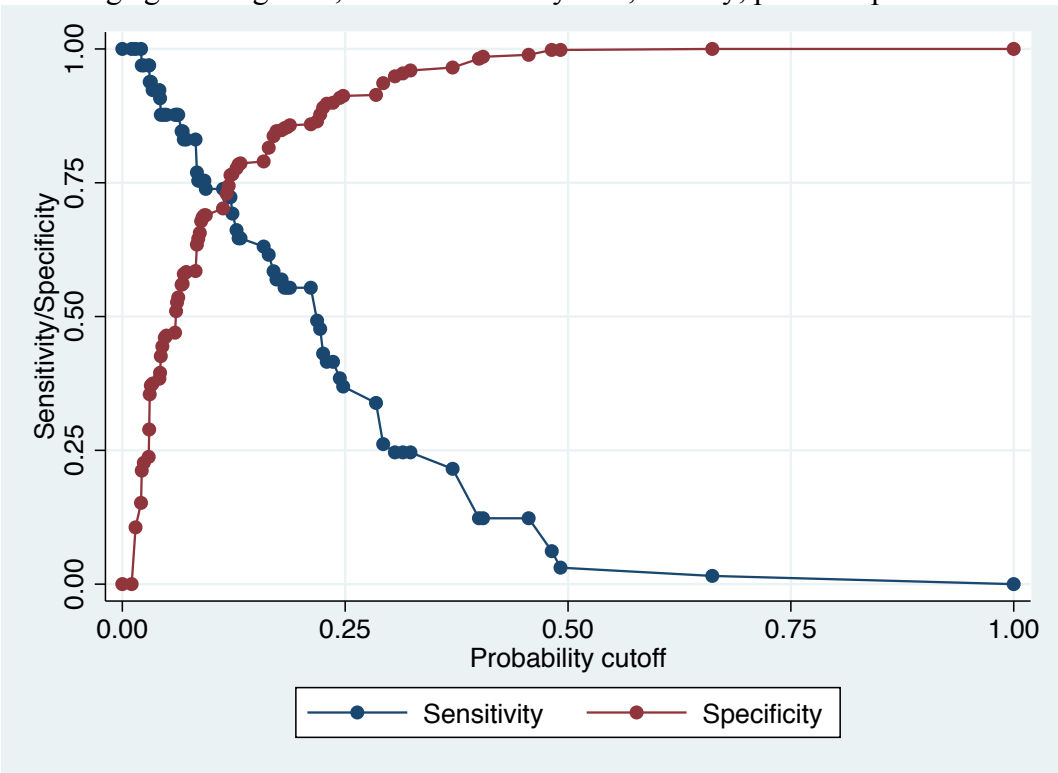


Figure 5.18 – Sensitivity and specificity graph for factors predicting dependency including age at diagnosis, Purdue assembly task, anxiety, pain and postural instability



6 BIOCHEMICAL PREDICTORS OF PROGRESSION IN PARKINSON'S DISEASE

This chapter investigates whether the proposed biochemical biomarkers can predict outcome in Parkinson's disease. The primary outcomes are cognition, motor impairment and disability as measured by the MOCA, UPDRS III and the Schwab and England activities of daily living questionnaire respectively. Each of the proposed biomarkers were analysed to see if they predicted baseline and longitudinal outcomes.

6.1 MEASURES OF PROGRESSION

A more detailed breakdown and description of the progression markers is given in chapter 5.1 with a summary of the outcomes for the participants included in the biochemistry study is given in Table 6.1. The demographics and outcome measures at each visit are very similar to the whole cohort, as would be expected with the large sample of 632 (approximately 2/3 of the whole cohort).

