**Chapter no 2**

**Parkinson's disease: basic pathomechanisms and a clinical overview**

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**Abstract:**

PD is a common and a debilitating degenerative movement disorder. Numbers of PD patients are increasing worldwide and as yet there is no cure for the disease. The majority of existing treatments target motor symptom control. PD is generally of unknown aetiology but over the last 18 years the impact of the genetic contribution to PD has been appreciated. Significant discoveries have been made, which have advanced our understanding of the pathophysiological and molecular basis of PD. In this chapter we outline current knowledge of the clinical aspects of PD and the basic mechanistic understanding.

**Key words:** Parkinson's disease, genetic risk, environmental factors, clinical features, treatment, pathology, Lewy body, prion-like mechanisms, neuroinflammation, autophagy/lysosomal dysfunction.

## 2.1: Introduction

The disease described by James Parkinson in 1817, which still bears his name 200 years later, is the most common degenerative movement disorder. Lifetime prevalence of Parkinson's disease (PD) is 0.2%, and increases significantly with age, so that incidence of PD is 0.1-0.3% and the prevalence is 1% over the age of 60 years (1, 2). Numbers are rising globally, creating mounting pressure on social care resources (3, 4).

PD is diagnosed clinically, with an appropriate history and according to established diagnostic criteria, such as the Queen Square Brain Bank (QSBB) criteria (5). The QSBB criteria list bradykinesia as an essential feature, alongside either rigidity or tremor. Objective motor features of PD are thought to emerge relatively late in the disease process when more than 50% of dopaminergic neurons have been lost in the substantia nigra (6). Symptomatic treatment is predominantly dopaminergic and is efficacious for most, but no known drug slows or halts inevitable neuronal loss. It is believed that disease may be too far advanced at the point of clinical diagnosis to be affected by potentially neuroprotective treatments (7). Over time, progressive cell loss leads to increasing physical disability, often in parallel to cognitive impairment, with treatment failing to provide adequate control in the advanced stages. Debilitating non-motor symptoms also feature throughout the disease and addressing these symptoms is a critical unmet need.

Besides the common sporadic form of PD, there also exist mutations in specific genes that are inherited in a Mendelian fashion in around 10% of patients. Over the past decade and a half several advancements have been made to understand the basic biology and pathobiology of the proteins associated with genetic PD. Several of the cases carrying gene mutations have been described neuropathologically and we have gleaned important information from these studies. How and why specific dopaminergic (DAergic) cells die is a matter of continued debate but evidence suggests several common pathogenic pathways that are involved in both sporadic and genetic PD. In this chapter we provide an overview of the clinical features, aetiological factors, neuropathology and common pathogenic mechanisms of sporadic and genetic PD and discuss current and future treatment prospects.

## 2.2: Risk factors

Genetic risk

It used to be thought that the majority of PD cases were entirely sporadic but over the last two decades a number of genetic causes and risk factors have come to light. There are a number of confirmed monogenic causes for PD, many of which have led to advances in the understanding of the mechanistic abnormalities that result in neurodegeneration (see Table 2.1). However the

genetic basis of PD extends more widely than monogenic causes. Genome wide association studies have identified a further 28 risk loci in large studies of unrelated cases and controls (8). Many of these can be linked to putative disease mechanisms or are supported by the findings of candidate gene studies in PD and other neurodegenerative diseases, increasing confidence that identified associations are real. The total heritability of PD is estimated to be approximately 30%, meaning there are many more genetic risk factors still to find and identified risk loci and monogenic forms account for only about 5-10%. (9, 10).

*Place table 2.1 here.*

Beyond causation, a large proportion of the heterogeneity of PD may be genetically determined, such as age of onset, emergence of dementia, and motor fluctuations, as well as explain the variability in response to drugs (for examples see Table 1) (11-13). Of the confirmed monogenic forms of PD, most are rare and do not account for elevated risk at a population level, whereas others are more common and may have different features to idiopathic PD.

Mutations in the LRRK2 gene are the commonest known genetic cause for PD and the G2019S mutation occurs in 4% of hereditary and 1% of sporadic PD. LRRK2-related PD demonstrates age-dependent penetrance, meaning that only a proportion of carriers will develop PD during life (14). Incidence varies according to ethnicity and is highest in north African Arabs and Ashkenazi Jews. The G2385 variant is a common risk factor in Asian populations (15).

Gaucher's disease (GD) is the most common lysosomal storage disorder and results from a deficiency in the enzyme glucocerebrosidase. It follows Mendelian recessive inheritance patterns and is most commonly found in Ashkenazi Jews. An association between GD and parkinsonism was observed in reports of GD patients who began to manifest clinical features of PD (16) and an excess frequency of GBA (glucosidase beta acid) mutations was observed in PD patients (17). Heterozygous GBA mutations are associated with an increased odds of PD with a ratio of 5.43 (95% confidence interval (CI) 3.89 to 7.57) (18). Large studies have shown that GBA mutations are common in Ashkenazi Jews, occurring in 15% of patients and 3% of controls (18). In unselected PD patients, 3.5% carry disease-associated GBA mutations compared to <1% of controls (18).

The genetic architecture of PD is continually expanding and is becoming increasingly complex. It conveys influence not only on risk of PD diagnosis but also heterogeneity observed within the disease.

Environmental risk factors

Risk of PD diagnosis is associated with a variety of environmental factors that have been determined in observational studies (19). Several of disease-specific cohorts have been established to study the role of environmental factors in PD (and in some cases interaction with genetic factors) (20-23).

One of the most studied associations is the link between PD and pesticide exposure (including proxies such as farming occupation, rural living and well water drinking) (19, 24). Rotenone and paraquat have been studied specifically and are used to create animal models of PD. Traumatic brain injury, particularly if recurrent or sufficient to cause loss of consciousness has emerged as an important risk factor for neurodegenerative disease and can give rise to the clinical syndromes of parkinsonism, dementia and motor neurone disease (25). Susceptible individuals include sportspersons or ex-serviceman. Head injury has been observed as a minor but significant risk factor for PD in observational studies (26).

