ICICLE-PD

(Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation – Parkinson's Disease)

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INTRODUCTION

Study Aims and Objectives

The aim of this study is to better understand the anatomical, biochemical and genotypic mechanisms determining the transition from Parkinson"s disease (PD) to dementia associated with PD (PDD). In doing so, we will determine clinical features associated with a high risk of incident dementia and establish putative biomarkers predictive of dementia.

These aims will be delivered through the following objectives:

1. Accurate characterisation of two independent cohorts of incident parkinsonism using a range of assessments, applying strict epidemiological criteria at presentation.

2. Follow up of these cohorts using the same assessments to identify patients who develop PDD and those factors that predict its evolution.

3. Establish a simplified panel of tests that can be used to predict PDD in order to develop a platform for studies investigating agents designed to help treat this aspect of PD.

Study Background

With increased population life expectancy over the last two centuries and the development of effective anti-parkinsonian drugs it is now recognised that the cumulative incidence of dementia in Parkinson"s disease (PD) may be as high as 80% (1, 2). A person with PD is 5-6 times more likely to dement than an age-matched control without PD (3). Dementia associated with PD (PDD) is associated with increased mortality (4), reduced quality of life (5), increased caregiver distress (6) and is a major risk factor for nursing home placement (7), with important implications for costs of care. Furthermore, dementia constrains effective medical and surgical management of the movement disorder. Hence, these symptoms have serious social and health economic consequences, as well as posing unique therapeutic challenges.

When disease-modifying treatments become available for cognitive failure in PD, prompt identification of individuals at high risk of dementia will assume great importance. It is therefore vital and a matter of urgency that improved clinical predictors and biomarkers are determined to accurately identify “high risk” individuals who would benefit most from the early use of these novel treatments. Furthermore, since the underlying pathophysiological process underpinning PDD is likely to be heterogeneous (8), characterising the clinical and/or biomarker correlates of this heterogeneity means that future treatments may be better targeted. For example, the use of “transferable” anti-amyloid strategies from Alzheimer trials may be more appropriate in some, but not all, individuals with PDD.

Clinical Predictors of PDD

Patient demographics & motor features

Increasing age is the most important risk factor currently identified for PDD. Worsening motor disability is also predictive of dementia and is synergistic with current age (9). A non-tremor dominant motor phenotype is over-represented in PDD (10) and, when present at onset, is associated with a four-fold increased risk of dementia (11). In addition, longer disease duration and male gender have also been

associated with increased risk of PDD with less well-established risk factors being low educational attainment and current smoking habit (contrasting with the well established inverse association of smoking and PD) (12-14).

Eye movement measures

Impairments in reflexive saccades are minimal when either cortical (Alzheimer's disease, AD) or nigrostriatal neurodegeneration (PD) exists solely (15); however, they become prominent with combined cortical and sub-cortical neurodegeneration as occurs in PDD (and also in DLB). However, saccadic latency, unlike velocity or duration, appears to be controlled at the cortical level and may therefore be sensitive to cognitive decline (16) and can be easily assessed and quantified in the clinic, using a new head-mounted portable device. The feasibility of this approach has been shown in Huntington"s disease (another basal ganglia disorder) (17, 18) as well as in PD subjects on and off L-dopa (19). Although this latter cross-sectional study highlighted high inter-subject variability and overlap of PD and control saccadic latency distributions, longitudinal data collection and analysis may be more important as a biomarker for disease progression, and cognitive decline in particular, given the diffuse cortical and subcortical pathology of the latter.

Cognitive & psychiatric features

Deficits in auditory verbal learning and nonverbal reasoning, picture completion, Stroop interference and verbal fluency have all been independently associated with an increased risk of cognitive failure in PD (20). Two simple bedside tests, pentagon copying and semantic fluency, may be predictive of cognitive decline and dementia in that deficits in these tasks at presentation are associated with PDD at both 3 and 5 years follow up (11)). In contrast, frontostriatal executive tasks are not predictive of PDD and may be more dopaminergic in aetiology (11, 21).

It remains uncertain whether depression is an independent risk factor for dementia in PD although there is an association between apathy and cognitive dysfunction, particularly executive impairment (22). It is unknown though whether apathy is independently predictive of dementia. Visual hallucinations in PD predict more rapid cognitive deterioration (23) and psychosis requiring antipsychotic therapy has been associated with the development and progression of dementia, while the use of atypical antipsychotics may have adverse disease modifying effects, for unknown reasons (24).

Neurophysiology

Parkinson’s disease (PD) is a complex, multi-system disorder affecting non-motor as well as motor characteristics. The pathophysiology of PD extends beyond dopaminergic pathways. For example, cognitive dysfunction and loss of attentional control is evident early in the disease process, underpinned by a loss of cholinergic transmission. This complex pathophysiology underpins the decline in cognitive performance in PD. Cholinergic activity can be estimated indirectly with short latency afferent inhibition (SAI). SAI is abnormal in degenerative dementias where there is a profound loss of cholinergic transmission, and is sensitive to changes in PD gait under medication. To date, there have been no studies looking at the relationship between cholinergic activity (as measured by SAI), cognition and CSF amyloid in PD.

Vascular risk factors (including autonomic features)

The role of vascular risk factors in the aetiology of dementia in PD is uncertain. In a prospective study of 177 people with PD, cerebrovascular risk factors were not associated with incident dementia (25). Data on risk factors was however based on clinical interview and examination, while there were low numbers of patients affected by some risk factors, reducing statistical power of several analyses. Raised levels of plasma homocysteine may be relevant (see below). Although one small MRI study suggested that deep white matter hyperintensities may contribute to PDD, cerebrovascular risk factors were apparently similar between demented and non-demented PD groups (26). Clinicopathological studies using retrospectively acquired clinical data initially suggested that cognitive impairment on PD may be largely independent from coexistent vascular pathology, except in cases in with severe cerebrovascular lesion burden (27). More recently, however, studies have implied an association between cerebral amyloid angiopathy with cognitive decline in PD, particularly where AD-type pathology is prominent (28).

Differences in sustained attention are highly significant between PD subjects with and without orthostatic hypotension (OH) (29). The exact relationship between autonomic abnormalities and cognitive decline is unclear, however, as to whether it is a predictor or causally related to the dementia of PD.

Sleep and related features

Virtually all people with PD will develop sleep disturbance at some stage of their illness (30). Excessive daytime somnolence and REM sleep behaviour disorder may be predictive of PDD, but the data to support both is relatively weak at present (31). To date, case series and retrospective case notes analysis have suggested that increasing age, male sex, disease duration and medication increase the risk of sleep disturbance (32). There have been no longitudinal studies to determine which components of sleep disturbance worsen over time, in which patients and whether particular PD subtypes, such as those with early PDD, are more prone to sleep problems.

Other clinical features and drug-related effects

Dementia *may* be associated with weight loss in PD, together with worsening of parkinsonism, age at diagnosis and emergence of visual hallucinations (33), but the predictive value of antecedent weight loss is not known. Anticholinergic drug use may be a risk factor for PDD, and prolonged use of these agents has been associated with an increased frequency of cortical plaques and tangles in non-demented PD patients (34). Poor response to L-dopa and hallucinations on dopaminergic treatment may predict dementia (35, 36), with L-dopa-induced elevation in plasma homocysteine levels possibly contributing to cognitive failure (see below). Conversely, amantadine may delay and attenuate dementia in PD (37).

In summary, several clinical features may predict the development of dementia associated with PD. However, problems arise in the interpretation of many of these studies from a lack of replication and inadequate study design (for example, poorly characterised cohorts, small sample size or cross-sectional rather than longitudinal observations). Furthermore, many factors have been studied in isolation, making it difficult to establish whether they are overlapping, additive or synergistic regarding dementia risk. Therefore, identifying clinical features and creating a model that robustly predicts dementia would permit stratification of patients according to risk, and hence assist trial design for putative disease modifying agents as well as aetiological factors.

Imaging Predictors of PDD

Structural magnetic resonance imaging (MRI)

MRI techniques can determine patterns and rates of cerebral atrophy (for example, voxel-based morphometry) or cortical function in response to activating paradigms, based upon the paramagnetic properties of blood flow in response to cortical activation (functional MRI, fMRI). There is frontal grey matter loss in PD (38) compared with control subjects and a nearly 4-fold increased rate of whole-brain atrophy in PDD compared with non-demented PD and controls (39). Furthermore, rates of cerebral atrophy in PD correlate with global measures of cognitive decline in some (40) but not all studies (39) implying that MRI may be a useful technique in predicting preclinical onset of dementia in PD. Diffusion imaging changes in frontal areas and olfactory tracts have been described as early changes in PD and, more recently, widespread changes in fractional anisotropy were found to be more prominent in those with non-tremor dominant PD (41), and so may indicate a predictor of subsequent cognitive decline.

Functional MRI

Reduced frontostriatal neural activity has been identified using fMRI in patients with early PD (42, 43). In addition the COMT val_{158}met genotype influences frontoparietal activity during both planning (44) and attentional set-shifting in patients with PD.

Positron emission tomography (PET)

In subjects with PDD studied using cerebral 2-[$_{18}$F]fluoro-2-deoxy-D-glucose positron emission tomography ([18]FDG-PET), early studies reported reduced glucose metabolism in frontal and temporoparietal association areas, a pattern similar to that seen in AD (45). Using co-registration with MR to account for changes due to underlying atrophy, there is decreased blood flow in posterior parieto-occipital regions, especially the precuneus (BA7), in PDD and DLB but not in AD where changes in posterior cingulate were observed instead (46). No decline in perfusion is seen over a one-year period in established dementia, suggesting that perfusion changes may occur early in the disease before dementia develops (47), possibly preceding atrophy as in other neurodegenerative disorders such as Huntington‟s disease (48). In a small cross-sectional study of non-demented, but cognitively impaired, PD subjects reduced glucose metabolism was demonstrated on [18]FDG-PET in posterior parietal and temporal cortical grey matter (49).

With the advent of PET ligands capable of binding to □-amyloid, it has become possible to study cortical amyloid burden in neurodegenerative dementias. As might be predicted, using 11C-PIB-PET, mean cortical levels of amyloid are two-fold increased in AD (50) and by 60% in dementia with Lewy bodies (DLB) (51). Evidence thus far for PDD, where motor disease duration preceded the onset of dementia by many years, suggests that group mean cortical amyloid load is not significantly elevated although 20% of individuals showed an Alzheimer pattern of increased 11C-PIB uptake (52). It is not known, however, whether cortical deposition of amyloid protein is a time-dependent process (thus different in DLB and PDD) or a common feature of these two types of dementia. Indeed, amyloid may play a greater role the shorter the time of onset of dementia in PDD and this hypothesis is supported by a recent clinicopathological study (53), where cortical amyloid and □-synuclein levels were greater in DLB and PDD of shorter prior disease duration, compared with PDD with a longer history of motor symptoms prior to dementia onset. 11C-PK11195 is a tracer that binds to peripheral benzodiazepine receptors (PBR) on

activated microgial cells. It uptake is thus linked to cerebral inflammation. This will provide further information on pathophysiology and the potential link between amyloid deposition and inflammation. Determining cortical amyloid burden in PDD and the whether this protein affects the clinical phenotype is clearly of vital importance in determining whether anti-amyloid strategies are likely to be beneficial in PDD.

Genetic Predictors of PDD

Protein aggregation genes

The genetic and biological basis underlying the heterogeneity in cognitive profile, including the development of PDD, remains largely unknown (8). Unlike AD, PDD is not clearly associated with APOE polymorphisms (54). There is preliminary evidence that inherited genetic variation influences the rate of cognitive decline and the development of PDD (55) and that common variation in both tau (MAPT) and alpha-synuclein (SNCA) genes influences susceptibility to sporadic PD, with the greatest risk being conferred by the combination of the MAPT H1/H1 and SNCA rs356219 G/G genotypes (odds ratio 2.14, 95% confidence interval 1.57 − 2.92, p=2x10^{-6}). Furthermore, the MAPT H1/H1 genotype was associated with a greater rate of cognitive decline in 109 incident PD patients prospectively followed up over a 3.5-year period (p=1x10^{-4}). For 15% of H1/H1 homozygotes, but none of the H2 carriers, the rate of cognitive decline was sufficient to develop dementia within the follow up period (p=0.015). Age, a recognized risk factor for cognitive impairment in PD, was strongly correlated with cognitive decline in H1 homozygotes (p=2x10^{-5}) whereas, remarkably, cognitive scores remained on average unchanged in H2 carriers, regardless of age (p=0.37). Multivariate analysis confirmed significant effects of *MAPT* genotype and age on change in MMSE per year, as well as a significant age□*MAPT* genotype interaction, even after correction for potential confounding variables. However, these results only just reached statistical significance, raising the possibility of a false positive (type 1) error. The next critical step is therefore to replicate these findings on the new prospective cohort.