Table 6.1 – Summary of demographics and outcome measures

	Visit 1 0 months	Visit 2 18 months	Visit 3 36 months	Visit 4 54 months
Numbers attended	632	514	287	83
Age (mean (SD))	67.5 (10.0)	69.0 (9.7)	70.2 (9.4)	70.8 (10.6)
Gender (female n (%))	224 (35.3)	187 (36.4)	104 (36.2)	29 (34.9)
Disease duration since diagnosis (median (IQR))	1.03 (0.46-2.03)	2.76 (2.1-3.7)	4.49 (3.73-5.54)	6.46 (5.44-7.36)
MOCA (median (IQR))	25 (23-27)	25 (22-27)	25 (22-27)	24 (21.5-27.5)
MOCA category[a]: • Normal • MCI • Dementia	 430 (67.5) 108 (17.0) 99 (15.4)	 327 (64.9) 76 (15.1) 101 (20.0)	 183 (65.8) 45 (16.2) 50 (18.0)	 48 (60.0) 12 (15.0) 20 (25.0)
UPDRS III (median (IQR))	25 (18-34)	27 (20-36.5)	32 (25-42)	29.5 (20-42.5)
Schwab and England ADL (median (IQR))	90 (90-90)	90 (80-90)	90 (80-90)	80 (80-90)
Functional dependency based on S&E scale:				
• Needs some assistance with ADL (n (%))	104 (16.4)	106 (20.9)	77 (27.0)	32 (39.0)
• Not completely independent (n (%))	51 (8.0)	77 (15.2)	61 (21.4)	17 (20.7)

[a]Cognition (MOCA) categories were also used in the regression model for analysis: normal cognition 24-30, mild cognitive impairment 22-23, dementia 0-21

6.2 APOLIPOPROTEIN A1

Table 6.2 - Linear mixed effects model for ApoA1 (standardised then analysed as a 5-level ordinal variable) using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

PD (n=632)	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
MOCA	0.30 (-0.03, 0.63), p=0.07	-0.01 (-0.11, 0.09), p=0.82
UPDRS III	-0.93 (-2.08, 0.22), p=0.11	0.003 (-0.39, 0.39), p=0.99
Schwab and England	1.53 (0.52, 2.53), p=0.003	-0.26 (-0.65, 0.12), p=0.18

Lower Apolipoprotein-A1 predicted an increase in disability at diagnosis, and may have predicted cognitive impairment (see Table 6.2). However, lower levels did not predict a more rapid decline over time and the fixed effect was small. The addition of statin use made little difference to the model.

Despite previous positive results published in the literature, this data suggests that ApoA1 is not strongly associated with a more rapid evolution to dementia.

6.3 C-REACTIVE PROTEIN

Table 6.3 - Linear mixed effects model for CRP (normalised, standardised and then analysed as a 5-level ordinal variable) using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

PD (n=632)	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
MOCA	-0.12 (-0.42, 0.18), p=0.43	-0.04 (-0.13, 0.05), p=0.42
UPDRS III	-0.12 (-1.16, 0.92), p=0.82	0.11 (-0.24, 0.45), p=0.55
Schwab and England	-1.09 (-2.02, -0.17), p=0.02	-0.03 (-0.38, 0.32), p=0.85

The results suggest that an elevated CRP may be inversely related to functional ability, but the effect is small and may reflect reverse causation (i.e. increased inflammatory processes driven by disability) (see Table 6.3). While published studies have suggested that CRP may be associated with a more rapid progression, these data provide strong evidence against that supposition. The large variability in CRP results, due its sensitivity for any inflammation or infection in the body, makes this less hopeful as an independent predictor of prognosis.

6.4 URIC ACID

Table 6.4 - Linear mixed effects model for uric acid (standardised and then analysed as a 5-level ordinal variable) using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

PD (n=632)	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
MOCA	0.16 (-0.17, 0.50), p=0.33	-0.01 (-0.11, 0.09), p=0.80
UPDRS III	-0.63 (-1.80, 0.55), p=0.30	0.18 (-0.22, 0.57), p=0.38
Schwab and England	-0.26 (-1.30, 0.78), p=0.63	-0.06 (-0.45, 0.33), p=0.76

While uric acid did not predict prognosis when considering the whole cohort together (see Table 6.4). Analysing each gender separately, lower levels of uric acid were associated with a slightly lower MOCA score at diagnosis (coefficient estimate 0.53 (95% CI 0.11, 0.94; p=0.02). The gender differences in uric acid levels are not fully understood, but the association between PD and uric acid compared to controls has both been reported greater (Shen et al. 2013, Gao et al. 2016) and lesser (Wen et al. 2017) in men. Even still, this fixed effect was modest and uric acid did not predict more rapid progression. The addition of medications which modulate uric acid levels to the model made little difference.

6.5 VITAMIN D

Table 6.5 - Linear mixed effects model for vitamin D (normalised and adjusted for seasonality, standardised and then analysed as a 5-level ordinal variable) using disease duration from diagnosis as time variable, adjusting for age, gender and their interactions with time.