Some environmental exposures appear to convey a protective effect. The inverse relationship between cigarette smoking and PD was first recognised over 50 years ago, with multiple studies since confirming this association (19). Whether these lifestyle exposures offer true neuroprotective properties or whether negative association arises through avoidance as part of an early PD personality change (reverse causality), are yet to be determined. Another consistent negative association exists between levels of serum urate and PD, suggesting a protective effect (27-29). Finally, there are a number of drugs for which negative. associations with PD have been reported in observational studies, including calcium channel blockers, non-steroidal anti-inflammatories and statins, and clinical trials to explore repurposing of some of these agents are underway (29-31).

## 2.3: Clinical features

Historically, PD was viewed primarily as a disorder of movement, but increasing recognition of the wide heterogeneity of PD and the importance of non-motor features has been building for several years (32, 33). Many non-motor features such as cognitive impairment and sphincter disturbance substantially increase care needs, along with associated care burden and financial implications. However their impact is experienced throughout the disease and may precede diagnosis based on motor features in some, if not the majority, of cases. As with so many neurological diseases, much of the information upon which a diagnosis is based, comes from careful attention to the specifics of the symptoms reported in the clinical history.

Motor features

Bradykinesia describes sequential loss of rate or amplitude with a repetitive movement, and should be differentiated from persistently slow movements (such as pyramidal slowing seen in multiple sclerosis or primary lateral sclerosis) or persistently low-amplitude movements (such as those seen in with finger tapping in progressive supranuclear palsy) with repetitive testing (34, 35). In the clinical setting bradykinesia is demonstrated by asking patients to repeatedly tap the index finger on the thumb or touch each finger onto the thumb (36). It is different to hypokinesia (small movement) and akinesia (absence of expected voluntary movement including slow reaction time), but these may frequently be present alongside bradykinesia (35). Foot tapping and

stamping can also be used similarly to demonstrate limb bradykinesia and these, and additional movements are measured in the unified Parkinson's disease rating scale (UPDRS) (37).

Appendicular rigidity can be differentiated from the high tone of spasticity because it persists through the full range of movement and is not velocity dependent. It is often best identified at the wrist and can be elicited by Froment's manoeuvre in a patient that is fully relaxed. The classic tremor of PD occurs at rest, with a frequency of 4-7Hz, and can be brought out by distracting the patient or occupying/stressing them with a task (such as reciting months of the year backwards). Postural and kinetic tremor may also be frequently observed but are less specific for PD. The three core features (bradykinesia, rigidity and tremor), when present, tend to manifest asymmetrically and often persist in this asymmetric fashion throughout the disease course.

Postural instability and impaired righting reflexes may be observed relatively early, but if marked from the outset and resultant in frequent falls, should prompt exploration and investigation for an alternative cause (e.g. atypical parkinsonism such as PSP or multiple system atrophy). In most patients with PD, motor features ought to be objectively improved by levodopa (5). Not infrequently there can be discrepancy between objective and subjective l-dopa responsiveness. L-dopa responsiveness is supportive criterion for PD, but sub-optimal response may be observed in 5-10%. Distinction ought to be drawn between 'sub-optimal response' and 'intolerance to levodopa' due to side effects. Doses should be increased to 1g per day of l-dopa before a conclusion of non-l-dopa responsive parkinsonism is made, otherwise 'l-dopa intolerant' is a more appropriate description. L-dopa responsiveness may be formally determined through an l-dopa challenge.

Motor fluctuations emerge in approximately 40% of patients on l-dopa between 6-9 years after diagnosis and are characterised predictable 'wearing off' before the next dose, unpredictable 'on'-'off' fluctuation, and sudden 'off' periods (38). Involuntary movements, which may be choreic (dyskinesia) or dystonic, occur in approximately 20% of patients on l-dopa in the same time period. Determinants of the emergence of motor fluctuations included disease duration and dose of levodopa, whereas dyskinesia are best predicted by treatment duration (38). For these reasons some clinicians used to withhold treatment with l-dopa because it was felt that there was a 'honeymoon period' of positive benefit before a gradual shift to increasing detrimental effects. This theory is largely discredited now and choice of optimum first treatment is based on alternative factors (see treatment section).

Non-motor features

Non-motor symptoms are experienced throughout the disease and there is substantial evidence that suggests that they can also predate diagnosis by several years (19). Olfactory disturbance is a common finding in patients with PD, occurring in up to 80% of cases (39). There is also evidence that hyposmia (which describes impaired smell as opposed to absent smell) may precede the

onset of motor features of PD (40-42) Some genetic forms of PD are less likely to have hyposmia as a feature such as PD caused by LRRK2 and parkin (43-45). Sleep disorders are very common in PD and include excessive daytime somnolence (EDS), reversal of the sleep wake cycle, insomnia and REM sleep behavior disorder (RBD) (32). RBD is characterised by vigorous enactment of dreams, and occurs in up to 3% of the normal population and approximately 30% of patients with PD. Subjects with polysomnographically diagnosed RBD are at high risk of being diagnosed with a neurodegenerative disorder, not just PD but also dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) (46). In patients with established PD, RBD is more common in those with cognitive impairment and hallucinations (47). Anxiety and depression have complex bi-directional relationships with PD in that they are symptoms of the disease with neuropathological correlates, but can also significantly worsen the movement symptoms of PD when present and uncontrolled. Anxiety is associated with worsening tremor or dyskinesia, worsening of speech and poor performance on cognitive tasks (32, 48). Depression, with psychomotor slowing, can bear features of PD, affect performance on cognitive tests and contribute to apathy, which is also common in PD (32). Both can affect sleep, which may have further detrimental effects on symptom control overall.

Autonomic dysfunction of some description is virtually ubiquitous in subjects with PD (32, 33). Constipation is the most common non-motor symptom and can be refractory to conventional treatment. There is substantial evidence for involvement of enteric nervous system in PD, and some evidence suggesting that involvement precedes diagnosis by a number of years (49, 50). Postural hypotension is also frequently encountered either as consequence of disease or as a side effect of medication, or both (32). Although very common in moderate to advanced PD, early objective postural hypotension in the context of parkinsonism, ought to prompt consideration of alternative diagnoses such as MSA (51). Similarly bladder complaints and erectile dysfunction are common non-motor manifestation of PD, but also MSA (32, 51).