Cell death/mitochondrial genes

There is emerging evidence that the mitochondrial respiratory chain plays a key role in the pathogenesis of neuronal loss in neurodegenerative diseases, including PD, AD and DLB. Focal deficits of respiratory chain function have been documented in PD and AD brains (56, 57), while an inhibitor of respiratory chain complex I can cause a neurodegenerative phenotype in rats and humans (58, 59) associated with the accumulation of □-synuclein, a major constituent of Lewy body pathology. Somatic mitochondrial DNA (mtDNA) mutations accumulate in PD and AD brains, and recent evidence implicates a major mitochondrial interaction between both apolipoprotein E and amyloid precursor protein – both strongly associated with AD pathology and dementia (60-63). Mitochondria contain their own DNA (mtDNA), which codes for 13 essential components of the respiratory chain. MtDNA is inherited down the maternal line, and there is increasing evidence that inherited genetic variation of mtDNA is important for a number of multifactorial late-onset diseases, including PD (64-66) and DLB (67, 68). A meta-analysis of the available data on PD (n = 1685 PD cases, N = 2782 controls) provides compelling evidence that mtDNA haplogroups play an important role in determining the risk of PD. Haplogroup-specific polymorphisms account for <10% of the mtDNA coding region and work is in progress to determine whether it is the

haplogroup-defining polymorphisms *per se* that are advantageous, or whether other polymorphisms on the same haplogroup background are actually responsible for the association that has been observed.

Other Biomarker Measures

Blood

In recent years, telomere length in human peripheral blood cells has gained attention as a possible biomarker of aging and age-associated diseases. Telomeres, the DNA-protein structures that define and protect the ends of all linear chromosomes, shorten with cellular aging *in vitro* and with increasing human age *in vivo*. In cross-sectional studies, short peripheral blood cell telomere length has been associated with an increased prevalence of various age-related diseases, including AD (69), while shorter telomere length is predictive of post-stroke dementia (70). In the latter study, telomere length measured within 3 months of stroke was a significant predictor of risk for dementia within 2 years after stroke. Patients whose telomere length was below the median were nearly five times more likely than others to experience development of dementia by 2 years (70). One recent study in PD indicated that, contrary to the telomere attrition observed in several aging-related diseases, shorter telomeres are not associated with an increased risk of PD (71). Another small study in Japanese male PD patients (ages 47-69) did not find a statistical difference in the mean telomere length of peripheral leukocytes between the PD patients and control participants, although the mean telomere lengths were only shorter in the PD patients (72). There was a significant PD-associated decrease in the telomere lengths. These observations were taken to suggest that telomere shortening is accelerated in PD patients in comparison to the normal population. To the best of our knowledge, telomere length has not been explored as a biomarker for *dementia* in PD and would seem a logical target to study.

Elevated plasma homocysteine levels are associated in the general population with atherosclerosis, vascular disease, depression and dementia (73). L-dopa may elevate plasma homocysteine. It is unknown whether raised plasma homocysteine is an independent risk factor for cognitive impairment in PD, with some (74, 75), but not all (76), studies finding an association. In general however, these studies have been small and cross-sectional in design, and so have been unable to resolve this issue.

CSF

PD patients with mild cognitive impairment may have lower levels of cerebrospinal fluid (CSF) □ amyloid$_{1-42}$ compared with PD patients and normal cognition (77). Whilst CSF studies have been performed in PDD, they have been cross-sectional and mainly concerned with differentiating this dementia from DLB and AD (78-81). A study of CSF biomarkers (□ amyloid$_{1-42}$, A□42, total tau, T-tau and phosphorylated tau, P-tau) in patients with mild cognitive impairment yielded a sensitivity of 95% and specificity of 83% for detection of incipient AD (82). Furthermore, low levels of CSF A□42 have recently been shown to predict cognitive decline over 8 years among older women without dementia (83). It is not known, however, whether CSF analysis has predictive value for the development of PDD, or indeed whether CSF profile can inform regarding the pathological basis of the dementia and its clinical phenotype but will form part of this study.

In summary, several ancillary investigations may be of benefit in, first, predicting the development of dementia in PD and, second, informing as to the nature of the underlying pathophysiology. Currently, the utility of these studies is simply not known and they remain primarily of research interest. Were biomarkers to be confirmed as predictive of PDD this would facilitate the production of a diagnostic algorithm, with the aim of increasing the positive predictive value beyond clinical observations. Furthermore, if biomarker profile was shown to correlate with clinical dementia phenotype and pathological findings these investigations could also assist with optimal targeting and monitoring of putative disease-modifying therapies such as anti-amyloid strategies.

Study Hypotheses

Our over-arching hypothesis is that PDD is predictable in the earliest stages of the disease as this disorder is associated with more extensive Lewy body (LB) pathology. We postulate that a distinct phenotype will be evident both clinically and by using a range of imaging paradigms, including structural and functional imaging with MRI and PET, as the extensive pathology that underlies this disorder will produce more atrophy (structural MRI); under-activation of posterior cortical networks (fMRI) and greater load of protein deposits (PET). The cause of PDD is largely genetic in origin with polymorphisms in critical genes (e.g. synuclein; tau) causing a subtle but definite increase in intracellular protein interactions, which in time leads to the formation of LBs, cellular dysfunction and death. Specific study hypothesises are that:

1. Patients at high risk of PDD can be recognised clinically by their old age, non-tremor dominant motor features and more pronounced saccadic abnormalities with cognitive deficits in frontoparietal tasks, along with orthostatic hypotension and psychiatric deficits.
2. Patients with early symptomatic RBD and excessive daytime somnolence are more likely to develop PDD.
3. Vascular risk factors and blood pressure dysregulation will predispose towards more rapid cognitive decline in PD.
4. Frontal atrophy on serial MR imaging, will be associated with subsequent cognitive decline in PD. In contrast, the structural imaging changes shown to be predictive of AD (atrophy of the hippocampus and entorhinal cortex, metabolic changes in the posterior cingulate) will NOT be predictive of PDD (84).
5. Diffusion changes, with reduced fractional anisotropy and increased diffusivity, in fronto-striatal regions will be seen in PD but when severe and more widespread (i.e. also affecting temporal and parietal regions) will predict cognitive decline.
6. Different profiles of cognitive deficits will have unique, but predictable, activation patterns using fMRI and tasks that we have shown to target frontostriatal, temporal and parietal lobe function (e.g. (85)). Such fMRI patterns may therefore also be predictive of PDD and, we hypothesize, will contrast with those changes shown recently to be associated with AD (86).
7. An abnormal [18]FDG-PET scan, as a marker of cortical dysfunction, will be predictive of PDD.

8. Cortical deposition of amyloid protein will be a time-dependent process with higher cortical [11]C-PIB burden in PDD of shorter prior disease duration, compared with PDD with a longer history of motor symptoms prior to dementia onset.
9. The MAPT H1/H1 genotype will be associated with a greater rate of cognitive decline in incident PD patients after correction for potential confounding variables.
10. Genetic variants linked to mitochondrial haplogroups are associated with risk of developing PD and that these, or other variants, may influence the PD phenotype. This influence may be direct or indirect via interaction with nuclear genetic variants.
11. Shorter peripheral blood cell telomere length will be predictive of cognitive decline and PDD.
12. Raised plasma homocysteine in people with untreated PD will be an independent risk factor for cognitive impairment.
13. Low β amyloid$_{1\text{-}42}$ levels in non-demented PD subjects at baseline will predict early onset PDD, and that elevated CSF T-tau and P-tau levels will predict a temporal lobe pattern of cognitive deficit, with greater hippocampal atrophy on MRI scanning.
14. Abnormal short latency afferent inhibition (SAI) at 18 months follow-up will be associated with low baseline CSF amyloid levels, reflecting greater underlying cholinergic dysfunction and Alzheimer-related pathology.
15. Abnormal SAI at 18 months follow-up will correlate with falls.
16. Abnormal SAI at follow-up will correlate with cognitive dysfunction.

METHODS

Recruitment of Study Subjects with Parkinson's disease

We will attempt to identify every new case of parkinsonism within Cambridgeshire and Newcastle upon Tyne/Gateshead, including all patients presenting with any extrapyramidal symptoms and signs (tremor, rigidity, bradykinesia, micrographia, loss of dexterity, hypomimia, reduced arm-swing, or parkinsonian gait). All newly diagnosed PD patients seen by specialists based in secondary care in Newcastle upon Tyne and Gateshead will be asked to participate in the study. In addition, patients with a new diagnosis of PD attending clinics provided by Northumbria Healthcare NHS Trust, City Hospitals Sunderland and County Durham and Darlington Foundation Trust will be asked by their local PD specialist if they wish to be contacted by the ICICLE-PD study team. Participants from Newcastle upon Tyne and Gateshead will be included in both incidence and clinical studies while participants recruited who live outside this catchment area will only be included in the clinical aspect of the study. Patients in Cambridgeshire will also be recruited from a parallel study taking place at the Cambridge Centre for Brain Repair (Parkinsonism: Incidence and Cognitive Heterogeneity in Cambridgeshire, REC Reference No: 07/H0302/138) who have given their consent to be contacted about other studies. The population denominator in our proposal (incidence study) is a total of 1 million, comprising 450,000 (N. East) and 550,000 (Cambridgeshire). Non-identifiable patient data will be collected in all newly diagnosed PD patients to improve case ascertainment and reduce Type 1 error (see Data Management).

The total recruitment period to ascertain incident PD cases will be 31 months (including the three month "run-in" period outlined below) with follow up assessments performed every 18 months in each person recruited. Assuming a non-consent rate of 20%, we expect to recruit approximately 350 PD cases. This figure is based upon the *actual* numbers of cases recruited to the CAMPAIGN study (since this is directly comparable to our current proposal (21)). Each participant will thus be re-assessed at least once, with the majority of participants being seen twice, over the 5 year study period. Allowing for a 20% mis-diagnosis rate for PD, the increased recruitment will yield 25-30 PDD cases during the study itself.

We will confirm and refine the diagnoses following assessment, defining PD according to UK Parkinson‟s Disease Brain bank criteria (87). Where uncertainty exists, diagnosis will be decided by expert consensus. A label of „unspecified parkinsonism‟ will be applied to those cases in whom insufficient information is available to precisely classify them. These cases will be invited to participate in the study and followed up so that the diagnosis can be reconsidered at a later date and to maximise numbers for case follow up. Details regarding date of symptom onset and date of diagnosis will be noted.

Exclusion criteria will comprise:

1. People suspected of parkinsonism prior to the onset of the study on the basis that they are prevalent rather than incident.
2. People who do not possess a working knowledge of English (defined as insufficient to perform the neuropsychological assessments and questionnaires in the opinion of the assessor).
3. Patients with significant memory impairment at presentation (defined as MMSE score < 24), or meeting DSM IV criteria for dementia at presentation. By published standards these patients will

not, by definition, have idiopathic PD. Patients with significant memory impairment at presentation (defined as MMSE score < 24), or meeting DSM IV criteria for dementia at presentation. By published standards these patients will not, by definition, have idiopathic PD.

4. Patients who do not have the capacity to consent to be involved in the study (as assessed by criteria laid out in the MHA code of practice, section 4-3).
5. The following parkinsonian disorders:
    (i) dementia with Lewy bodies (DLB), according to revised consensus criteria (88)
    (ii) exposure to dopamine receptor blocking agents at the onset of symptoms, leading to a diagnosis of 'drug-induced parkinsonism'
    (iii) repeated strokes or stepwise progression of symptoms, leading to a diagnosis of 'vascular parkinsonism'
    (iv) a diagnosis of progressive supranuclear palsy, multiple system atrophy, or corticobasal degeneration, according to accepted diagnostic criteria (89)

See Appendix 1 for a summary of the recruitment flow to ICICLE-PD.

Sampling and Case Ascertainment via Primary Care

- We will discuss ICICLE-PD with appropriate research officers (*i.e.* Primary Care Research Network, PCRN, Comprehensive Local Research Network, CLRN, Dementia and Neurodegenerative Diseases Network, DeNDRoN) covering relevant Primary Care Trusts (PCTs) and secondary care systems to specify detail of processes and standard operating procedures.

- PCT approval will be needed to work with the Primary Care Research Network and DeNDRoN staff. We will approach all practices covered by the relevant PCTs in the region of Newcastle-Gateshead (e.g. North of Tyne, South of Tyne and Sunderland PCT) and Cambridgeshire. Eligibility of participants for incidence estimation will be those living within particular PCT geographical boundaries (i.e. Newcastle-Gateshead & Cambridgeshire). The population estimates for these PCTs during the years of recruitment will provide the population denominators for incidence estimations.