PD (n=632)	**Intercept coefficient: estimate (95% CI), p-value**	**Random slope coefficient: estimate (95% CI), p-value**
MOCA	0.15 (-0.15, 0.46), p=0.33	0.03 (-0.06, 0.12), p=0.47
UPDRS III	-1.04 (-2.10, 0.02), p=0.05	0.10 (-0.25, 0.45), p=0.57
Schwab and England	2.01 (1.08, 2.94), p<0.001	-0.12 (-0.46, 0.22), p=0.49

Vitamin D appeared to be inversely associated with motor function and disability at diagnosis, although the association is small (see Table 6.5). The analyte did not then predict a more rapid progression over time. Despite the evidence of differences between cases and controls in the literature, and the possible role that vitamin D may play in the pathogenesis, this study provides evidence that it is not a prognostic factor.

6.7 SUMMARY

None of the potential biochemical markers predicted prognosis. As would be expected given the results of the mixed effect models, none of the tested analytes predicted new onset dementia or dependency using the same methods as for the clinical factors in chapter 5.5 (data not shown).

This study adds considerable evidence to the literature on each of these analytes. Conflicting evidence for the use of vitamin D and uric acid in multiple studies has led to uncertainty over their utility to predict outcomes in early PD, despite the precious resources used to investigate these. Pilot studies investigating EGF, ApoA1 and CRP have been promising, but these results have failed to be replicated in this large prospective cohort. Therefore, this study should guide future research towards other candidates.

7 CONCLUSIONS

7.1 MAIN FINDINGS

Poor performance on three simple clinical tests performed early in PD (the Purdue assembly task, phonemic fluency and cube copying) has the ability to predict early dementia with a good specificity. Similarly, poor performance on the Purdue assembly task and the presence of pain, anxiety and postural instability predicts early loss of independence. The likelihood of both of these outcomes also increases with age. A poor performance on each of these clinical assessments are independently associated with PD compared with controls, including the age at onset, adding strong evidence to their relationship with the underlying disease processes. Each of these tests are brief, non-specialist and require very little in the way of resources.

The tested serum biochemical markers (ApoA1, uric acid, vitamin D, EGF, and CRP) were not associated with rapid progression in PD. While the level of serum uric acid was associated with PD compared to controls, the differences were not enough to be useful diagnostically. Thus, this study provides high quality evidence that these biomarkers are not sufficiently useful as biomarkers for diagnosis or prediction of prognosis in early Parkinson's disease.

Until the recent publication of inception cohorts, there has been a dearth of high quality data on the natural history of early PD. Neurodegeneration offers particular challenges in clinical trials. These include the combination of slow progression over time and the lack of a validated biomarker. To date, there have been no successful trials to modify progression in PD, potentially (at least in part) due to the inability of clinical trials to detect changes during the trial period. Expense limits the size and follow-up duration of these studies. Coupled with this lack of biomarkers, disease heterogeneity has also

caused challenges with mechanistic studies and clinical trial design for disease modifying therapies.

Using the predictive model to predict early dementia developed in chapter 5.5.1, one could restrict participants included in a clinical trial for cognitive impairment to those who performed poorly on a combination of the Purdue assembly task, phonemic fluency and the cube copying task. For example, a 70 year old man who performed well at all three tasks would have a 4% (95% CI 2%-7%) risk of developing early dementia, compared to an estimated 63% (95% CI 43-83%) risk if they performed poorly on all three tasks (see Figure 7.1). While 35% (n=170) of the cohort were 'good performers', there were few 'poor performers' who performed badly in every test (1.5%, n=7). However, as shown in Figure 7.1 a combination of poor performance on two tests may substantially enrich a sample with at risk patients (15%, n=74). Alternatively, one could restrict trials aimed at preserving global function to those who performed poorly on the Purdue assembly task with symptoms of postural stability, anxiety and pain (see Figure 7.3). Thus a sample enriched with high risk participants would be more likely detect a smaller signal. One potential limitation of these models is the possibility that these 'rapid progressors' may be a subtype of PD and thus not generalisable, but the ability to determine more homogenous trial cohorts would be a strength.