<u>Cognitive</u>

The prevalence of dementia in all PD patients is approximately 30% (52)and at 8 years disease duration, approximately 50% of patients with PD will have developed dementia (53). Risk factors for developing dementia are older age, older age at diagnosis of PD, non-tremulous phenotype with more marked gait involvement, as well as the presence of RBD and visual hallucinations.(52)
The pattern of PD dementia (PDD) is heterogeneous and whilst it often includes involvement of fronto-subcortical attentional-executive domains, visuospatial function and memory can also be impaired (54). An associated (if not identical) synucleinopathy, DLB mirrors the features of moderate to severe PDD with global deficits in memory, attention, language, psychomotor performance, and executive functions, with severe deficits in visuospatial and visuoconstructive abilities (52). Frank cognitive impairment evident at the point of diagnosis, or predating motor features, defines DLB as opposed to PDD, in which cognitive impairment emerges at least one year after motor features (55).

Key features of these synucleinopathy-related dementias are fluctuation and associated visual hallucinations. The content of hallucinations can be fairly predictable including observing small animals or insects, or non-threatening apparitions of people, and extracampine phenomena (56). Insight is frequently retained early on, but if not addressed insight can be lost (54). Confusion, disorientation and hallucinations may be unmasked at times of intercurrent illness. Delusions may also occur and again can be characteristic including Othello syndrome (spouse infidelity), Capgras' syndrome (replacement by an imposter) and Cothard's syndrome (death) (57).

In recent years, the notion of mild cognitive impairment occurring in early PD and even pre-diagnosis has been proposed. MCI is recognisable in 20-25% of non-demented patients with PD (58). There is evidence to suggest subtle cognitive change in patients soon after diagnosis that largely does not affect daily living but can be a harbinger for subsequent dementia. Definitions for MCI in PD have been developed (59) and case finding helps underpin biomarker initiatives that will support neuroprotective drug trials aiming to prevent overt dementia in PD.

Cognitive impairment is also differentially observed in the various genetic causes of PD. Perhaps partly due to younger age of onset or restricted pathology, cognitive impairment is less common in parkin-related PD. LRRK2-related PD does not appear to be associated with cognitive impairment, expect perhaps in the those cases found to have Lewy pathology at post-mortem (60). Mutations in the GBA gene (particularly those that also cause Gaucher's disease) tend to be associated with greater cognitive impairment (61, 62). As well as these moderate-to-big effect mutations, small effect, common variants can also influence risk of dementia such as the H1 haplotype of MAPT and variants in APOE may also play a part (53, 57).


Diagnosis

The diagnosis of PD remains a clinical one that can be made confidently and accurately in most situations where established motor features and atypical features are not elicited in either the history or examination. Traditional diagnostic criteria such as the QSBB criteria are used worldwide to define cases and differentiate individuals with PD from those with mimics and alternatives (5). In the UK, national guidelines encourage that suspected patients be referred untreated to a movement disorders specialist for assessment and potentially further investigation (63). Whilst there are no diagnostic tests for PD per se, in specialist centres, supportive investigations are not infrequently undertaken. CT imaging of the brain is unlikely to yield much additional information other than to rule out structural lesions resulting in symptomatic parkinsonism. MR imaging can be useful to rule out features associated with atypical parkinsonism such as a reduction in midbrain to pons ratio/hummingbird sign/morning glory sign characteristic of PSP or the 'hotcross bun' sign/ atrophy of the middle cerebellar peduncles or pons/ signal change in the posterior putamen seen in

MSA (36, 64).  There exist few MR imaging markers characteristic of PD but some have been proposed and await further replication/validation.

In some regions of Europe, transcranial sonography is used in the work up of parkinsonian disorders, with hyperechogenicity of the substantia nigra a reasonably sensitive and specific sign of PD (65). The cost, low risk and widespread availability of ultrasound makes TCS an attractive modality to consider, but there remains scepticism due to an absence of an MR correlate for the lesion identified, lack of the understanding about what the lesion represents, and difficulty with visualisation in older women and patients of South-East Asian descent.

Functional imaging with 123I-FP-CIT SPECT and 123I-beta-CIT SPECT characterise the availability and/or function of pre-synaptic dopamine transporters. DaTSPECT is probably the closest thing to a diagnostic test for PD but is expensive and can be 'normal' in the early stages of PD (66). The interpretation of DaTSCANs is somewhat subjective and quantitative measures are being pursued, but currently false positives and negatives do occur (67). The situation has been made more complicated by the realisation that some subjects with clinically defined PD, have normal DaTSCANs. These subjects have been described as SWEDDs (scans without evidence of dopaminergic deficit). The natural history of SWEDDs is incompletely understood (68).  Some clearly go on to manifest progressive parkinsonism, and frequently in these subjects the DaTCSAN subsequently becomes abnormal. Other remain symptomatic but may not progress and sometimes later receive a revised diagnosis of dystonic tremor or similar. Furthermore, as yet, DaTSCAN cannot reliably differentiate PD from alternative causes of parkinsonism including MSA and PSP. Current guidelines in UK recommend DaTSCAN where there is diagnostic uncertainty such as cases of isolated tremor, and to delineate when parkinsonism may have an alternative aetiology (i.e. vascular or drug induced) (63). Additional tests such as formal levodopa challenge, neuropsychometry, genetic and objective smell testing, and other imaging modalities such as PET and MIBG, can all have a role in refining the diagnosis of PD.

## 2.4. Biomarkers/Pathology

2.4.1. Neuropathology of Sporadic PD

The gold standard of PD diagnosis is neuropathological confirmation at autopsy. The vital pathological feature of PD upon post-mortem is the severe loss of pigmented (neuromelanin) DAergic neurons of the substantia nigra (SN). Exponential neuron loss specifically occurs in the ventrolateral tier of the SN (6) whose neuronal projections extend to the dorsal putamen. The loss of nigrostriatal system also results in reduction of tyrosine hydroxylase (the rate-limiting enzyme of dopamine synthesis) and the Dopamine transporter (DAT) indicating loss of nerve terminals (69). Synaptic DA loss in the putamen leads to the typical motor features of PD. Cell loss can occur quite rapidly and recent work suggests that depletion of DAT is complete by 4 years post-diagnosis (70). Neuron loss in PD is not restricted to the SN alone but also occurs in the locus

coeruleus, nucleus basalis of Meynert, dorsal motor nucleus of the vagus, and the amygdala (71).