- For the first three months of the study we will establish the set up of systems using PCT, PCRN and DeNDRoN staff as appropriate for referral of all suspected case of parkinsonism by GPs into the neurological services. This could also include routine checking of electronic records by NHS Staff within practice.

- If the patient expresses interest in the study the research network nurses, in close liaison with practices, will arrange to see the possible research recruit plus any next of kin as appropriate and follow case recruitment procedures (i.e. explaining the study, giving materials etc) in greater detail.

Secondary Level Recruitment

People who are diagnosed with parkinsonism are managed in both areas by neurologists, geriatricians and general practitioners (GPs). The neurology services are provided at regional neurology units in Cambridge and Newcastle, which include clinics specifically devoted to Parkinson‟s disease. There are further neurological outpatient facilities provided in District General Hospitals in both regions. At least

one consultant neurologist and their staff serve each hospital. Geriatric inpatient and outpatient facilities are also provided at all of these hospitals. At least seven specialist Parkinson"s disease nurses work with Parkinson"s disease inpatients and outpatients within the Cambridgeshire and relevant North East areas. Incident cases will be detected using four sources, with written requests for notification of patient details sought at 3-monthly intervals: (i) all GPs; (ii) all neurologists; (iii) all geriatricians and (iv) all Parkinson"s disease specialist nurses.

For the clinical study only we will extend secondary level recruitment to patients attending clinics provided by Northumbria Healthcare NHS Trust, City Hospitals Sunderland and County Durham and Darlington Foundation Trust. Secondary care specialists based in these additional locations who have experience in the diagnosis and management of Parkinson"s disease will be encouraged to refer all newly diagnosed PD patients to the study. Where in doubt, referral to the nearest movement disorders service as per normal clinical practice will be encouraged.

Frequent personal visits will be made to all individuals among sources ii–iv, to increase participation. We also seek the help of DeNDRoN staff to assist with recruitment to this study (both Newcastle and Cambridge are covered by DeNDRoN local research networks). The study will also be advertised through presentations given to local branches of the Parkinson"s Disease Society. Patients referred by GPs to the study will be seen in NHS outpatient clinics for consultant opinion and management advice. The referral of *untreated* patients will be encouraged throughout to all ascertainment sources. From these clinics, potential participants will be given written information about the study and what it entails. After a period of not less than 24 hours, they will be contacted to determine whether or not they wish to consider taking part. If they wish to consider involvement further, they will be invited to attend the relevant clinical trials unit in Cambridge or Newcastle.

Assessment of Incidence

Once the primary care and secondary care level recruitment is running smoothly the formal incidence study will be initiated so that all those identified as meeting study criteria between specified dates will be included. These will relate to the estimated population at risk in those geographical areas.

Incidence data will be collected from both; subjects who have been assessed as part of the clinical study and subjects who have declined participation. In the latter instance, non-identifiable patient data for the purpose of the incidence (epidemiology) study will be collected from the respective sources stated above. Non-identifiable data that will be collected include subject initials, date of birth, gender, ethnicity and the use of dopamine transporter imaging. When suitable, computerised medical records will be used to help ascertain the above data. This will be done via the referral source (i.e. clinician) and may involve the input from already established research networks (e.g. DeNDRoN). All data collected for the incidence study will be stored on secure, password protected servers only accessible by the study team (see data management).

Study Visits and Procedures

After providing written, informed consent, the following baseline assessments will be performed in all participants:

A] Diagnostic and Demographic Evaluation

1. A detailed history of disease, level of education, current and all previous occupations; ethnic origin; family history of neurological disease and psychiatric illness; full drug history; significant co-morbidity, including a structured proforma for vascular risk factors; and referral source.

2. A standardized neurological assessment, looking specifically for features of other extrapyramidal diseases (e.g. progressive supranuclear palsy, multiple system atrophy and corticobasal degeneration). Other assessments will include the new version of the Movement Disorders Society-Unified Parkinson‟s Disease Rating Scale (MDS-UPDRS) (90), timed motor tests for hand-tapping and walking, and the PDQ-39 (91) to assess the patients‟ quality of life.

3. Demographic information of caregivers/informants will also be taken, including: level of education, current and previous occupations, ethnic origin and caregiving history. Physical health will be measured using the The Duke OARS (Older Americans Resources and Services) Physical Health Checklist (132). Comorbidities in mental health will be measured by the Hospital Anxiety and Depression Scale (HADS) (133).

B] Assessment of Clinical Features Hypothesised to be Predictive of, or Relevant to, Incident Dementia

1. Motor phenotype will be defined according to a well established method (92).

2. A 12 lead resting ECG will be recorded. Blood pressure will be measured with standardised semi automated sphygmomanometers. Three seated blood pressure readings will be taken after 5 minutes‟ rest and the reading taken as the average of the 2nd and 3rd reading. American Association of Neurology and British Hypertension Society (BHS) guidelines will be followed for the measurement of OH, which require a three-minute stand following a 10-minute supine rest period (93). A subject is defined as having OH if a drop in systolic blood pressure (SBP) after standing is greater than or equal to 20mm Hg or if SBP falls to less than 90mm Hg.

3. Weight and height will be recorded, for calculation of body mass index.

4. All participants will be asked to complete an extended form of the Pittsburgh Sleep Quality Index (PSQI) (94) and the Epworth Sleepiness Scale (95) to assess sleep quality and disturbance, and excessive daytime somnolence, respectively.

5. Cognitive function will be assessed using standardized, previously validated tests; the mini mental state examination (MMSE) (96), the Montreal Cognitive Assessment (MoCA) (128, 129), the National Adult Reading Test (NART) (97) as a measure of premorbid IQ, a test of verbal fluency for words starting with the letters F, A and S for 1 min each (98), and a test of verbal fluency for animals in a 90-s period (99) (tests sensitive to frontostriatal and frontoparietal impairment) (100). The pentagon copying item within the MMSE will be graded using a modified 0-2 rating scale, as a measure of visuoconstructional function (11, 101). The Montreal Cognitive Assessment has proven sensitivity and is more specific in detecting mild cognitive impairment in Parkinson‟s disease (128, 129). The NART will only be used as a measure of premorbid IQ for native English speakers. The

Parkinson‟s Disease Everyday Cognition Questionnaire (PD-ECQ) will be used to measure cognitive dysfunction in the early stages of Parkinson‟s disease (See Appendix 4). The short version of the Informant Questionnaire on Cognitive Decline in the Elderly (IQ-CODE) will be used to measure caregiver observations of cognitive dysfunction (134). Short Form The following subsets from the CANTAB battery (102, 103) will also be used: (i) Pattern (PRM) and spatial recognition memory (SRM), differentially sensitive to impairment of temporal and frontal lobe function, respectively (104). (ii) The modified ('one touch') version of the Tower Of London task, a test of planning requiring working memory (105) which has been shown to activate a fronto-parietal-caudate circuitry (106, 107) (iii) PAL; paired associates learning; a visuospatial test of learning and memory that is sensitive to both temporal and frontal damage and has been shown to predict Alzheimer's disease 32 months before formal diagnosis in memory clinic or in the community (108, 109). (iv) MOT; Motor screening.

6. The Ferman “4-item questionnaire” and Cognitive Drug Research (CDR) battery will be used to assess cognitive fluctuations (110). Although not yet validated in PDD, informant endorsement of 3 out of 4 of the former has an 83% positive predictive value in discriminating DLB from AD. The CDR is not prone to observer bias and is of suitable test duration for the participants (111). The three tests in the CDR attention battery are Simple Reaction Time, Choice Reaction Time, and Digit Vigilance. Four composite measures are derived from these tasks including power of attention and continuity of attention (112). The Spatial Working Memory test will also be performed.

7. Neuropsychiatric assessment will comprise the Neuropsychiatric Inventory (NPI-D) (113), the Geriatric Depression Score (GDS-15) (114), and the North East Visual Hallucinations Inventory (NEVHI, (115)). The NEVHI comprises four sections: trigger questions (7 items), frequency and severity (5 items), phenomenological questions (23 items) and questions referring to hallucinations in other modalities and emotions and behaviours associated with visual hallucinations (15 items). 2 questions from the Mayo Sleep Questionnaire with proven sensitivity and specificity for REM-sleep behaviour disorder will be incorporated in the assessment (126). Further quality of life questions, in the form of semi-structured interviews and the Parkinson’s Disease Carer Questionnaire (PDQ-C), will be added for participants and their carers (130, 131).

8. A newly developed portable head-mounted saccadometer (Ober Consulting, Poznan) will be used to present targets and measure eye movements by changes in the binocular infra-red scleral reflectance (116). The device is comfortable to wear and requires no particular skill to set it up. It can even be used in the home as no stabilisation of the head is necessary. The equipment allows saccadic latencies to be recorded within 15 minutes, and is automatically calibrated using a small number of preliminary trials. Latency data will be downloaded onto a laptop running LatencyMeter (116), which deletes results from spurious eye movements. Subjects will be asked to track the movement of a red laser dot as rapidly as possible. The tracking task involves a step task paradigm with no gap or overlap of stimuli. After a random fore-period (0.5-1 s) the central fixation is extinguished as either the left or right target, chosen at random, is turned on, remaining on until a saccade has been performed or for a maximum of 2 s (19).

9. The Unified Dyskineasia Rating Scale (UDysRS) will be used to assess disability and impairment from dyskinesias (135). It includes an evaluation of on and off dyskinesias, and observational measures during simple activities of daily living. It also includes patient-based evaluations of dyskinesia. The Scwab and England Activity of Daily Living measures a person's ability to perform daily activities in terms of speed and independence. It includes a scale rating activity from 100%, complete independence, to 0%, bedridden (136).

C] Imaging Studies

1. MR studies

MR studies will be undertaken in all subjects recruited from both sites who consent to undergo brain imaging. We will adopt a common MR protocol in both sites for data acquisition. In Newcastle all subjects will be imaged on a 3 Tesla Philips Intera Achieva scanner within the Newcastle University Magnetic Resonance Centre and in Cambridge on a Siemens Tim Trio scanner within the MRC Cognition and Brain Sciences Unit. The following sequences will be obtained:

a) Structural imaging will include whole brain volumetric (T1 weighted magnetisation prepared rapid acquisition gradient echo (MP-RAGE) Repetition Time 9.6 ms, Echo Time 4.6, Flip Angle = 8$_o$ ms 150 sagittal slices 1.2 slice thickness; 240x240 Field of View; SENSE = 2; Acquisition time 4 minutes) and FLAIR sequences; Repetition Time 11000 ms; Inversion time 2800 ms; echo time 125 ms; 50 axial slices 3mm thick; 240x240 Field of View; Imaging Time 4:25 minutes.

b) Diffusion Spin Echo EPI sequence 2x2 mm voxel size; slice thickness 2.5 mm; 256 x 256 Field of View; 50 slices; SENSE = 2; Repetition Time 5541 ms; TE 71; Half-scan factor 0.681; Flip Angle 90$_o$ EPI factor = 67 Diffusion b=0 & 1000 ; 64 directions. One average: imaging time 6:38.

c) We will determine if it is feasible to detect differences in the structure and cortical connections of the pedunculopontine nucleus (PPN), given its potential role in determining motor phenotype (and possibly an influence upon cognition). We will perform high resolution diffusion tensor imaging from which maps of the tissue fractional anistropy (FA) will be calculated and used to determine the location of the PPN. To further assess the cellular composition of the PPN we will use Q-space diffusion imaging. We believe this method will be sensitive to altered cellular structure within the PPN. The Q-space scan will be restricted to a small number of slices encompassing the PPN and measurements will be made with diffusion encoding along the 3 principle directional axes defined from the calculated FA map.

d) fMRI imaging will comprise T2-weighted echo-planar images depicting blood oxygen-level dependent (BOLD) signal acquired during up to three 10 minute runs. Resting BOLD will also be performed. Each image will consist of 32 slices of 3 mm thickness with a 1mm interslice gap (descending slide order), with an in-plane resolution of 3 x 3mm. The TR will be 2 seconds. Slices will angled away from the orbits to avoid signal dropout due to magnetic susceptibility inhomogeneity.

Stimuli will presented on a computer screen visualised using a mirror positioned within the scanner during three tasks which we have previously shown to produce specific, performance-related activity in frontal and parietal-lobe regions (44, 117) and frontal and hippocampal regions (85, 118). The first is a problem solving task based on the Tower of London planning test. Participants are required to solve up to twelve visuospatial problems by working out how many moves are required to match two different

configurations of coloured stimuli. In a non-planning 'control' condition they simply have to count the number of stimuli on the screen and perform a simple subtraction between the two configurations. In both conditions a response is made by pressing a button with the right hand. The second task assesses episodic memory; prior to the scan, the participants are shown a series of pictures of obscure (e.g. not previously seen) abstract art and they are asked to remember a subset of those stimuli. In the test condition (during scanning) they are shown a series of abstract art stimuli, some previously viewed and some novel, and they are asked to indicate by pressing a button whether or not they recognise each stimulus. The third task, which assesses mental rotation abilities, is known to activate parietal cortex in healthy volunteers (Hampshire & Owen, unpublished pilot data). Participants are required to select which pattern is a rotated version of a target pattern from among four alternatives. All of these tests have been well validated and shown to be tolerated will by PD patients both in and out of the fMRI environment (44, 119).