Figure 7.1 – Estimated probability of early dementia (including 95% CI) comparing those who performed well on the Purdue assembly task, phonemic fluency and the cube copying task at baseline with those who performed poorly on all three tasks.

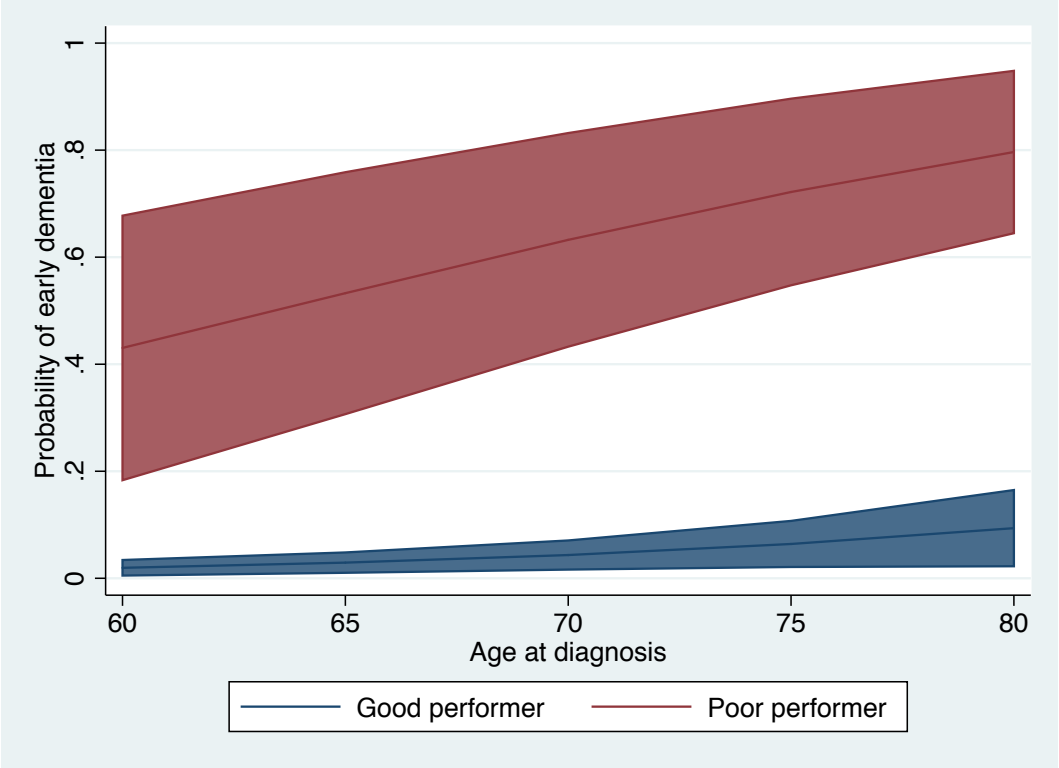


Figure 7.2 – Estimated probability of early dementia (including 95% CI) comparing those who performed well on the Purdue assembly task (assembly), phonemic fluency (fluency) and the cube copying task (CCT) at baseline with those who performed poorly on two tasks.