Another critical pathological finding for PD is the presence of intracellular, eosinophilic, fibrillar proteinaceous structures called Lewy bodies (LBs) in the SN neurons. In 1997, the main component of LBs was identified as alpha-synuclein (72). Besides LBs, fibrillar alpha-synuclein can be present within a range of neuronal structures including intraneuritic LBs, Lewy neurites, axonal spheroids and sometimes also in astrocytes (73-75). Lewy pathology is present in selected neuronal subtypes and affects unmyelinated axons more robustly (76). LBs are also seen in neurons containing glutamate, GABA, serotonin, noradrenaline, histamine and acetylcholine-containing projection neurons and also in the DAergic neurons of the hypothalamus (69, 77).

Cell death throughout the SN is not uniform. The A9 neurons of SN ventral tier are most vulnerable and typically die off first. These DAergic neurons are rich in neuromelanin, have low calbindin protein, reduced glycolytic enzymes and increased iron content, and it is thought that these factors could contribute to neuronal death (78). The converse is true for the DAergic neurons of the dorsal tier (A10 neurons) which are involved relatively late in the terminal stages of disease process (78).

It is still unknown whether formation of alpha-synuclein positive LBs are a cause or consequence of selective DAergic cell death. Consequently, it is unclear what the very early pathological or biochemical changes are, prior to alpha-synuclein accumulation in LBs and overt DAergic neuronal loss. In this respect, a few recent studies have described early changes associated with microglial activation as additional pathological features that occur in early stages of PD. T-cell infiltration accompanied by activated microglial and astrocytic accumulation occur around SN pars compacta region (79). However, the benefits of use of anti-inflammatory compounds in PD remain a controversial topic (80). Recent evidence also suggests that synaptic deficit precedes motor deficits in animal models of genetic PD (81). More profound understanding of the various early contributors of DAergic neuron death may lead to identification of novel restorative therapies.

Braak and colleagues proposed a now generally accepted pathological staging system for PD, which suggested that Lewy pathology occurs in discreet areas of the nervous system before progressing to involve the basal ganglia (82). The progression of PD can be loosely clinically and pathologically correlated to the six stages described by Braak and colleagues, although there is likely to be considerable variability (83, 84) (Table 2.2).

Stage 1 marks the onset of the disease process and involves the anterior olfactory nucleus, the olfactory bulbs and the dorsal motor nuclear complex of cranial nerves IX and X (DMV), which may account for early olfactory or autonomic dysfunction (82). Separately, alpha synuclein deposits have also been found in the gastrointestinal neuronal tissue in patients with established PD and

in subjects that underwent bowel biopsy pre-morbidly and were subsequently diagnosed with PD (50, 85, 86).

Braak stage 2 involves the locus coeruleus and subcoeruleus complex, the magnocellular area of the reticular formation and posterior raphe nucleus (82). Disease involvement in these areas could account for recognised sleep and mood disorders, including anxiety and depression, which have been reported to antedate motor disease. Stage 3 involves structures including the substantia nigra and amygdala, which may correspond with the onset of the classical motor features of PD. Stages 4 to 6 are defined by progressive involvement of cortical structures, perhaps accounting for the later features such as memory impairment, visual hallucinations and other visual disturbances, and change in personality.

Alternative staging criteria

The DLB consortium has revised consensus guidelines for pathological diagnosis of LB diseases (including PD) using semi-quantitative grading of lesion density that distinguish three pathological phenotypes - brain stem predominant, limbic/transitional and diffuse neocortical (55) (Table 2.2). However, both the Braak staging (82) and McKeith criteria (55) fail to include cases in which the brainstem is not affected, including those with predominant involvement of the limbic pathway and olfactory bulbs. More recently Beach et al (87) proposed a unifying system of staging that allows classification of all subjects with LB disorders. In this scheme, subjects were classifiable into one of the following stages: I: olfactory bulb only; IIa: Brainstem predominant; IIb Limbic predominant; III Brainstem and limbic; IV neocortical (87). Progression of these cases correlated well with worsening of mini mental State examination score and UPDRS. This staging scheme allows classification of a greater proportion of LB cases and is a likely improvement over the Braak and McKeith criteria. However, in order to be accepted more widely, the Beach classification system (87) needs to be replicated.

*Place table 2.2 here.*

## 2.4.2. Neuropathology of genetic Parkinson's disease

### *PARK1(SNCA)*: Alpha-synuclein

Thus far 6 different point mutations in the *SNCA* gene causal for a PD phenotype have been described. The *A53T, A30P, E46K* were described previously (88) with further new mutations *G51D, H50Q* and *A53E* identified within the last two years (89-93). In addition there are the alpha-synuclein duplication and triplication cases (88) that manifest a PD phenotype. The presence of extra copies of the alpha-synuclein gene results in overexpression of the protein thus linking up-regulated alpha-synuclein levels to PD clinical symptoms. In this regard, Poulopolos et al (94) have described the neuropathology of all the genetic PD cases described until 2012. All of the *SNCA* cases have severe loss of SN and locus coeruleus neurones together with substantial LB pathology (Braak stage 6 in

most cases). In majority of the cases, the hippocampus was also affected heavily by Lewy pathology.

The more recently discovered mutations, the *G51D* and the *H50Q* also mirror previously described phenotype of alpha-synuclein mutations associated with severe neuronal loss in the SN and PD Braak stage 6 alpha-synuclein pathology. In some cases, locus coeruleus and the dorsal motor nucleus of the vagus also demonstrated neuronal loss and accumulation of alpha-synuclein pathology (90, 95). The *G51D* mutation cases develop disease symptoms relatively early compared to the two *H50Q* mutations described (92, 95). The neuropathology of two other patients harbouring *G51D* mutation has also been described.  One patient was French and had a history of PD, and the other Japanese in origin with apparently sporadic manifestation (91, 96). The French patient had very rapid disease progression and post-mortem examination showing marked neuronal loss in SN and the presence of LBs in the brain stem. Lewy pathology was also distributed in superficial layers of cortical laminae in the same case (91).

The *A53E* mutation was identified in a Finnish patient who was diagnosed with atypical Parkinson's disease at 36 years of age (93). Neuropathological diagnosis performed at the age of 60 years showed abundant alpha-synuclein pathology present throughout the brain and spinal cord. Prominent alpha-synuclein lesions were present in both neurons and in oligodendrocytes. The basal ganglia and cortical structures, especially the superficial and deep laminae were severely affected by pathology. These features reflect those seen in *G51D* patients. Oligodendroglial alpha-synuclein inclusions, the hallmarks of MSA are also seen in some subjects with alpha-synuclein multiplications (97, 98), *G51D* and *A53T* mutations. Intriguingly, few cases also harbour tau or TDP-43 inclusions.