Quality assurance and harmonisation of scanner sequences between sites will operate throughout the study. This will ensure comparable data is obtained between centres whilst providing the potential to develop and improve sequences that yield equivalent or better data in the study group. To facilitate the comparison of images obtained between Newcastle and Cambridge MR machines, imaging of a control cohort of up to 20 subjects will be performed in both centres (i.e. Newcastle-Gateshead and Cambridgeshire). Selection for participants in the control group will be base on (i) age; ≥18 years (ii) Cognitively intact (iii) No known cerebral disease/ abnormality (iv) The ability of the participant to travel between centres for scans within a one month period.

2. PET Studies

a) [18F]FDG PET will be performed in a minimum of 80 subjects aged 65 years or over (40 each in Newcastle and Cambridge) recruited sequentially to avoid bias and to increase the external validity of the PET findings. The scanned subjects will be classified independently as having a “high” or “low” risk of incident dementia according to clinical measures other than age (specifically, semantic fluency, pentagon copying, motor phenotype).

Newcastle subjects will be scanned locally, whilst Cambridge subjects will be scanned at the Hammersmith Hospital, London. All subjects will be scanned after injection of 185 mBq [18]FDG using either a GE CT PET Discovery or Siemens Biograph 40 camera with 5mm and 4.2mm resolution, respectively, after image reconstruction according to a previously reported protocol (50). Parametric maps of absolute regional cerebral glucose metabolism (rCMRGlc) will be generated with spectral analysis using an arterial input function. For region of interest (ROI) analysis of [18F]FDG scans all the individual images are co-registered to their corresponding MRIs and then normalised to MNI stereotaxic space. Function of the anterior and posterior cingulate cortex, thalamus, striatum, frontal, temporal, parietal and occipital cortical regions will be sampled, as will that of hippocampus, amygdala, parahippocampal gyrus, primary motor and sensory cortices. A between group comparison of parametric rCMRGlc images will be performed employing statistical parametric mapping (SPM05) to localize significant changes in mean [18F]FDG uptake at a voxel level using a threshold of $p<0.001$ with

an extent threshold of 50 voxels. ANCOVA is applied to remove the confounding effects of global on regional uptake variance.

b) A sub-group of subjects (n of 20-30 predicted from dementia conversion rate – see below*) (to delete)* recruited in Cambridge will undergo [18]FDG, [11]C-PK11195 and PIB PET scanning upon fulfilling Task Force Criteria for PDD as part of the Memory impairment in Parkinson‟s disease: A PET study into the deposition of amyloid in Parkinson‟s disease. This study has been approved by the Charing Cross Research Ethics Committee (REC reference 08/H0711/135). Cortical glucose metabolism, inflammatory changes and amyloid load in these subjects will be compared with additional prevalent PD cases with longer disease durations prior to onset of dementia. In view of the relatively small numbers in this sub-study we will undertake a preliminary analysis only of cognitive and neuropsychiatric features of PDD patients as correlated with [18]FDG, [11]C-PK11195 and PIB PET findings, to determine whether cortical metabolic profile and amyloid burden might be predictive of clinical phenotype. A cohort of PD patients with risk factors for developing dementia but without dementia whom have been recruited in Cambridge will also be offered a chance to participate in the above study.

Subjects will be scanned after injection of 370 mBq ^{11}C-PIB using a GE CT PET Discovery camera with 5mm resolution after image reconstruction, according to a previously reported protocol (50). Target region to cerebellar ^{11}C-PIB uptake ratio images are created by dividing a mean 60-90 minute tracer uptake image by the integral 60-90 minute uptake value of cerebellar grey matter. Target to cerebellar ratios at these times provide a blood flow independent measure of specific ^{11}C-PIB retention that is easy to calculate and reflects amyloid binding, is robust, rests on minimum assumptions and does not require arterial sampling. Individual 60-90‟ uptake RATIO images are co-registered to the corresponding MRI scan. Both co-registered RATIO images and MRI scans are subsequently spatially normalized to the T1 MRI template in MNI space using the default settings in SPM. MRI images are segmented into grey matter, white matter and CSF using SPM, and grey matter images thresholded at 50% probability. The binarised grey matter map is then convolved with a probabilistic brain atlas in order to sample the following regions: frontal, temporal and parietal association cortices, anterior and posterior cingulate gyrus, striatum, thalamus, cerebellum, hippocampus, amygdala and parahippocampal gyrus, primary motor, primary sensory and primary visual cortex. Clusters of significant differences in mean 60-90 minute ^{11}C-PIB region to cerebellar uptake ratios between PDD and control subjects are localised at a voxel level using SPM05. Spatially normalized RATIO images will be interrogated using a threshold of $p<0.00001$ with an extent threshold of 200 voxels to detect significant change without applying ANCOVA or proportional scaling.

c) As a comparator to our PD patients, consenting control participants will also undergo PET scanning to enable us to determine whether changes observed in PD participants are reflective of the underlying disease process. [18F]FDG PET will be performed in a minimum of 20 control subjects aged 65 years or over. They will be recruited sequentially to avoid bias and to increase the external validity of the PET findings. Newcastle subjects will be scanned locally, using the methods described above.

D] <u>Genetic and Functional Studies</u>

Our primary aim is to generate a biobank for future research studies. The methods of extraction and storage are designed to ensure that we maximise the potential use of serial clinical data, enabling hypothesis-driven research at the DNA, RNA, and protein level. Specific investigations planned at this stage of the programme will focus on cognitive decline and mitochondrial mechanisms, which are our areas of specific expertise and interest. Our previous work implicates the *H1/H1 MAPT* genotype in cognitive decline. We will replicate this study and determine the H1 sub-haplotypes responsible for the association. We will also determine whether other genetic risk factors for cognitive decline and psychiatric features interact with *MAPT*, including *APOE,* and the *GSK3B, MTHFR*, *TBP,* 5HT(*SCL64A*), *ADRA2*, *COMT*, *DRD2*, *CHRM2*, and the recently described glucocerebrosidase (*GBA)*, *LRRK2* and chromosome 2 loci associations with DLB. All patients who test positive for one of the Mendelian forms of PD will be excluded from any further analysis of our data, although we do not anticipate that this will be significant numbers based on our previous LRRK2 studies (120). As itemised in schedule of visits, 20-30mls of venous blood will be collected on 2 occasions and separated into various tubes; polypropylene, EDTA and a PAXgene Blood RNA tubes. The blood tubes and subsequent biological samples will be labelled with a unique study code to ensure patient anonymity in the laboratory. The code will be kept securely and separate from the samples. 5mls will be snap frozen at -80$_{o}$C in two separate aliquots for future studies. RNA will be extracted from one 5 ml sample using the trizol-based spin-column RNA kit and stored at -80$_{o}$C. The remaining 5 mls will be separated into serum and buffy coat by centrifugation at 10,000 rpm for 5 minutes. Total genomic DNA will be extracted from the remaining 2.5 mls using Quiagen extraction kit. The DNA will be split into 10 aliquots that will be snap frozen and stored in -80$_{o}$C freezers. The serum will also be split into aliquots, snap frozen and stored at -80$_{o}$C.

Genotyping will be carried out in Cambridge (Taqman, assay on demand) and Newcastle (Sequenom Mass Array) using two independent methods with 10% duplicate genotyping for each SNP. Logistic regression will be used to determine the relative risk of cognitive decline attributable to each genetic variant, whilst controlling for potential confounding variables such as age, gender and co-morbid features. Any genetic associations will be studied further at the gene expression level in RNA extracted from the PD cohort, and in post-mortem brain from the Newcastle Brain Tissue Resource, and the pathology and neurochemistry will be compared in brains from patients and controls with and without the putative genetic modifier.

The same blood sample will also be used to study telomere length. These samples will be processed in the laboratory of Professor Thomas von Zglinicki, Campus for Ageing and Vitality, Newcastle University, according to well established methods (70).

Mitochondrial superoxide generation and cellular peroxide levels in ex-vivo primary lymphocytes from all available study participants will be measured by flow cytometry (127). Mitochondrial membrane potential and mitochondrial mass will also be measured using fluorescent indicator dyes. Related information on oxidative stress-mediated damage at the cellular (telomere length) and systemic (isoprostane levels in serum) level will also be measured. Together, they will allow a comprehensive characterisation of oxidative stress and stress-dependent damage in the study participant.

E] Other Blood and CSF Studies

1. Blood Sampling

A fasting lipid profile and blood glucose will be taken during the assessment, with the subject fasted from midnight. Breakfast will be provided thereafter.

Consistently non-fasted total plasma homocysteine levels will be measured in nominated laboratories and processed according to recent recommendations (121). As far as possible, samples will be collected in the morning. Samples will be cooled on ice until centrifugation, which will be performed as quickly as possible, to remove blood cells. Medication will not be withheld for this sampling.

Serum from patients (obtained as described above under "Genetic Studies") will also be stored in a -80$_0$C freezer for future metabonomic studies (the value and power of this approach is currently being investigated through a PDS project grant to Dr R Barker and will potentially give added value to the programme). Repeat random blood tests for uric acid, red cell folate, B12 and homocysteine levels will be done at 18 months +/- 36 months if participants consent to this. Additionally, a fasting sample for lipid profile, glucose, genetic studies and metabonomics will be taken. The subject will fast from midnight and breakfast will be provided following the blood sample.

2. CSF Studies

All subjects agreeing to participate will be approached to undergo lumbar puncture (LP) for CSF analysis, including A□42, T-tau and P-tau$_{181}$. This will comprise a sub-study. Information about the procedure will be provided in the participant information sheet and consent will be taken before the assessment should the individual wish to participate in this optional procedure.

If agreeable, participants will attend fasted in the morning and if taking antiparkinsonian medications, participants will be asked to withhold these from midnight where possible, prior to the procedure (a majority of people will be untreated at this stage). Eight mls of CSF will be removed using standard aseptic LP technique with the person in the lateral decubitus position. Paired serum samples will also be obtained via venesection. The morbidity from this day case procedure is extremely low, with an incidence of "moderate headache" reported in <1% of subjects (82).

All participants who agreed to consent to lumbar puncture at initial presentation will be separately consented again for a repeat procedure at 18 and/or 36 months as an optional component of the longitudinal study. Information about the procedure has provided in the initial information sheet and can be provided again if necessary. The protocol for collecting and storing CSF will be as per initial visit.

CSF will be withdrawn into polypropylene tubes, centrifuged at 2000g for 10 minutes (at 4˚C) subaliquotted into 500 □l siliconized tubes and frozen to -80$_0$C within 30 minutes. Batched frozen samples will be analysed locally and also sent to Dr Brit Mollenhauer (Kassel, Germany) for analysis. (122).

F] Brain Tissue Donation

All participants will be approached regarding future Brain Tissue Banking in a Human Tissue Authority-approved facility, either in Newcastle (the Newcastle Brain Tissue Resource), or in London (either Imperial College PDS Brain Bank, or Queen Square Brain Bank) at their first follow up visit (scheduled

for ~18 months). This will involve a separate consent process and follow well-established standard operating procedures for approval and consent conducted according to the principles of Good Clinical Practice and guidelines issued by the Human Tissue Authority.

G] Neurophysiology- Short Latency Afferent Inhibition (SAI)

SAI will be undertaken in subjects recruited from Newcastle who consent to undergo SAI studies. These studies will not be performed in those subjects with contraindications to transcranial magnetic stimulation (for example, epilepsy; cranial implant; presence of a deep brain stimulator; presence of a pacemaker). The SAI technique is non-invasive and comprises an inhibition of the motor-evoked potential observed when transcranial magnetic stimulation is delivered with a 2-8ms delay, after a peripheral nerve input has reached the somatosensory cortex. Trans Magnetic Stimulation (TMS) will be performed to elicit a motor response in the dorsal interrosei muscle in the hand for right and left sides. Surface electromyography (EMG) electrodes placed over the dorsal interrosei muscle in the hand will record the response (the motor-evoked potential) (MEP). A sensory conditioning stimulus will be applied to either the median nerve or the digital nerve prior to TMS, to inhibit the MEP. Differences in TMS response before and after nerve stimulation will be recorded. This will be obtained at baseline and follow-ups in control subjects and at follow-ups in those with PD for those that consent.