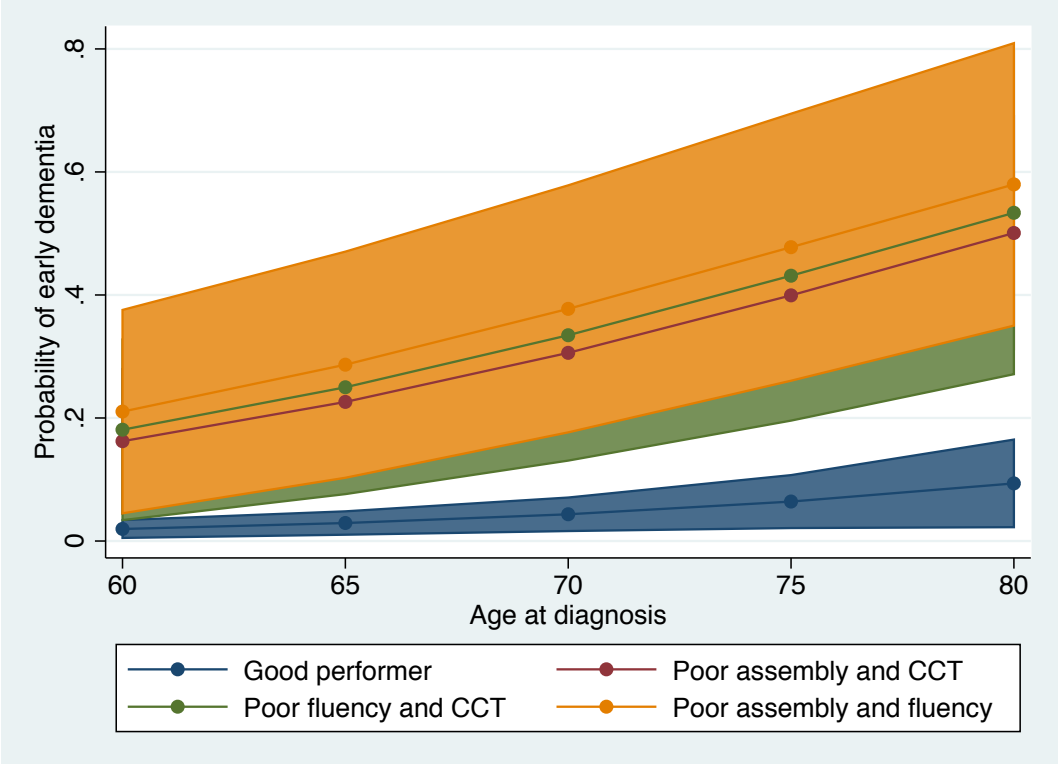
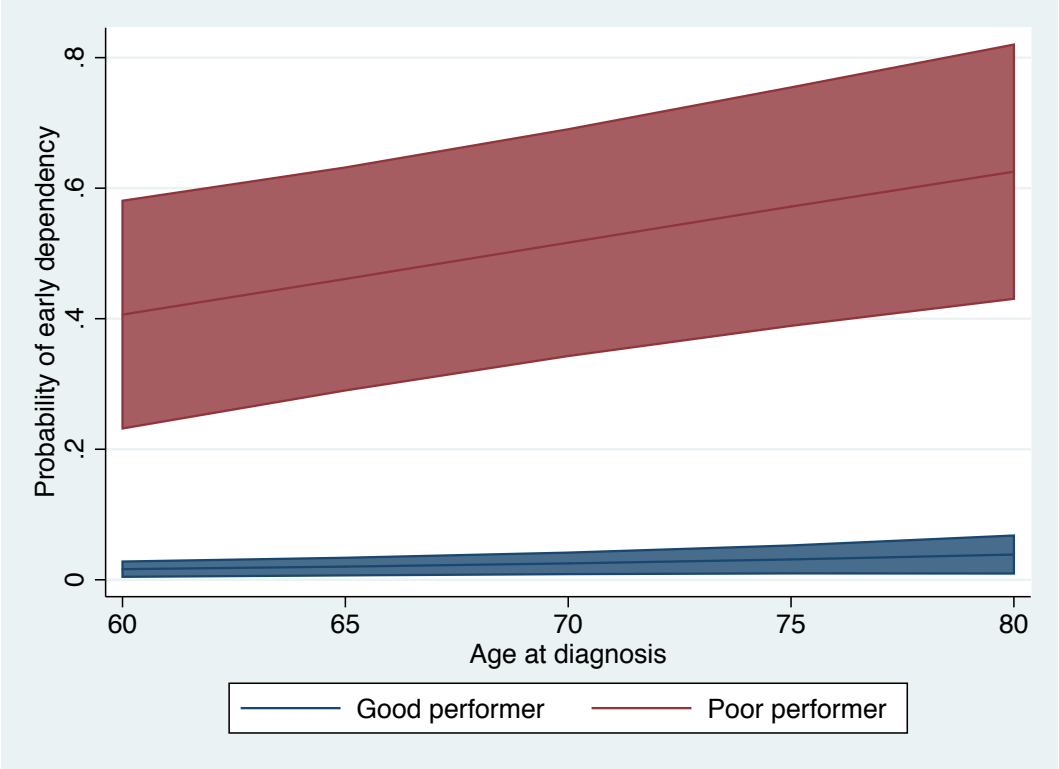


Figure 7.3 - Estimated probability of early disability (including 95% CI) comparing those who performed well on the Purdue assembly task and lacked postural instabilty, anxiety and pain, compared to those who did badly on the task and suffered all those symptoms