In summary, all SNCA mutation cases that have come to post-mortem demonstrated presence of Lewy Body pathology but some cases also have overlapping tau or TDP-43 pathologies. Some of the mutations also contain abnormal alpha-synuclein deposits in the oligodendroglia.

*PARK8*: Leucine rich repeat kinase 2 (LRRK2)

The LRRK2 protein harbours two enzymatic activity domains, the GTPase and kinase at its core and their synergism is an integral part of LRRK2 function (99, 100). The most prevalent mutation, *G2019S*, occurs in the kinase domain of the protein, and increases kinase activity, which is central to LRRK2s role in PD pathogenesis. Currently LRRK2 kinase inhibitors are being developed for potential use in clinic (101-103).

Several LRRK2 mutation cases have now come to post-mortem and the neuropathology is heterogeneous, although the majority harbour Lewy bodies. In the absence of Lewy body pathology, the range of pathologies detailed for LRRK2 mutations includes pure nigral degeneration (ND), ND with ubiquitin-only pathology, ND with alpha-synuclein positive GCI's, ND accompanied with NFTs resembling PSP pathology (94). The common thread that links all LRRK2 mutation cases is inevitable loss of DAergic neurons in the SN and locus

coeruleus. Gliosis is also a common feature observed in all cases. The LB pathology reported in autopsies corresponded to Braak stages 3-6 with variable tau pathology in cortical areas. The majority of the Sagamihara kindred (the original LRRK2 family) with the *I2020T* had pure ND, however one case presented ND-GCI whilst another case displayed brain stem LBs. It is intriguing to note that the cases without *G2019S* have more severe nigral neuronal loss (94). Even patients in the same family with the *R1441C* mutation can have widely variable pathology (104). Patients with *R793M* or *L1156P* had TDP-43 inclusions in the temporal cortex whilst 1 patient with *R1441C* mutation harboured TDP-43 pathology in the SN (105, 106). Our group has recently published a case with *G2019S* mutation and a rare tau mutation without LBs but harbouring TDP-43 pathology (107).

*PARK2:* Parkin

Loss of function homozygous and compound heterozygous mutations in the *PARK2* gene are the most common cause of early onset autosomal recessive PD worldwide (108). A small number of cases with *PARK2* mutations have now been examined at post-mortem. DAergic cell loss in the SN was the major common pathological feature in all cases, yet most lacked LB pathology. Some also had cell loss in the locus coeruleus. Tau inclusions resembling those present in PSP, such as neurofibrillary tangles (NFTs) and thorn shaped astrocytes are present in few reported cases (94). Recently Doherty *et al* (109) reported five cases with compound heterozygous PARK2 mutations at autopsy. All cases had severe depletion of DAergic neurons in the ventral tier of the SN and moderate gliosis. Immunohistochemistry with alpha-synuclein revealed dissimilarity with typical PD cases. Only three out of five cases had sparse and restricted LB pathology. Some tau positive neuropil threads were also noted. It is clear from the published cases that PARK2 mutations represent a separate neuropathological paradigm and clinically too these are rather different. These cases may suggest that formation of LB inclusions are not a prerequisite of nigral cell loss and parkinsonism.

*PARK7* (DJ-1) and *PARK6* (PINK1)

Homozygous mutations in the DJ-1 and PINK1 genes are rare causes of early onset PD (88). The pathology of DJ-1 mutation cases remains obscure, as no case has yet been reported at post-mortem.  For PINK1, pathology from only one compound heterozygote (containing a deletion and a splicing mutation) has been described in the literature (110). Autopsy findings included significant neuronal loss in the SN pars compacta region as seen in PD but with relative sparing of the locus coeruleus.

*GBA* (Glucocerebrosidase)

Several groups have now reported autopsies of PD patients carrying heterozygous mutations in GBA (61, 111). Loss of biochemical activity of GBA has been reported in the mutated patients (111, 112). Majority of the GBA mutated PD cases have LB pathology (94) with some reports suggesting GBA

mutation status associated with widespread cortical LBs (113, 114). Tau pathology is present in some GBA mutations (94). Overall pathological observations to-date overlap significantly with those observed in sporadic PD.

## 2.5: Shared pathogenic mechanisms between sporadic and genetic PD

The exact aetiology of both sporadic and genetic PD remains undetermined and the precise mechanisms leading to specific DAergic cell loss are yet to be elucidated. There is ample evidence in the literature suggesting shared pathogenic mechanisms. Some of these are discussed below.

### 2.5.1 Evidence of oxidative stress, protein and DNA oxidation in PD

Oxidative stress is a common underlying pathogenic mechanism shared with both sporadic and genetic forms of PD. Evidence from post-mortem studies indicate the presence of markers for reactive oxygen species (ROS) (115). Brain tissue is particularly susceptible to damage caused by ROS because of high amounts of polyunsaturated fatty acids and low antioxidant activity (115). Oxidative damage in PD can be widespread and is not just restricted to the SN. At the cellular level there is evidence for accumulation of 4 hydroxy 2- nonenal (HNE)-protein adducts and protein carbonyls in PD brains (116, 117). High levels of lipid peroxidation markers are also observed during aging (116). Additionally, peroxynitrite is formed from the reaction of superoxide and nitric oxide and this can advance nitration of key proteins that are involved in the pathogenesis of PD. In support of this, nitration of alpha-synuclein is increased in Lewy bodies and Lewy neurites in PD patients (118). Several of the proteins that are associated with genetic PD impact on pathways that leads to excessive ROS generation in a feed-forward manner (115), creating a damaging environment within the brain. These include mutations in *SNCA*, *PARK6*, *PARK2* and *PARK8*. In post-mortem PD brains, greater amounts of oxidised DJ-1 are also observed (119, 120). Oxidative stress can lead to altered phosphorylation status of LRRK2 which may affect downstream signalling (121). Whether the phosphorylation status of LRRK2 is altered in post-mortem brains remains to be determined.

Oxidative damage can also affect nucleic acids. Elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OHDG) and 8-hydroxy guanine in PD SN and levels of 8-OHDG were found to be elevated in the urine of early stage PD patients (115, 122). Mitochondrial DNA from PD patients also showed increase in 8-OHDG levels in PD SN along with elevated expression of DNA repair proteins (123).