Follow Up and Diagnosis of Dementia

Clinical Studies

The diagnosis of PD will be re-validated every 18 months, at which time each subject will also be reassessed using the same battery of clinical measures described above [see A and B]. We have chosen this time interval because, first, the rate of global cognitive decline in non-demented PD subjects is slow (between 0.3 and 0.5 MMSE point per year, (11, 123), making more frequent assessments insensitive to change, and, second, because the predicted number of 175 participants per centre (Cambridge and Newcastle) with recruitment period extending over 30 months, means that this frequency of assessment is feasible for all subjects from the date of study entry. All participants will therefore undergo baseline and either one or two follow up assessments over the 5-year programme.

MR Studies

MR studies will be repeated on all subjects at 18 months using identical protocols to those described above for structural imaging, diffusion spin echo imaging and fMRI (see C1). We estimate 90% of subjects will agree to repeat imaging. Serial volume changes will be undertaken using the boundary shift method to determine rates of brain atrophy and voxel compression analysis after fluid registration to infer the regional distribution of the atrophic pattern. The utility of specific measures for prediction in individual cases will be determined by calculating sensitivity, specificity and positive predictive values.

Diagnosis of Dementia

A diagnosis of dementia will be made according to new Task Force Criteria for PDD (124), with operationalisation of these criteria according to newly formulated guidelines (125). The criteria permit classification of PDD at “probable” and “possible” levels. The “traditional” method of MMSE score less than 24 and fulfilment of DSM-IV criteria for dementia will also be applied to all cases to permit

evaluation of both methods and also inter-study comparison, since the latter criteria have been widely used to date. Standardisation of the DSM-IV criteria will be according to previously described methods (11), to lessen the reliance upon subjective judgements. For incident cases of dementia, unless interim medical documentation is available, time of dementia onset will be assumed to be the mid-point of the interval between assessments.

Recruitment of Study Subjects for Control Group

The recruitment of a control group to the ICICLE-PD study will provide an essential comparator for cross-sectional analyses of ICICLE-PD data and relevant sub-studies, and will allow us to control for the effects of normal ageing. We will therefore recruit an age-matched control group of 110 subjects from the North East that are demographically comparable (similar in age, sex and educational level) to participants in ICICLE-PD.

As in participants with PD, assessment of controls will be performed at 0, 18 and 36 months. Tests that will be administered to controls are outlined on page 29. Participants will be given the option of participating in other aspects of ICICLE-PD that include blood tests (including genetic and proteomic studies) and imaging (functional and structural MRI brain scan), together with related studies of sleep, gait and cholinergic function (SAI). MRI brain imaging will be performed on the first 50 participants who consent. Participants who consent for brain imaging will be approached at 18 months to undergo a second MRI scan.

Inclusion criteria for the control group will be as follow:

- Age range ≥ 45 years from any educational background.
- English as a first language or fluent command of the English language as defined by the assessor.
- Ability to mobilise independently without the use of a walking aid.
- No history of dementia/ significant cognitive impairment, movement disorder (including essential tremor) or current significant mood disorder.

Exclusion criteria:

- MMSE <24 or dementia as defined by DSM IV criteria
- Significant vascular co-morbidity (i.e. stroke disease)
- Patients who do not have the capacity to consent to be involved in the study (as assessed by criteria laid out in the MHA code of practice, section 4-3).

Recruitment strategy for control group:

- The Primary Care Research Network (PCRN) covering relevant Primary Care Trusts (PCTs) (e.g. North of Tyne, South of Tyne & Sunderland PCTs) will be primarily responsible for identifying potential control participants via research active general practices. The PCRN have considerable experience of recruiting such cohorts and will adhere to good research practice principles, including confidentiality etc, in identifying such subjects.

- Participants will also be recruited from the local hospital trusts in the region (e.g. Newcastle-upon-Tyne City Hospitals) via appropriate study awareness methods and advertisements.
- Participants may also be recruited via the Public Engagement Team based at the Institute for Ageing and Health at Newcastle University. The latter have established networks and partnerships with organisations such as VOICENorth, Age Concern, etc.
- An advert to be placed in the local paper, advertising for healthy volunteers.
- After receiving the names of potential participants who have given their permission to be approached, the ICICLE-PD research team will contact these individuals and provide more study information and give an opportunity to ask questions. After a further period of not less than 24 hours, potential control subjects may then consent to participate.
- We will obtain the participant‟s consent to contact their partner or caregiver. Upon gaining consent we will seek to meet their partner/caregiver. Should this not be possible they will be contacted via telephone whereby relevant observer/ informant based information will be acquired as per protocol (i.e. relevant partner/caregiver based questionnaires will be administered)

Sampling and Case Ascertainment via Primary Care

- We will discuss ICICLE-PD with appropriate research officers (*i.e.* Primary Care Research Network, PCRN, Comprehensive Local Research Network, CLRN, Dementia and Neurodegenerative Diseases Network, DeNDRoN) covering relevant Primary Care Trusts (PCTs) and secondary care systems to specify detail of processes and standard operating procedures.
- PCT approval will be needed to work with the Primary Care Research Network and DeNDRoN staff. We will approach all practices covered by the relevant PCTs in Newcastle/Gateshead and Cambridgeshire. Eligibility of participants for incidence estimation will be those living within particular PCT geographical boundaries. The population estimates for these PCTs during the years of recruitment will provide the population denominators for incidence estimations.
- For the first three months of the study we will establish the set up of systems using PCT, PCRN and DeNDRoN staff as appropriate for referral of all suspected case of parkinsonism by GPs into the neurological services. This could also include routine checking of electronic records by NHS Staff within practice.
- If the patient expresses interest in the study the research network nurses, in close liaison with practices, will arrange to see the possible research recruit plus any next of kin as appropriate and follow case recruitment procedures (i.e. explaining the study, giving materials etc) in greater detail.

Statistical Analysis

A minimum sample size of 30 is required to ensure robustness of standard statistical summary measures, for example mean, SD, correlation. It also allows approximation to normality in some statistical tests. Conversion rate to dementia is assumed to be 10% at a mean 3.5 years (SD=0.7) follow

up (11) so that recruitment of 300 to 320 cases is required. 320 cases will allow some dropout and for variation around the mean conversion rate and follow up time.

The study assessments will result in data of a binary, categorical and continuous nature. Cognitive decline will also be captured as a binary (demented vs. non-demented) and as a continuous variable (i.e. deterioration in cognitive scores), allowing more powerful statistical techniques to be used.

The resulting sample of cases will be unbalanced with 30 dementia and 270 non-dementia cases. This sample size will give 80% power to detect a difference in proportions (between dementia and non-dementia groups) of approximately 30% (20% v 50%) assuming a type 1 error rate of 5%. At a lower proportion of 25%, this sample size will give 80% power to detect a difference in proportions of approximately 25% (0% v 25%) assuming a type 1 error rate of 5%. The sample size will also give 80% power to detect a difference in a continuous measurement of approximately 60% of the SD assuming a type 1 error rate of 5%. A smaller difference corresponds to greater sensitivity; the sensitivity increases with the multivariable approach, so multivariable tests are expected to be more powerful.

Baseline measures for all cases will be summarised for informative purposes using univariate and multivariate descriptive statistics and will be tabulated and presented graphically for clarity. Time series data showing the changes over time between baseline, 18 month and 36 month follow up measurements will be analysed and illustrated. As incident dementia cases occur, individual baseline, 18 month and 36 month follow up measurements will be compared in order to illustrate significant differences using either chi-squared, Mann-Whitney or students t-tests as appropriate, depending upon whether the data is normally or non-normally distributed. Multivariable analysis, for example binary logistic regression will be used to compare combinations of characteristics between dementia and non-dementia groups; outputs may be given in terms of odds ratios.

Many of the test results will have synergy and give variables which are correlated with each other. If multiple univariate tests are carried out there will be a high risk of false positives. The critical values applied to multiple univariate tests will be adjusted using an appropriate technique, such as the sequential Bonferroni technique (see, for example Rice WR (1989) "Analyzing tables of statistical tests", Evolution 43:223-5) to limit the overall experiment-wise error to a suitable level, e.g. 0.05 or 0.10. In practice this means that markers will have to show a highly significant predictive ability to be considered important and clinically useful. We anticipate that such markers will be found; for example, a non-tremor dominant motor phenotype when present at onset has previously been associated with a four-fold increased risk of dementia.

It is a general recommendation to have at least 30 cases to be able to carry out basic statistical tests. This, however, is a guideline and many analyses can be carried out with fewer cases, depending upon the nature of the variability shown in the cases and the type of statistical tests applied. Sample sizes of 30 PDD and 270 non-demented PD cases have been chosen to illustrate power calculations as a guideline.

It is not uncommon for principal components and other multivariate analyses to be performed in situations where there are many more variables, p, than cases, n, i.e. p>>n (see for example *Change in Marine Communities - an approach to statistical analysis*. Plymouth Marine Lab 1994. NERC UK). All the sample variance can be explained by the first n-1 principal components. None of the p variables are

ignored but they are represented by a smaller number (<n) of principal component variables. It is expected that there will be correlations within the variables and this makes the dimension reduction due to principal components likely to be meaningful. Predictive equations may make use of a subset of the principal components.

Multiple regression will be used when the dependent variable is a continuous measure of dementia. Suitable factors and covariates in the analysis will be drawn from the measurements collected. Principal components and factor analysis will be used to reduce the dimensionality of the measurements and obtain representative features that are meaningful combinations of the original variables. Cluster analysis and other data mining methods will be used to look for patterns in the data with the aim of understanding the relationship between study assessment measurements and incident dementia.

Project Management and Timetable

Study Organisation and Management

The study will be run by a Project Management Group (PMG), comprising the Principal Investigators from the three centres, together with a nominated patient and/or carer member of the PDS Research Network and a nominee of the PDS (UK). The PMG will meet twice per year and will be responsible for ensuring progress of the study in relation to administrative, clinical and academic issues.

In addition, the wider research group (including the PMG and all research workers involved in the study in the three centres) will constitute the ICICLE-PD Study Group. All published output from the study will acknowledge this study group. From years 3-5 of the programme, the Study Group will meet on an annual basis to discuss the data acquired and proposals for analysis, further studies etc. These meetings will be synchronised with meetings of the PMG for economy of time and expense.

Data Management

Patient identifiable data will be collected in trial folders, stored in secure, lockable filing cabinets in research facilities in Newcastle and Cambridge that are locked and alarmed out of hours. Imaging data (i.e. MRI & PET) to be analysed together with colleagues external to source data centres will be shared via anonymous image files placed on to CD/DVD or via a secure password protected university servers. To decrease the likelihood of Type 1 error (e.g. patients representing to the study via multiple referral sources), non-identifiable patient data (date of birth, gender and patient initials) may be kept on a secure electronic database in Newcastle and Cambridge. All electronic databases, using study numbers and non-identifiable data, will be held according to Caldicott and local Research Governance Guidelines. Clinical data will be acquired and stored on secure databases in Cambridge and Newcastle. The databases will be similar on both sites, to facilitate combining datasets for analysis.

The database will include ICICLE-PD and its relevant sub-studies. e.g. „ICICLE-GAIT" (REC ref 09/H0906/82) and „Characterisation of sleep disturbance in an incident cohort of Parkinson"s disease and its relationship to disease phenotype" (REC ref 08/H0906/148). This will enable efficient data analysis to be performed. The access of ICICLE-PD data will remain separate from its relevant sub-studies to protect the confidentiality of participants who have not consented to participate in any sub-study.

Genetic, other biomarkers and imaging data will be stored on separate secure databases in each centre, but, where studies adopt common protocols (e.g. MRI scanning), there will be prior agreement over the format used and the information collected by post-processing. Analysis of FDG PET scans undertaken in Newcastle and London will be led by Professor Brooks‟ team and by local teams in Newcastle.