7.2 CONSISTENCY WITH EXISTING LITERATURE

7.2.1 CLINICAL PREDICTORS

7.2.1.1 Factors predicting dementia

Age remains the single most robust risk factor for Parkinson's progression. Older age is the strongest risk factor for developing the disease. It is associated with cognitive impairment and autonomic dysfunction by the time of diagnosis, and subsequently more rapid progression to dementia and dependency. This finding remains robust across all well-designed published studies. The reasons for this are thought to relate to the concurrence with other age-related pathology, such as amyloid and tau deposition in the case of cognitive impairment, causing a synergistic effect. Older age is also associated with an increased cerebrovascular burden which may diminish the brain 'reserve', thus increasing the rate of progression, but this was not shown in this cohort in the early stages. In this cohort, there was a trend that mood disturbance was linked to younger age, the reason behind this is not clear. It may be a direct result of the condition or, perhaps, associated with a reaction to developing a chronic disabling disease. It is possible that younger patients are more susceptible to this as they may perceive a greater burden of the disease.

Visuo-spatial and fluency impairment have been demonstrated to be robust predictors of early cognitive decline in the Campaign and CARPA cohorts (Velseboer et al. 2016), but there are some differences in the findings of these studies. While the underlying pathology which drives both phonemic and semantic fluency are similar, the relative performance may help localise between fronto-striatal (phonemic fluency) and posterior cortical (semantic fluency) deficits (Williams-Gray et al. 2007). In contrast to these other cohorts, this cohort found both semantic and phonemic fluency predicted dementia with similar results, although phonemic fluency had a slightly stronger association. An

important difference in these studies is their use of more stringent criteria for defining dementia, with more in depth neuropsychological assessments available. They did report that both forms of fluency predicted MCI, while only semantic fluency impairment predicted dementia. It may be that the criteria in this study for dementia, based solely on a global cognitive score, is less specific for dementia. In support of the relevance of posterior cortical dysfunction driving cognitive impairment, poor performance on the cube copying task predicted early dementia (also the pentagon copying task, although this was a weaker association). This test of visuo-spatial and constructional ability is thought to represent parietal lobe dysfunction. It remains unclear, however, how much these tasks are influenced by subcortical cholinergic and dopaminergic systems or whether cortical Lewy-body deposition is primarily responsible. This study adds weight to the hypothesis of posterior cortical impairments predicting dementia, although it was unable to discern the potential difference in outcome from frontal executive impairments.

This clinical predictor model compared favourably with the results of the PPMI cohort, despite different assessments. Using only clinical variables (hyposmia, RBD, depression and UPDRS III), they are able to predict cognitive impairment with an AUC of 0.74 (95% CI 0.68-0.81) which improved to 0.80 (95% CI 0.74-0.87) with the addition of imaging and laboratory measures. This was similar to the results of this study which only used clinical variables (AUC 0.81, 95% CI 0.74-0.89), despite their criteria also including MCI in addition to dementia. A key difference between the cohorts is the much younger population in the PPMI cohort (mean age 61), who would be at lower risk of early cognitive impairment.

While hyposmia, depression and RBD in this cohort predicted a slight difference in MOCA at diagnosis, they did not predict a more rapid decline. The mechanisms by which motor impairment and dementia are linked is probably due to global progression

(i.e. multi-system impairment) rather than an individual process. Primarily, the Purdue task is a measure of dexterity but can be used as representative of motor dysfunction. It correlates strongly with the UPDRS III total motor score (OR 1.45, $p<0.001$ in logistic regression model adjusting for age and gender, analysing UPDRS III as quintiles). As they are closely associated, it is likely the global motor score was not selected due to collinearity. A number of subtyping studies have also found an association between rapid evolution of cognitive and motor symptoms (van Rooden et al. 2010, Fereshtehnejad et al. 2017). The ability of the Purdue pegboard task to predict dementia has been shown before in a smaller study of more advanced PD (Anang et al. 2014). The task itself correlates well with reduced putamen functional connectivity (Simioni et al. 2016), which may not be related to cognitive dysfunction in itself. The ability of the Purdue pegboard task to predict prognosis is likely an indication of a globally faster neurodegeneration affecting both motor and NMS, further indicated by its ability to predict dependency. It is possible that Purdue assembly task is better for predicting dementia than other measures of motor function as it does require a degree of executive functioning and thus reflect cognitive impairment also.