Several antioxidant enzymes play a major role in maintaining a healthy brain devoid of ROS. These are superoxide dismutases, catalases, peroxiredoxins and gluthathione. Glutathione is endogenously produced and plays an important role in antioxidant defence mechanisms and is finely regulated in neurons (115). Altered glucose metabolism through the pentose phosphate pathway in PD (124) could affect GSH production.

Dopamine oxidation also creates harmful dopamine adducts such as dopamine quinones in the SN of PD patients (115, 125). The generation of these species

along with an age-related decrease in the anti-oxidant molecule glutathione can be detrimental for neuronal survival (115, 125). The lysine (K) and tyrosine residues that are modified by dopamine in PD are depicted in Fig 2.1. Other factors that could contribute to aggravated ROS production are neuromelanin, increased calcium, increased iron levels and also neuroinflammation (115). Clearly, there are multiple risk factors that emerge with age and in neurodegeneration, which may lead to elevation in oxidative stress, leading to cellular dysfunction and cell death. Despite encouraging results from animal models, attempts to use antioxidant as potential treatments for PD have so far been unsuccessful (115). The reason for this is not entirely clear but one explanation could be that the antioxidant products may not be effective against all kinds of deleterious oxidants.

*Place Fig 2.1 here.*

2.5.2 Protein aggregation (alpha-synuclein aggregation)

The physiological function of the small 14kDa alpha-synuclein protein remains ambiguous. Alpha-synuclein has highest expression in the brain more specifically in the DAergic neurons in the SN (126) and localises to nerve terminals (127). The monomeric protein can be subdivided into three main regions: the first 1-60 amino-acids (aa) forms the N-terminal alpha-helical region with 11 imperfect repeats of hexameric motif (KTKEGV), aa 60-95 forms the hydrophobic non-amyloid component (NAC) region and finally the C-terminal acidic tail (FIG 2.1) (128). Alpha-synuclein has a strong propensity to self-aggregate in vitro and the normally unstructured molecule adopts an alpha-helical structure in presence of lipids (128). Several factors also modulate the conformational behaviour of alpha-synuclein and it can form several different forms of aggregates from oligomers to amyloid-like fibrils (128).

The toxicity of alpha-synuclein is a matter of continuing debate with several studies suggesting toxic oligomeric forms being the most harmful species (129). Mutations in SNCA have been linked to autosomal dominant PD. Almost all of the missense mutations identified to date are present in the alpha-helical region of the N-terminal domain (Fig 2.1). More recently two further point-mutations of questionable pathogenicity have been identified, the *A18T* and *A29S* to be associated in sporadic PD cases (130). The *A30P, E46K* and *A53T* SNCA mutations have accelerated aggregation properties in vitro compared to wild-type alpha-synuclein (128). A recent study has shown that *H50Q* increases and *G51D* decreases alpha-synuclein aggregation properties (131).

Recent evidence suggests that alpha-synuclein can exist in a dynamic equilibrium as a folded monomer along with an ordered tetramer that is resilient to fibrillisation (132). Any stimuli that perturb this equilibrium could lead to further monomer formation which has a random structure and this could further propagate fibrillisation. Local cellular factors such as altered pH, stress thresholds, neuronal activity patterns, altered proteostasis and even advancing age contribute to selective vulnerability of cell loss (133). Oxidatively damaged or post-translationally modified (phosphorylated/nitrated) alpha-synuclein is

present in aged brains as well as in PD (125) (Fig 2.2). Using specific antibodies, pathophysiologically relevant alpha-synuclein oligomers were found in the PD brain with LBs and LNs (134, 135). In addition, nitration of alpha-synuclein may lead to formation of dimers and oligomers which may hinder its ability to bind to membranes (136).

Alpha-synuclein is enriched in all neurons, yet LBs are formed in only some definite neurons. The reason for this is currently unknown however a probable explanation could be the intrinsic differences in the neurochemical milieu of the vulnerable neurons affected by alpha-synuclein pathology compared to unaffected regions. Understanding of how the neurons in unaffected regions respond to different stressful stimuli could be crucial and may provide important insight towards unlocking potential neuroprotective strategies.

Given the central role of various forms of alpha-synuclein in PD, attempts have been made at using passive or active immunisation as a valid treatment for PD. Two vaccine candidates PD01A and PD03A are now in clinical testing phase after showing promise of improved motor features in animal models (137). The outcomes of these trials are keenly awaited.

*Place Fig 2.2 here*

## 2.5.3 Role of extracellular alpha-synuclein in PD: prion-like propagation of alpha-synuclein pathology in PD?

Cell to cell propagation of pathological alpha-synuclein was first proposed by Braak and colleagues who put forward the hypothesis of pathological spread from one region to another, along defined anatomical pathways, which may originate from the gut (82, 85).  Recent concepts suggest prion-like transmission although the forms of alpha-synuclein responsible remain contentious. A landmark discovery was evidence showing transmission of alpha-synuclein pathology from host to graft neurons in a PD patients transplanted with non-diseased neurons for successful cell replacement treatment (138, 139). Experimental evidence for prion-like transmission was provided by Luk *et al* (140) who showed a single injection of synthetic alpha-synuclein fibrils led to cell-cell transmission of LB-like pathology in anatomically interconnected regions. This resulted in progressive loss of DA neurons and accompanying motor deficits in mice (140).

Under experimental conditions, it was shown that both monomeric and aggregated alpha-synuclein could be secreted from neuronal cells via unconventional endoplasmic reticulum/Golgi-independent exocytosis (141) under non-stressed conditions but is increased under mitochondrial, lysosomal and proteasomal dysfunctions (142). Under stressful conditions, more aggregated alpha-synuclein species are exocytosed (142). Pre-formed alpha-synuclein fibrils and oligomers can be internalised by endocytosis by neurons in culture (143) and these could act as further seeds of aggregation. In line with this an early report showed the presence of alpha-synuclein in the CSF and plasma of PD and control patients (144). The source of plasma alpha-synuclein is debatable

as red blood cells could be a major source of alpha-synuclein in blood (145) whereas in the CSF alpha-synuclein is most likely derived from neurons (146). The prion-like propagation of pathogenic alpha-synuclein is an exciting area of research, however caution needs to be maintained whether data from in vitro and in vivo animals could be fully extrapolated in PD. This model opens up novel opportunities for therapeutic development to combat pathogenic alpha-synuclein species (142).