Time Schedule, Study Milestones and Deliverables

SUMMARY OF VISITS FOR PARKINSON'S DISEASE SUBJECTS

Visit 1 (Week 0)

Informed consent

Weight and height

Vital signs (*incl.* lying & standing BP)

Diagnostic and demographic evaluation (including MDS-UPDRS II & III, see Appendix 2, timed motor tests)

12-lead ECG

PDQ-39

Cognitive function assessments

Neuropsychiatric Inventory (see Appendix 3)

GDS-15

NEVHI

Ferman 4-item questionnaire

CDR attention battery

Visit 2 (Weeks 1-4)

CANTAB computerised test battery

PD ECQ

Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale

Montreal Cognitive Assessment (MoCA)

Saccadometry

Blood sampling for fasting full blood count, lipid profile, blood glucose, telomere length, metabonomic & genetic studies (total ~ 20-30ml)

MRI brain scanning *incl.* fMRI studies

Visit 3 (Weeks 8-16):

Non-fasting blood specimens for red cell folate & vitamin B12 levels, plasma total homocysteine concentration, uric acid and glucose

Lumbar puncture

Visit 4 (Within 4 months of initial assessment)

[18F]FDG PET in 40 consecutive subjects aged > 65 years in each site

Visit 5 (1ST REVIEW at ~18 months)

Weight

Intercurrent history and diagnostic re-evaluation (including MDS-UPDRS II & III, timed motor tests)

PDQ-39

Vital signs (*incl.* lying & standing BP) & 12-lead ECGCognitive function assessments

Neuropsychiatric Inventory

GDS-15

NEVHI

Ferman 4-item questionnaire

CDR attention battery

Random bloods for RCF, B12, homocysteine, uric acid

Visit 6 (1st REVIEW at ~18 months) (to complete 1st REVIEW within 4 months from commencement when possible)

Fasting bloods for genetics, telomere length, metabonomics & genetic studies

CANTAB computerised test battery

PD ECQ

Montreal Cognitive Assessment (MoCA)

PSQI and Epworth Sleepiness Scale

Saccadometry

Approach for brain tissue donation

Short latency afferent inhibition (SAI)

Visit 7 (1st REVIEW at ~ 18 months)

MRI brain scanning (within 4 months from 1st review)

Visit 8 (1st REVIEW at ~18 months)

Lumbar puncture (within 4 months from 1st review)

Subsequent Reviews at ~ 18 month intervals (with assessments done within 4-month time window when possible)

Weight

Intercurrent history and diagnostic re-evaluation (including MDS-UPDRS II & III)

PDQ-39

Vital signs (*incl.* lying & standing BP)

PSQI and Epworth Sleepiness Scale

Cognitive function assessments (incl MoCA & PD-ECQ)

Neuropsychiatric Inventory

GDS-15

NEVHI

Ferman 4-item questionnaire

CDR attention battery
CANTAB computerised test battery
Repeat blood sampling for homocysteine, genetics, telomere length, metabonomics
Random bloods for RCF, B12, homocysteine, uric acid
12-lead ECG
Scale of Quality of Life of Care-givers (SQLC)
Semi-structured quality of life interviews
Parkinson's Disease Carer Questionnaire (PDQ-C)
Caregiver/Informant demographic evaluation
OARS Physical Health Checklist (for caregiver/Informant)
Hospital Anxiety and Depression Scale (HADS) (for caregiver/Informant)
IQ-CODE
Schwab and England ADL Scale
Unified Dyskinesia Rating Scale (UDysRS)

Subsequent reviews from ~72 months after baseline assessment (Visit 1, Week 0)
Intercurrent history and diagnostic re-evaluation (including MDS-UPDRS II & III)
Schwab and England ADL Scale
Unified Dyskinesia Rating Scale (UDysRS)
Ferman 4-item questionnaire
Cognitive function assessments (incl MoCA, MMSE, Verbal Fluency, Semantic Fluency)
PDQ-39
GDS-15
Repeat blood sampling for genetics, telomere length, metabonomics
Neuropsychiatric Inventory
IQ-CODE

From 1st December 2015 all participants will complete a reduced battery of testing (54 month and 72 month assessments)

Subsequent reviews ~ 18 month intervals
Intercurrent history and diagnostic re-evaluation (including MDS-UPDRS II & III)
Schwab and England ADL Scale
Unified Dyskinesia Rating Scale (UDysRS)
Ferman 4-item questionnaire
Cognitive function assessments (incl MoCA, MMSE, Verbal Fluency, Semantic Fluency)
PDQ-39
GDS-15
Repeat blood sampling for genetics, telomere length, metabonomics
Neuropsychiatric Inventory
IQ-CODE

From 1st December 2016, there will be no further blood sampling.

Clinic only assessment: Subsequent reviews ~90 months from baseline and at ~18 month intervals thereafter

Assessments for PD participants approximately 90 months after the baseline assessment will take place at their usual movement disorders clinic using a basic assessment which includes routine clinical assessments and questions:

An intercurrent history including full drug list, dementia status using a structured proforma.

MDS-UPDRS III

Cognitive function assessments (incl MoCA and MMSE)

Schwab and England ADL Scale

IQ-CODE

SUMMARY OF VISITS FOR CONTROL SUBJECTS

Visit 1 (0 weeks)

Informed consent

Screening & clinical assessment (including MMSE)

Weight and height

Vital signs (*incl.* lying & standing BP)

12-lead ECG

Cognitive function assessments (incl CANTAB computerised test)

GDS-15

MoCA

Verbal fluency

NART

ESS

PSQI

Sleep diary (for 2 weeks in conjunction with actigraphy)

Saccadometry

Random bloods (homocysteine, urate, B12/folate, full blood count)

Visit 2 (~1-8 weeks)

Fasting bloods (lipid profile, glucose, genetic & proteomic studies)

Gait assessment and cognitive tests (including CDR computerised cognitive tests)

MRI brain scanning (structural and functional studies)

Short latency afferent inhibition (SAI)

Visit 3 (1st REVIEW~18 months) 32

Clinical review (including MMSE)

Weight and height

Vital signs (*incl.* lying & standing BP)

12-lead ECG
Cognitive function assessments (incl CANTAB computerised test)
GDS-15
MoCA
Verbal fluency
NART
ESS
PSQI
Sleep diary (for 2 weeks in conjunction with actigraphy)
Saccadometry
Random bloods (homocysteine, urate, B12/folate, full blood count)

Visit 4 (1st REVIEW~18-20 months)
Fasting bloods (lipid profile, glucose, genetic & proteomic studies)
Gait assessment and cognitive tests (including CDR computerised cognitive tests)
MRI brain scanning (structural and functional studies)
Approach for brain tissue donation
Scale of Quality of Life of Care-givers (SQLC)
Short latency afferent inhibition (SAI)

Subsequent Reviews at ~ 18 month intervals (with assessments done within 4-month time window when possible)
Clinical review (including MMSE)
Weight and height
Vital signs (*incl.* lying & standing BP)
12-lead ECG
Cognitive function assessments (incl CANTAB computerised test)
Neuropsychiatric Inventory
GDS-15
MoCA
Verbal fluency
ESS
PSQI
Sleep diary (for 2 weeks in conjunction with actigraphy)
Random bloods (homocysteine, urate, B12/folate, full blood count)
Fasting bloods (lipid profile, glucose, genetic & proteomic studies)
Gait assessment and cognitive tests (including CDR computerised cognitive tests)
Approach for brain tissue donation
Caregiver/Informant demographic evaluation
OARS Physical Health Checklist (for caregiver/Informant)

Hospital Anxiety and Depression Scale (HADS) (for caregiver/Informant)

Semi-structured quality of life interviews

IQ-CODE

Schwab and England ADL Scale

[18F]FDG PET in 40 consecutive subjects aged > 65 years in each site

<u>Subsequent reviews from ~54 months and ~72 months after baseline assessment (Visit 1)</u>

Intercurrent history

Cognitive function assessments (incl MoCA, MMSE, Verbal Fluency, Semantic Fluency)

Neuropsychiatric Inventory

GDS-15

Repeat blood sampling for genetics, telomere length, metabonomics

IQ-CODE

Schwab and England ADL Scale

Unified Dyskinesia Rating Scale (UDysRS)

Gait assessment

From 1st December 2016, there will be no further blood sampling.

"~" denotes approximate timing for the study visit/ assessment, as correction may be made according to exact timing of the visit and participant preference/convenience will be paramount, given this is not an interventional study. Timing of visits and tests may vary based on participant consent and comfort.

ETHICAL AND CLINICAL ISSUES

Dementia associated with PD is an emotive topic and not one that many health care professionals who deal with PD, nor the patients and carers themselves, often feel comfortable talking about. Euphemisms like “memory problems” or “cognitive impairment” therefore abound. We are acutely aware that many patients may not wish to know what their disease holds for them in the future, since there is currently no effective intervention. We believe that the means of recruitment, using community-based incident cohorts will minimise bias, while the extensive experience of both Cambridge and Newcastle in dealing with these issues will provide a sensitive approach to explaining the rationale for the study and obtaining informed consent. Careful explanation, including the fact that this research will help us to better understand the mechanisms underpinning dementia, as well as to identify more accurately “high risk” patients for trials of disease modifying treatment, should allay concerns as to the clinical applicability of the programme.

Participation in this study will have no direct implications for clinical care. The range of assessments that will be carried out every 18 months is more extensive than would routinely be performed and *may* have implications for improving management in some cases. Consent will be sought at study entry to permit us to inform the participant‟s GP (or other relevant health care professional, if necessary) should information come to light during the study that may have consequences for management and/or treatment. Furthermore, we appreciate that not all participants will necessarily wish to agree to undergo every component of the protocol. Thus, for procedures such as lumbar puncture, genetic testing, short latency afferent inhibition etc separate consent forms will be produced to allow a person to “opt in or out” of each component.

For measures of quality of life and wellbeing, participants will be asked questions relating to situations that they may feel are mildly upsetting or may feel distressed. Written consent will first be obtained from all participants and their carers, and they will be made aware of possible effects via the Patient Information Sheet. It will be made clear to participants that if they feel too distressed, they are able to stop at anytime and can refuse to answer the questions. They will also be given a list of contacts of appropriate health professionals if they should need help or advice (e.g. GP, PD consultant, PD nurse help-line).

Data sharing

We will share anonymised data (referenced only with study number) with approved collaborators both nationally and internationally (inside and outside of the EU) for scientifically sound, peer reviewed studies. Data sharing offers a more open approach which allows us to maximise the impact of the study for the health and wellbeing of the population.

Data sharing will be managed by our data management committee according to the following procedures:

1. Collaborators interested in accessing data from the study will send the data management committee an expression of interest, for example, using data request from (see Appendix 5 as an example) or via research platforms data portals.
2. The committee will then review the data request. If required, the data management committee may request changes to the proposed study by collaborators. The data management committee may then approve or reject the proposed study.
3. A data use agreement will be drafted, and once terms and conditions are agreed by all parties concerning the use and analysis of the data, the agreement will be signed by all parties (see Appendix 6 as an example).
4. As agreed by data managing committee and collaborators, and according to signed data agreement forms, anonymised data will be transferred to the collaborators.

Data will be securely transferred to collaborators. Data will be securely stored by collaborators for a fixed duration, as stated in the signed data use agreement. Only anonymous and unidentifiable data will be sent.

Glossary of Terms

Cognitive impairment: difficulty with thought processes that often include memory problems, but may also include difficulty with planning and execution of tasks, spatial awareness, etc.

Incident: newly diagnosed cases with a condition (as opposed to cases already known to exist i.e. prevalent) – usually expressed as number of cases per 100,000 population per year

Cohort: group of patients / study participants

Pathophysiology: the underlying pathological, neurochemical and genetic processes that collectively lead to the development and expression of a condition.

Functional magnetic resonance imaging (fMRI): a technique that provides a measure of activation or function of a particular part or parts of the brain in response to an experimental condition (e.g. moving a hand, trying to add up figures). fMRI relies upon the paramagnetic properties of blood and the fact that blood flow increases to a particular part of the brain when it is used.

Positron emission tomography (PET): a technique that involves injecting a low dose of radioactivity (positron emitting isotope, produced by a cyclotron) attached to a tracer of interest. This tracer enters the brain and provides information about function (or abnormalities) in the brain. Thus, PET can provide a map of metabolic activity in the brain, and highlight areas that are abnormally under-active. Additionally, the technique can demonstrate abnormal accumulation of proteins like amyloid in the brain.

Cortex: the covering of the brain (grey matter) that is sub-specialised into different functions depending upon location. Parts of the basal ganglia, deep-seated collections of grey matter within the brain, connect to different cortical regions via a series of circuits, or loops. These loops are functionally segregated and have different roles (e.g. motor control).

Short latency afferent inhibition (SAI): a non-invasive technique that provides a measure of cholinergic activity, an important neurotransmitter in the brain that is disrupted in PD and thought to be correlated with aspects of cognitive function. It involves stimulation of a peripheral nerve and stimulation of the motor cortex in the brain, using transcranial magnetic stimulation (TMS). The motor evoked potential of the muscles in the hand are then recorded using electromyography (EMG).