The largest study (n=1,350 in the discovery cohort) looking at prediction of cognitive impairment had several differences from this study (Liu et al. 2017). While this replicated age as being predictive, their findings of years of education, motor exam score, sex and depression were not replicated in this study. They also found the GBA mutations were predictive, but these were not tested in this study due to low number of carriers. A low baseline cognitive score was predictive, but this was not tested in this study as the cognitive score was also the outcome. Their cognitive risk score, with this increased follow-up and large sample has the most accurate predictive model to date with an AUC of 0.88 (95% CI 0.79-0.94). The key differences in study design between these studies are the inclusion of more advanced PD (up to 12 years from disease onset)

and a longer follow-up period (up to 12.8 years), which may explain these differences. This study focussed exclusively on cognitive impairment in the earlier stages of PD.

7.2.1.2 Factors predicting loss of independence

There are far fewer published studies examining the factors associated with dependency. Age at diagnosis, Purdue assembly task, anxiety, pain and postural instability predicted new dependency at the first follow-up visit in this cohort. The association between age and both motor impairment and cognitive impairment is well established. In addition, age is associated with increasing co-morbidity which can cause disability independently of PD. Baseline cognitive tasks, perhaps surprisingly, did not predict early dependency. This may be because at this early stage of the disease, motor function is more limiting. An alternative hypothesis would be that the Purdue assembly task is already predicting those who would become cognitively impaired in the model and so the addition of other cognitive predictors does not add further value.

The inclusion of both the Purdue assembly task and postural instability implies their independence in predicting disability. This fits with the mechanisms of postural instability differing from the dopamine responsive symptoms (including bradykinesia and rigidity) which affect dexterity. The loss of postural stability correlates strongly with gait difficulty and falls, thus its severity would impair the ability to perform activities of daily living. This association of axial impairment linked with progression has been repeatedly found in studies from (Hoehn et al. 1967) to more recent incident cohorts (Macleod et al. 2016, Velseboer et al. 2016). The mechanism of how mood disturbance may link to disability is less clear. Depression has been linked to a worse outcome (Liu et al. 2017, Schrag et al. 2017), but not anxiety. There is a strong correlation between the two mood disorders, but in this cohort anxiety had more predictive value. Each are disabling but they may also affect the outcome measure itself, as people suffering mood disturbance may give a more negative opinion on questionnaires.

Chronic pain is disabling in itself. This study measured pain as a symptom but did not distinguish between different causes. Parkinson's itself causes pain but there are many other causes in this age group, commonly including musculo-skeletal disorders such as osteoarthritis. While pain was more common in the PD group than controls, indicating its association with the disease itself, it is not clear whether pain generated by the condition or co-morbid conditions causing pain is responsible for the increased risk of disability.

7.2.2 BIOCHEMICAL PREDICTORS

There was insufficient evidence to support the use of the tested biomarkers as being useful for diagnosis or prognosis in Parkinson's. None of the potential biochemical markers predicted prognosis as regards motor, cognitive and functional impairment. This adds considerable evidence to the literature as a 'negative' study. All studies, including this one, have limitations but despite these it remains a well-designed study. Identifying which biomarkers are not useful is important for the use of future resources.

Vitamin D deficiency has been linked with PD in multiple studies and systematic reviews (Lv et al. 2014, Wang et al. 2015, Rimmelzwaan et al. 2016), although some results are conflicting, with meta-analyses demonstrating differences between cases and controls. Many of these studies have included advanced PD, so it is not clear whether the lower Vitamin D levels are linked to consequences of the condition (i.e. due to loss of mobility), rather than the underlying mechanisms. It is also possible that multiple small cross-sectional studies had found associations where none exist. Publication bias, despite recent efforts to improve publication of all well-designed studies, can lead to false positive results in meta-analyses.

Similarly, studies investigating uric acid have had conflicting results. This study did show an association between Parkinson's and lower uric acid levels at baseline ($p=0.02$), but the differences between the groups were not sufficient to be used diagnostically. This is consistent with a potential role of uric acid as an anti-oxidant and free radical scavenger, reducing the oxidative stress causing dopaminergic loss (Church et al. 1994, Toulorge et al. 2016). However, there was significant overlap between the cases and controls, so while there may be a role of uric acid in the disease mechanisms of PD, it would be part of a more complex picture.