2.5.4 Mitochondrial dysfunction in the pathophysiology of PD

Mitochondrial dysfunction appears to be an important contributory factor in the pathogenesis of both sporadic and genetic PD. Environmental toxins such as MPTP, rotenone, some herbicides and pesticides that are known to impair respiratory chain function have been used in experimental in vivo models of PD (147).

Clinical studies have shown mitochondrial complex 1 activity is deficient in the SN of PD patients (148). Higher levels of mitochondrial DNA deletions are observed in PD brains (149) and are also associated with ageing, with sharp increases in deletions seen approximately 70 years (149, 150). Additionally, PGC1-alpha, a key regulator of mitochondrial biogenesis was found to be decreased in PD patients (151).

Several of the recognised PD genes affect mitochondrial function; including *SNCA, PARK2, PARK6, PARK7* and *PARK8* (152). Mutations in these genes in animal models show mitochondrial dysfunction, although this may not always accompany DA cell loss. Fission and fusion are two opposite highly dynamic processes that maintain mitochondrial organisation (153). This process controls mitochondrial morphology as well as subcellular location and function. Mutations in PD genes control fusion or fission; mutations in *PINK1/PARK2* lead to mitochondrial fusion whereas mutations in *LRRK2* and *DJ-1* promote mitochondrial fission. PD patients carrying *PINK1/PARK2* mutations exhibit aggravated mitochondrial fragmentation. DJ-1 deficient patients displayed aberrant mitochondrial morphology (154). In mice expressing doubly mutated alpha-synuclein and *PARK2 KO*, severe mitochondrial morphological and functional abnormalities were noted in the SN coinciding with reduced complex 1 activity (155).

The most common of the LRRK2 mutations, the *G2019S* interacts with dynamin related protein 1 (drp1) to promote mitochondrial fragmentation which could be reduced by overexpression of the fusion machinery protein Mfn2 (154, 156). iPS DAergic cells derived from *G2019S* mutation fibroblasts showed increased mitochondrial fission, abnormal autophagy and neuronal damage (157). Treatment with P110, a selective inhibitor of Drp1 was shown to reduce mitochondrial fragmentation and impairment and reversed anomalous autophagy (157).

Mitochondria-assisted autophagy is termed mitophagy (158). It mostly occurs in defective mitochondria following damage or stress. Mitophagy is essential for the

functioning of a healthy cell by maintaining mitochondrial quality control and energy demand of the cell (159). Altered mitophagy is apparent in both sporadic and genetic PD (153). Neurotoxins such as MPP+, rotenone or 6-hydroxydopamine exposure to cultured cells demonstrated an increased number of autophagosomes together with cell death (153).  Two proteins linked with genetic PD, PINK1 and PARK2 play a major role in the mitophagy pathway (159). PINK1 is a key signalling molecule that acts upstream of parkin and is responsible for recruiting parkin in the damaged mitochondria. The failure of PINK1/parkin mediated mitophagic processes leads to accumulation of damaged mitochondria, which can increase ROS production leading to cell death (159). DJ-1 knockdown in cells also results in decreased membrane potential, increased ROS and mitochondrial fragmentation and reduced autophagy (153). Interestingly PINK-1/parkin overexpression can rescue mitochondrial defects induced by DJ-1 knockdown suggesting DJ-1 acts upstream of the PINK1/parkin mitophagy pathway (153, 160). Accumulating evidence indicates that several PD-related genes may converge at the autophagy-mitophagy intersection pathway highlighting that this may hold promise for future PD therapeutics.

## 2.5.6 Impairment of the Ubiquitin-proteasome system (UPS)

A variety of evidence points to impairment of UPS in sporadic forms of PD (118, 161). 20/26S proteasome subunits are reduced in the SN of sporadic PD patients (162). In cell models, misfolded or excess levels of alpha-synuclein inhibit UPS leading to impaired protein clearance (163). Systemic exposure of protease inhibitors in rats caused striatal dopamine depletion and DAergic cell death, indicating degeneration of nigrostriatal pathway. Markers of apoptosis, inflammation and LB-like inclusions were present in the SN (162).

Some of the proteins associated with genetic PD are also linked with the function of the UPS. Alpha-synuclein can be degraded both by proteasomes and autophagy (164). Thus alpha-synuclein deubiquitination enzyme USP9X is reduced in PD frontal cortex (165). Components of the UPS, including UCHL1, proteasomal subunits, ubiquitin and parkin (a ubiquitin E3 ligase) are present in LBs indicating their involvement in LB biogenesis (163). Fine-tuning of hydrolase activity of UCHL1 and ligases are necessary for smooth degradation of protein molecules, whilst mutations in UCHL1 and parkin are likely causes of genetic PD indicating that UPS is intricately involved in genetic PD (161, 166). DJ-1 in its oxidised state can prevent alpha-synuclein fibrillisation (167) suggesting excessive oxidation of DJ-1 can enhance alpha-synuclein aggregation. Our understanding of the UPS pathway and its role in PD pathogenesis is still incomplete and more investigations are warranted in relevant cellular models.

## 2.5.7. Dysfunction of autophagy/lysosomal pathway

Another emerging mechanistic concept in the pathways to PD is the dysfunction of the autophagy/lysosomal system associated with alpha-synuclein, LRRK2, GBA, VPS35, ATP13a2 and to some extent also in sporadic PD (168). Alterations in lysosomal, proteasomal and chaperone-mediated autophagy markers are observed in PD patients (169, 170). Alpha-synuclein levels are associated with

impaired macroautophagy (171) whilst dominant point mutations in SNCA abrogate the function of lysosomes thus decreasing autophagic flux (172). Under physiological conditions alpha-synuclein associates in the regulation of vesicle fusion events through SNARE complexes, thus linking alpha-synuclein with the autophagy pathway (173). GBA is intimately linked with the lysosomal pathway and mutations in GBA that cause PD disrupts lysosomal function (168). Indeed, ambroxol is being sought as a potential small molecule drug to target GBA dysfunction (174). A number of studies link the function of LRRK2 with the autophagy pathway (168) but the exact pathway is unclear. VPS35 and ATP13a2 are components of the retromer pathway and lysosomal protein respectively. Loss of function of both these proteins leads to lysosomal abnormalities (168).