References

1. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sørensen P. Prevalence and characteristics of dementia in Parkinson disease. Arch Neurol 2003;60:387-392.
2. Galvin JE, Pollack J, Morris JC. Clinical phenotype of Parkinson disease dementia. Neurology 2006;67:1605-1611.
3. Hobson P, Meara J. Risk and incidence of dementia in a cohort of older subjects with Parkinson's disease in the United Kingdom. Mov Disord 2004;19:1043-1049.
4. Levy G, Tang MX, Louis ED, Côté L, Alfaro MA, Mejia H, *et al*. The association of incident dementia with mortality in PD. Neurology 2002;59:1708-1713.
5. Playfer JR. Depression, cognition and quality of life in parkinsonian patients. Age Ageing 1999;28:333-334.
6. Aarsland D, Larsen JP, Karlsen K, Lim NG, Tandberg E. Mental symptoms in Parkinson's disease are important contributors to caregiver distress. Int J Geriatr Psychiatry 1999;14:866-874.
7. Aarsland D, Larsen JP, Tandberg E, Laake K. Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. J Am Geriatr Soc 2000;48:938-942.
8. Emre M. Dementia associated with Parkinson's disease. Lancet Neurology 2003;2:229-237.
9. Levy G, Schupf N, Tang MX, Cote L, Louis ED, Mejia H, et al. Combined effect of age and severity on the risk of dementia in Parkinson's disease. Ann Neurol 2002;51:722-729.
10. Burn DJ, Rowan EN, Minett T, Sanders J, Myint P, Richardson J, *et al*. Extrapyramidal features in Parkinson's disease with or without dementia and dementia with Lewy bodies: a cross-sectional comparative study. Mov Disord 2003;18:884-889.
11. Williams-Gray CH, Foltynie T, Brayne CEG, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. Brain 2007;130:1787-1798.
12. Glatt SL, Hubble JP, Lyons K, Paolo A, Troster A, Hassanein RES, et al. Risk factors for dementia in Parkinson's disease: effect of education. Neuroepidemiology 1996;15:20-25.
13. Cohen OS, Vakil E, Tanne D, Nitsan Z, Schwartz R, Hassin-Baer S. Educational level as a modulator of cognitive performance and neuropsychyatric features in Parkinson disease. Cogn Behav Neurol 2007;20:68-72.
14. Levy G, Tang MX, Cote LJ, Louis ED, Alfaro B, Mejia H, et al. Do risk factors for Alzheimer's disease predict dementia in Parkinson's disease? An exploratory study. Mov Disord 2002;17:250-257.
15. Mosimann UP, Müri RM, Burn DJ, Felblinger J, O'Brien JT, McKeith IG. Saccadic eye movement changes in Parkinson's disease dementia and dementia with Lewy bodies. Brain 2005;128:1267-1276.

16. Carpenter RH. The neural control of looking. Curr Biol 2000;10:R291-R293.
17. Ali FR, Michell AW, Barker RA, Carpenter RH. The use of quantitative oculometry in the assessment of Huntington's disease. Exp Brain Res 2006;169:237-245.
18. Antoniades CA, Altham PM, Mason SL, Barker RA, Carpenter R. Saccadometry: a new tool for evaluating presymptomatic Huntington patients. Neuroreport 2007;18:1133-1136.
19. Michell AW, Xu Z, Fritz D, Lewis SJG, Foltynie T, Williams-Gray CH, et al. Saccadic latency distributions in Parkinson's disease and the effects of L-dopa. Exp Brain Res 2006;174:7-18.
20. Janvin CC, Aarsland D, Larsen JP. Cognitive predictors of dementia in Parkinson's disease: A community-based, 4-year longitudinal study. J Geriatr Psychiatry Neurol 2005;18:149-154.
21. Foltynie T, Brayne CEG, Robbins TW, Barker RA. The cognitive ability of an incident cohort of Parkinson's disease patients in the UK. The CamPaIGN study. Brain 2004;127:550-560.
22. Pluck GC, Brown RG. Apathy in Parkinson's disease. J Neurol Neurosurg Psychiatry 2002;73:636-642.
23. Burn D, Emre M, McKeith I, De Deyn PP, Aarsland D, Hsu C, et al. Effects of rivastigmine in patients with and without visual hallucinations in dementia associated with Parkinson's disease. Mov Disord 2006;21:1899-1907.
24. Ballard CG, Perry RH, McKeith IG, Perry EK. Neuroleptics are associated with more severe tangle pathology in dementia with Lewy bodies. Int J Geriatr Psychiatry 2005;20:872-875.
25. Haugarvoll K, Aarsland D, Wentzel-Larsen T, Larsen J. The influence of cerebrovascular risk factors on incident dementia in patients with Parkinson's disease. Acta Neurol Scand 2005;112:386-390.
26. Beyer MK, Aarsland D, Greve OJ, Larsen JP. Visual rating of white matter hyperintensities in Parkinson's disease. Mov Disord 2006;21:223-229. 36
27. Jellinger KA. Prevalence of cerebrovascular lesions in Parkinson's disease: a post-mortem study. Acta Neuropathol 2003;105:415-419.
28. Jellinger KA, Attems J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol 2008;115:427-436.
29. Allcock LM, Kenny RA, Tordoff S, Mosimann UP, Wesnes K, Hildreth AJ, et al. Orthostatic hypotension in Parkinson's disease: association with cognitive decline? Int J Geriatr Psychiatry 2006;21:778-783.
30. Dhawan V, Healy DG, Pal S, Chaudhuri KR. Sleep-related problems of Parkinson's disease. Age Ageing 2006;35:220-228.

31. Boddy F, Rowan EN, Lett D, O'Brien JT, McKeith IG, Burn DJ. Sleep quality and excessive daytime somnolence in Parkinson's disease with and without dementia, dementia with Lewy bodies and Alzheimer's disease: a comparative, cross-sectional study. Int J Geriatr Psychiatry 2007;22:529-535.
32. Körner Y, Meindorfner C, Möller JC, Stiasny-Kolster K, Haja D, Cassel W, *et al*. Predictors of sudden onset of sleep in Parkinson's disease. Mov Disord 2004;19:1298-1305.
33. Uc EY, Struck LK, Rodnitzky RL, Zimmerman B, Dobson J, Evans WJ. Predictors of weight loss in Parkinson's disease. Mov Disord 2006;21:930-936.
34. Perry EK, Kilford L, Lees AJ, Burn DJ, Perry RH. Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. Ann Neurol 2003;54:235-238.
35. Caparros-Lefebvre D, Pecheux N, Petit V, Duhamel A, Petit H. Which factors predict cognitive decline in Parkinson's disease? J Neurol Neurosurg Psychiatry 1995;58:51-55.
36. Apaydin H, Ahlskog JE, Parisi JE, Boeve BF, Dickson DW. Parkinson disease neuropathology: later-developing dementia and loss of the levodopa response. Arch Neurol 2002;59:102-112.
37. Inzelberg R, Bonuccelli U, Schechtman E, Miniowich A, Strugatsky R, Ceravolo R, *et al*. Association between amantadine and the onset of dementia in Parkinson's disease. Mov Disord 2006;21:1375-1379.
38. Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT. Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. Brain 2004;127:791-800.
39. Burton EJ, McKeith IG, Burn DJ, O'Brien JT. Brain atrophy rates in Parkinson's disease with and without dementia using serial magnetic resonance imaging. Mov Disord 2005;20:1571-1576.
40. Hu MTM, White SJ, Chaudhuri KR, Morris RG, Bydder GM, Brooks DJ. Correlating rates of cerebral atrophy in Parkinson's disease with measures of cognitive decline. J Neural Transm 2001;108:571-580.
41. Tessa C, Giannelli M, Della Nave R, Lucetti C, Berti C, Ginestroni A, *et al*. A whole-brain analysis in *de novo* Parkinson disease. Am J Neuroradiol 2008;29:674-680.
42. Lewis SGJ, Dove A, Robbins TW, Barker RA, Owen AM. Cognitive impairments in early Parkinson's disease are accompanied by reductions in activity in frontostriatal neural circuitry. J Neurosci 2003;23:6351-6356.
43. Lewis SGJ, Dove A, Robbins TW, Barker RA, Owen AM. Striatal contributions to working memory: a functional magnetic resonance imaging study in humans. Eur J Neurosci 2004;19:755-760.

44. Williams-Gray CH, Hampshire A, Robbins TW, Owen AM, Barker RA. COMT val158met genotype influences frontoparietal activity during planning in patients with Parkinson's disease. J Neurosci 2007;27:4832-4838.
45. Vander Borght T, Minoshima S, Giordani B, Foster NL, Frey KA, Berent S, *et al*. Cerebral metabolic differences in Parkinson's and Alzheimer's diseases matched for dementia severity. J Nucl Med 1997;38:797-802.
46. Firbank MJ, Colloby SJ, Burn DJ, McKeith IG, O'Brien JT. Regional cerebral blood flow in Parkinson's disease with and without dementia. Neuroimage 2003;20:1309-1319.
47. Firbank M, Burn D, McKeith I, O'Brien J. Longitudinal study of cerebral blood flow SPECT in Parkinson's disease with dementia, and dementia with Lewy bodies. Int J Geriatr Psychiatry 2005;20:776-782.
48. Andrews TC, Brooks DJ. Advances in the understanding of early Huntington's disease using the functional imaging techniques of PET and SPET. Mol Med Today 1998;4:532-539.
49. Hu MTM, Taylor-Robinson SD, Ray Chaudhuri K, Bell JD, Labbé C, Cunningham VJ, *et al*. Cortical dysfunction in non-demented Parkinson's disease patients: a combined 31P-MRS and 18FDG-PET study. Brain 2000;123:340-352.
50. Edison P, Archer HA, Hinz R, Hammers A, Pavese N, Tai YF, *et al*. Amyloid, hypometabolism, and cognition in Alzheimer disease. An [11C]PIB and [18F]FDG PET study. Neurology 2007;68:501-508. 37
51. Rowe CC, Ng S, Ackermann U, Gong SJ, Pike K, Savage G, *et al*. Imaging b-amyloid burden in aging and dementia. Neurology 2007;68:1718-1725.
52. Edison P, Rowe CC, Rinne J, Ng I, Kemppainen N, Nãgren K, *et al*. Amyloid load in Lewy body dementia, Parkinson's disease dementia and Parkinson's disease measured with 11C-PIB PET. Neurology 2007;68(Suppl 1):A98.
53. Ballard C, Ziabreva I, Perry R, Larsen JP, O' Brien J, McKeith I, *et al*. Differences in neuropathologic characteristics across the Lewy body dementia spectrum. Neurology 2006;67:1931-1934.
54. Jasinska-Myga B, Opala G, Goetz CG, Tustanowski J, Ochudlo S, Gorzkowska A, *et al*. Apolipoprotein E gene polymorphism, total plasma cholesterol level, and Parkinson disease dementia. Arch Neurol 2007;64:261-265.
55. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis S, Brown J, *et al*. Common variation in tau and alpha synuclein genes influences risk of developing idiopathic Parkinson disease and related cognitive impairment. Ann Neurol 2007;62:145-153.
56. Parker WD, Boyson SJ, Parks JK. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 1989;26:719-723.
57. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 1990;54:823-827.

58. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci 1985;36:2503-2508.
59. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 2000;3:1301-1306.
60. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, *et al*. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 2006;38:515-517.
61. Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, McKee AC, Beal MF, *et al*. Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. Genomics 1994;23:471-476.
62. Lin MT, Beal MF. Alzheimer's APP mangles mitochondria. Nat Med 2006;12:1241-1243.
63. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006;443:787-795.
64. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, *et al*. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 2003;72:804-811.
65. Ghezzi D, Marelli C, Achilli A, Goldwurm S, Pezzoli G, Barone P, *et al*. Mitochondrial DNA haplogroup K is associated with a lower risk of Parkinson's disease in Italians. Eur J Hum Genet 2005;13:748-752.
66. Pyle A, Foltynie T, Tiangyou W, Lambert C, Keers SM, Allcock LM, *et al*. Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. Ann Neurol 2005;57:564-567.
67. Chinnery PF, Taylor GA, Howell N, Andrews RM, Morris CM, Taylor RW, *et al*. Mitochondrial DNA haplogroups and susceptibility to AD and dementia with Lewy bodies. Neurology 2000;55:302-304.
68. van der Walt JM, Dementieva YA, Martin ER, Scott WK, Nicodemus KK, Kroner CC, *et al*. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. Neurosci Lett 2004;365:28-32.
69. Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R. Shorter telomeres are associated with mortality in those with APOE epsilon4 and dementia. Ann Neurol 2006;60:181-187.
70. Martin-Ruiz C, Dickinson HO, Keys B, Rowan E, Kenny RA, Von Zglinicki T. Telomere length predicts poststroke mortality, dementia, and cognitive decline. Ann Neurol 2006;60:174-180.
71. Wang H, Chen H, Gao X, McGrath M, Deer D, De Vivo I, *et al.* Telomere length and risk of Parkinson's disease. Mov Disord 2008;23:302-305.