Pilot studies investigating EGF, ApoA1 and CRP have been promising, but these results have failed to be replicated in this large well-phenotyped cohort. Therefore, this study should guide future research towards other potential biomarkers.

7.3 STRENGTHS AND LIMITATIONS

The large size of this early cohort is a particular strength, almost unparalleled, with only Tracking PD similar in scope. The inclusion of controls offers a direct comparison with an unaffected population to delineate disease specific symptoms and signs. The breadth of clinical phenotyping with a broad range of NMS sets modern studies of this type apart from many in the published literature. The use of validated tools with severity scales for NMS rather than screening questionnaires with binary outcomes allows for accurate characterisation of symptoms.

A major strength of this cohort is its reflection of a real-world population of PD. On key demographics, including age of onset, gender ratio and diagnostic features this cohort closely matches community based incident cohorts, but with a substantially larger cohort. The lack of stringent entry criteria and inclusion of patients with incident cognitive impairment is also a strength, as their exclusion may bias the findings to less aggressive PD subtypes. Importantly, co-morbidities and vascular risk was similar with the control population and expected rates in the wider population. This provides evidence against a sample biased toward a healthier cohort, as can be a potential limitation in studies based in secondary care and recruitment to clinical trials in general. Thus, this unselected cohort is highly representative of early PD, hence meaningful conclusions can be made.

There are several study limitations that need to be considered. Screening tests, such as global cognition scores, were used to assess some clinical features rather than more in depth assessments, due to time constraints. Whilst the attempt was made to adjust for

demographic differences between groups, there may still be residual confounding differences. All case control designs are susceptible to recall bias, unless using data recorded prior to disease onset. Despite the large number of participants, approximately 53% to 66% of eligible PD subjects in the Thames Valley did not agree to take part. The potential bias of mobility, co-morbidity, cognition and social constraints among non-participants could bias results, though we suspect this would (if anything) result in an under-estimate of the prevalence and severity of patient's symptoms. The lack of outcome of the participants who had withdrawn is likely non-random and may have led to a bias in the findings. Although, as the withdrawn participants are likely to have been more impaired or more rapidly progressing, the effect would again likely be an under-estimate of symptom severity.

The use of a global cognitive score to define dementia lacks the specificity of level 2 criteria, requiring either multi-domain impairment or the use of a measure of cognitive functional impairment. Using a lower cut-off score (MOCA <22) to increase specificity at the expense of sensitivity, helps to address this. The mixed effect models assumed a linear progression which may not be true, although over the short time period examined in the early stages this is unlikely to have had a meaningful effect on the results.

All the included participants had a diagnosis confirmed by a specialist in movement disorders. Most patients have remained under follow-up with the diagnosis reviewed at each visit. However, at the time of writing, very few patients have died with subsequent pathological confirmation of their diagnosis. One would expect that, despite repeated clinical evaluation, up to 10% could have an incorrect diagnosis. The application of novel criteria, which include NMS in addition to a non-motor diagnosis, may help in future, although they were unavailable when the cohort was recruited.

7.4 IMPLICATIONS FOR FUTURE RESEARCH AND PATIENT CARE

This model requires replication. As discussed in chapter 2 there have been numerous candidate biomarkers which have failed to be replicated across cohorts. Whether the proposed models remain the same across cohorts, or if there are some differences, will add robust evidence to this method of stratification. The addition of imaging and laboratory markers, preferably cheap and readily available tests, could improve the accuracy of the model as and when they are developed.

Subtyping methods are thought to help guide mechanistic research by delineating phenotypically similar groups. This greater homogeneity of symptoms is thought to have the potential to power laboratory and imaging studies. It may be that using prognostically similar subtypes, i.e. faster and slower progressors, may help uncover differences in cell biology.

The clinical assessments used to predict dementia and dependency are simple and readily translatable to a clinical setting for use in management and patients counselling. Clinicians would be able to use this model to counsel patients about their likely future risk of cognitive impairment and need for care. Many patients struggle with the uncertainty of prognosis. Predicting those at high risk of decline could allow patients to plan their future, financially and socially, in advance. At risk patients could potentially be followed up more closely. Early referral to memory clinics could lead to the early recognition and prompt initiation of dementia treatments.

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