72. Guan JZ, Maeda T, Sugano M, Oyama J, Higuchi Y, Suzuki T, *et al*. A percentage analysis of the telomere length in Parkinson's disease patients. J Gerontol A Biol Sci Med Sci 2008;63:467-473.
73. Smith AD. The worldwide challenge of the dementias: a role for B vitamins and homocysteine? Food Nutr Bull 2008;29(Suppl 2):S143-S172.
74. O'Suilleabhain PE, Sung V, Hernandez C, Lacritz L, Dewey RBJ, Bottiglieri T, *et al*. Elevated plasma homocysteine level in patients with Parkinson disease: motor, affective, and cognitive associations. Arch Neurol 2004;61:865-868. 38
75. Zoccolella S, Lamberti P, Iliceto G, Diroma C, Armenise E, Defazio G, *et al*. Plasma homocysteine levels in L-dopa-treated Parkinson's disease patients with cognitive dysfunctions. Clin Chem Lab Med 2005;43:1107-1110.
76. Hassin-Baer S, Cohen O, Vakil E, Sela BA, Nitsan Z, Schwartz R, *et al*. Plasma homocysteine levels and Parkinson disease: disease progression, carotid intima-media thickness and neuropsychiatric complications. Clin Neuropharmacol 2006;29:305-311.
77. Aarsland D, Mulugeta E, Bronnick K, Tysnes OB, Larsen JP, Alves G. Cognitive impairment in early and untreated Parkinson's disease: associations with cerebrospinal fluid levels of b-amyloid1-42, total tau, and phosphorylated (181p) tau protein. Mov Disord 2008;23(Suppl 1):S89.
78. Gómez-Tortosa E, Gonzalo I, Fanjul S, Sainz MJ, Cantarero S, Cemillan C, *et al*. Cerebrospinal fluid markers in dementia with Lewy bodies compared with Alzheimer disease. Arch Neurol 2003;60:1218-1222.
79. Mollenhauer B, Bibl M, Trenkwalder C, Stiens G, Cepek L, Steinacker P, *et al*. Follow-up investigations in cerebrospinal fluid of patients with dementia with Lewy bodies and Alzheimer's disease. J Neural Transm 2005;112:933-948.
80. Bibl M, Mollenhauer B, Esselmann H, Lewczuk P, Klafki H, Sparbier K, *et al*. CSF amyloid-□-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. Brain 2006;129:1177-1187.
81. Wada-Isoe K, Kitayama M, Nakaso K, Nakashima K. Diagnostic markers for diagnosing dementia with Lewy bodies: CSF and MIBG scintigraphy study. J Neurol Sci 2007;260:33-37.
82. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurology 2006;5:228-234.
83. Gustafson DR, Skoog I, Rosengren L, Zetterberg H, Blennow K. Cerebrospinal fluid beta-amyloid 1-42 concentration may predict cognitive decline in older women. J Neurol Neurosurg Psychiatry 2007;78:461-464.

84. Tam C, Burton E, McKeith I, Burn D, O'Brien J. Temporal lobe atrophy on MRI in Parkinson disease with dementia: a comparison with Alzheimer disease and dementia with Lewy bodies. Neurology 2005;64:861-865.
85. Dove A, Brett M, Cusack R, Owen AM. Dissociable contributions of the mid-ventrolateral frontal cortex and the medial temporal-lobe system to human memory. Neuroimage 2006;31:1790-1801.
86. Gould RL, Arroyo B, Brown RG, Owen AM, Bullmore ET, Howard RJ. Brain mechanisms of successful compensation during learning in Alzheimer disease. Neurology 2006;67:1011-1017.
87. Gibb WRG, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-752.
88. McKeith I, Dickson D, Lowe J, Emre M, O'Brien J, Feldman H, *et al*. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863-1872.
89. Litvan I, Bhatia KP, Burn DJ, Goetz CG, Lang AE, McKeith I, *et al*. SIC task force appraisal of clinical diagnostic criteria for parkinsonian disorders. Mov Disord 2003;18:467-486.
90. Fahn S, Elton RL, on behalf of the Movement Disorders Task Force Committee. Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, editors. Recent developments in Parkinson's disease. New York: Macmillan; 1987. p. 153-163.
91. Peto V, Jenkinson C, Fitzpatrick R, *et al*. The development and validation of a short measure of functioning and well being for individuals with Parkinson's disease. Qual Life Res 1995;4:241-248.
92. Jankovic J, McDermott M, Carter J, Gauthier S, Goetz C, Golbe L, *et al*. Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. Neurology 1990;40:1529-1534.
93. Kaufmann H. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure and multiple system atrophy. Clin Auton Res 1996;6:125-126.
94. Buysee DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatr Res 1988;28:193-213.
95. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-545.
96. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-198.
97. Nelson HE, O'Connell A. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. Cortex 1978;14:234-244. 39
98. Benton AL. Differential behavioural effects of frontal lobe disease. Neuropsychologia 1968;6:53-60.

99. Goodglass H. The assessment of aphasia and related disorders. Philadelphia: Lea and Febiger; 1972.
100. Miller E. Possible frontal impairments in Parkinson's disease: a test using a measure of verbal fluency. Br J Clin Psychol 1985;24:211-212.
101. Ala TA, Hughes LF, Kyrouac GA, Ghobrial MW, Elble RJ. Pentagon copying is more impaired in dementia with Lewy bodies than in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2001;70:483-488.
102. Sahakian BJ, Morris RG, Evenden JL, Heald A, Levy R, Philpot M, *et al*. A comparative study of visuospatial memory and learning in Alzheimer-type dementia and Parkinson's disease. Brain 1988;111:695-718.
103. Robbins TW, James M, Owen A, Sahakian BJ, McInnes L, Rabbitt PM. Cambridge Neuropsychological Test Automated Battery (CANTAB): A factor analytic study of a large sample of normal elderly volunteers. Dementia 1994;5:266-281.
104. Owen AM, Sahakian BJ, Semple J, Polkey CE, Robbins TW. Visuospatial short term recognition memory and learning after temporal lobe excisions, frontal lobe excisions or amygdala-hippocampectomy in man. Neuropsychologia 1995;33:1-24.
105. Owen AM, Sahakian BJ, Hodges JR, Summers BA, Polkey CE, Robbins TW. Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. Neuropsychology 1995;9:126-140.
106. Baker SC, Rogers RD, Owen AM, Frith CD, Dolan RJ, Frackowiak RSJ, *et al*. Neural systems engaged by planning: A PET study of the Tower of London task. Neuropsychologia 1996;34(515-526).
107. Cheesman AL, Barker RA, Lewis SJ, Robbins TW, Owen AM, Brooks DJ. Lateralisation of striatal function: evidence from 18F-dopa PET in Parkinson's disease. J Neurol Neurosurg Psychiatry 2005;76:1204-1210.
108. Swainson R, Hodges JR, Galton CJ, Semple J, Michael A, Dunn BD, *et al*. Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks. Dement Geriatr Cog Disord 2001;12:265-280.
109. Blackwell AD, Sahakian BJ, Vesey R, Semple JM, Robbins TW, Hodges JR. Detecting dementia: Novel neuropsychological markers of preclinical Alzheimer's disease. Dement Geriatr Cog Disord 2004;17:42-48.
110. Ferman TJ, Smith GE, Boeve BF, Ivnik RJ, Petersen RC, Knopman D, *et al*. DLB fluctuations: specific features that reliably differentiate DLB from AD and normal aging. Neurology 2004;62:181-187.
111. Ballard CG, Aarsland D, McKeith I, O'Brien J, Gray A, Cormack F, *et al*. Fluctuations in attention: PD dementia vs. DLB with parkinsonism. Neurology 2002;59:1714-1720.

112. Wesnes KA, McKeith IG, Ferrara R, Emre M, Del Ser T, Spano PF, *et al*. Effects of rivastigmine on cognitive function in dementia with Lewy bodies: a randomised placebo-controlled international study using the cognitive drug research computerised assessment system. Dement Geriatr Cogn Disord 2002;13:183-192.
113. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308-2314.
114. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, *et al*. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 1982;17:37-49.
115. Mosimann UP, Collerton D, Dudley R, Meyer TD, Graham G, Dean JL, *et al*. A semi-structured interview to assess visual hallucinations in older people. Int J Geriatr Psychiatry 2008;23:712-718.
116. Ober JK, Przedpelska-Ober E, Gryncewicz W, Dylak J, Carpenter RS, Ober JJ. Hand-held system for ambulatory measurement of saccadic durations of neurological patients. In: Gadja J, editor. Modelling and Measurement in Medicine. Warsaw: Komitet Biocybernityki i Inzyneierii Biomedycznej; 2003. p. 187-198.
117. Hampshire A, Owen AM. Fractionating attentional control using event related fMRI. Cerebral Cortex 2006;16:1679-1689.
118. Gould RL, Brown RG, Owen AM, ffytche DH, Howard RJ. fMRI BOLD response to increasing task difficulty during successful paired associates learning. Neuroimage 2003;20:1006-1019.
119. Owen AM, Beksinska M, James M, Leigh PN, Summers BA, Marsden CD, *et al*. Visuospatial memory deficits at different stages of Parkinson's disease. Neuropsychologia 1993;31:627-644.
120. Williams-Gray CH, Goris A, Foltynie T, Brown J, Maranian M, Walton A, *et al*. Prevalence of the LRRK2 G2019S mutation in a UK community based idiopathic Parkinson's disease cohort. J Neurol Neurosurg Psychiatry 2006;77:665-667. 40
121. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, *et al*. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3-32.
122. Olsson A, Vanderstichele H, Andreasen N, De Meyer G, Wallin A, Holmberg B, *et al*. Simultaneous measurement of beta-amyloid(1-42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005;51:336-345.
123. Burn D, Rowan E, Allan L, Molloy S, O'Brien J, McKeith I. Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies. J Neurol Neurosurg Psychiatry 2006;77:585-589.

124. Emre M, Aarsland D, Brown R, Burn D, Duyckaerts C, Mizuno Y, *et al*. Clinical diagnostic criteria for dementia associated with Parkinson disease. Mov Disord 2007;22:1689-1707.
125. Dubois B, Burn D, Goetz C, Aarsland D, Brown R, Broe A, *et al*. Diagnostic procedures for Parkinson's disease dementia. Mov Disord 2007;22:2314-2324.
126. Boeve B, Rochester M, Ferman T et al. Validation of a Questionnaire for the diagnosis of REM sleep behavior disorder. Neurology 58; Supp 3, 2002: A509
127. S. Ahmed, J.F. Passos, M.J. Birket, T. Beckmann, S. Brings, H. Peters, M.A. Birch-Machin, T. von Zglinicki, G. Saretzki, Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J Cell Sci 2008;121:1046-1053.
128. Zadikoff Cindy, Fox Susan H, Tang-Wai David F., Thomsen Teri, de Bie Rob M.A., Wadia Pettarusup, Miyasaki Janis, Duff-Canning Sarah, Lang Anthony E. and Maras Connie. A comparison of the Mini Mental State Exam to the Montreal Cognitive Assessment in identifying cognitive deficits in Parkinson‟s disease. Movement Disorders 2008; 23: 297-299.
129. Mamikonyan Eugenia, Moberg Paul J., Siderowf Andrew, Duda John E., Ten Have Tom, Hurtig Howard I., Stern Matthew B. and Weintraub Daniel. Mild cognitive impairment is common in Parkinson‟s disease patients with normal Mini-Mental State Examination (MMSE) scores. Parkinsonism & Related Disorders 2009; 15: 226-331
130. Glozman, J.M., Bicheva, K.G. and Fedorova, N.V. Scale of quality of life of caregivers (SQLC) Journal of Neurology 1998;245: S39-S41.
131. Jenkinson, C., Dummett, S., Kelly, L., Peters, M., Dawson, J., Morley, D. and Fitzpatrick, R. The development and validation of a quality of life measure for the carers of people with Parkinson's disease (the PDQ-Carer), Parkinsonism Relat Disord, 2012;18, 483-7.
132. Whitelaw, N.A. and Liang, J. The structure of the OARS physical health measures, Med Care, 1991; 294; 332-47.
133. Zigmond, A.S. and Snaith, R.P. The Hospital Anxiety and Depression Scale. Acta Psychiatrica Scandinavica, 1983; 67; 361-370.
134. Jorm, A.F. and Korten, A.E. Assessment of cognitive decline in the elderly by informant interview, *The British Journal of Psychiatry*, 1988; 152, 209-13.
135. Goetz, C.G., Nutt, J.G. and Stebbins, G.T. The Unified Dyskinesia Rating Scale: presentation and clinimetric profile, Mov Disord, 2008; 23, 2398-403.
136. Schwab RS, England AC. Projection technique for evaluating surgery in Parkinson’s disease. In: Gillingham FJ, Donaldson IML (eds), Third Symposium on Parkinson’s Disease, Edinburgh: E and S Livingstone 1969; 152–157.