## 2.5.8. Role of neuroinflammation

Chronic inflammation is a pathological feature of both sporadic and genetic PD (175, 176). It is increasingly recognised that environmental factors and age-related cellular changes occur to compromise the immune system and that this is most likely to happen in early phases of PD (175) and this could further perpetuate pathogenic progression of PD (177). Ageing in rhesus monkeys has been associated with increased activated microglia in the SN thereby rendering neurons vulnerable to degeneration (178). To this end, evidence of on-going neuroinflammation in affected regions has been shown through accumulation of pro-inflammatory cytokines such as Interferon gamma, TNF-alpha, Interleukin 6 and Interleukin 1beta in cerebrospinal fluid and post-mortem brain (175). A recent PET imaging study of PD patients showed evidence of neuroinflammation early in disease that may occur in parallel with DAergic terminal loss (179).

Many of the PD-linked genes also connect with aspects of neuroinflammation (176). Alpha-synuclein promotes a pro-inflammatory phenotype in microglia (180), in particular the oligomeric and fibrillar forms directly activate Toll like receptor 2 (176) to mediate an inflammatory signalling cascade. In a murine model of Thy-1 alpha-synuclein overexpression, microglial activation was noted firstly in the lower brain stem and later in the SN and these changes preceded neuronal loss (176, 181). In addition, nitrated alpha-synuclein can also serve as an antigen for peripheral lymphocytes that lead to further DAergic cell loss implying an adaptive immune response as a pathogenic factor (182). Microglial activation either by alpha-synuclein monomeric or pathogenic forms could increase ROS and this is likely to perpetuate further nigral neuron degeneration (183). Mice lacking interferon-beta (IFN-β) signalling caused defects in autophagy prior to accumulation alpha-synuclein containing LBs in brains, whilst IFN-β gene therapy in the human alpha-synuclein mouse model prevented DAergic neuron loss (184).

One of the multiple functions of the LRRK2 protein is its involvement in immune related pathways in cell and animal models (185). LRRK2 is a member of the RIPK family of proteins which have established roles in immunity (176). In addition to PD, LRRK2 is also linked with increased risk of Crohn's disease (186). LRRK2 is highly expressed in peripheral mononuclear cells and its expression is up-regulated by interferon gamma treatment and it is robustly expressed in

murine microglia and is enhanced by treatment with LPS (Reviewed in (176)). Furthermore, in murine macrophages LPS signalling activates TLR signalling to phosphorylate two key serine residues (S910 and S935) within the LRRK2 molecule (187). In addition, pathogenic LRRK2 mutations may regulate cytokine production (188) through TLR mediated downstream effects, although the precise mechanism remains to be elucidated.

Recent evidence indirectly links recessive PD genes, *PARK2, PARK6* and *PARK7* with immune function. All of these three genes are associated with abnormal cytokine production. *PARK2* null mice show increased susceptibility to LPS-induced dopamine neuronal loss in the SN (189) and peripheral macrophages from these mice display increased levels of TNF, IL-1beta and iNOS mRNA(190). Parkin may also play a role in infection; parkin deficient mice are more susceptible to tuberculosis infection (191) and *PARK2* polymorphisms are linked with leprosy infection in Indian population (192).

PINK1 is able to moderate T-cell mediated immunity (176). PINK1-deficient T-cells affects IL2 signalling pathways (193). In PINK1 null mice, treatment with LPS generated higher levels of interleukin and TNF-alpha (194). DJ-1 null mice demonstrate higher responses to LPS mediated cytokine expression (195). DJ-1 deficiency resulted in increased ROS and elevated nitric oxide production leading to increased dopamine neurotoxicity and reduced TREM2 expression in microglia, which could lead to pro-inflammatory conditions in the brain (196). In PD brain where there is a lack of DJ-1 (197) immunomodulatory processes may be altered. However, more detailed studies in relevant models will be required to gain further insight.

Inflammation has long been linked with PD but whether it is a cause or consequence of disease remains a critical question. The roles of *SNCA* and LRRK2 seem to suggest a more established link with neuroinflammation but the roles of parkin, PINK1, DJ-1 remains to be fully established.

## 2.6. Applications of induced pluripotent stem cells and a potential future for PD treatment

The problems that have hampered progress in identifying disease- modifying treatments for PD is the inability of the existing cellular and animal models to fully recapitulate disease pathogenesis. In general, the genetic mouse models are of limited use as they do not represent *in vivo* pathophysiological neurodegeneration in combination with protein aggregation (198), whilst the toxin models fail to represent the slow and progressive nature of DAergic loss or LB-like inclusions as seen in PD. The cellular models are mostly based around the use of immortalised tumour cell lines but the relevance of molecular findings from these are questionable from human disease point of view. Importantly, these models do not recapitulate genetic heterogeneity observed in human brain cellular subtypes, both neurons and glia.

The recent development of iPSC technology has greatly enhanced our ability to model disease in human cells. For PD it means that we can now model the

disease in dopaminergic neurons (199, 200). Over the past few years several different groups have produced iPSC from patients suffering from sporadic and genetic forms of PD. These studies have divulged several key disease phenotypes, such as alpha-synuclein accumulation and defects in mitochondria and autophagy (199). The developments of iPSCs unlocks the most relevant cellular platforms on which to base future disease modifying therapies. Challenges still remain in this field, as the cause of PD is multifactorial and DAergic cell death is caused by a culmination of events occurring both in the neurons and glia. The advent of new technologies such as isogenic corrected controls and efficient cell sorting and live cell imaging provide powerful tools for future drug discoveries using iPSCs. The prospect to study both symptomatic and asymptomatic mutation carriers allows the identification of disease-linked early diagnostic biomarkers and individualised treatments.

## 2.7 Treatment and management

Opportunities to develop novel therapeutics with disease-modifying intent have been listed along with descriptions of pathophysiological aberration above. As it stands however, current therapeutic strategies are not disease modifying and largely target symptom control. Modern management of PD is multidisciplinary, but the role of physiotherapists, psychologists, occupational and speech and language therapist, specialist nurses, community support workers and pharmacists will not be further discussed here.

The vast heterogeneity of PD means that tailored therapeutic regimens are required, which address not only symptoms and expectations but also fit into a lifestyle and daily routine (201). There is inevitable progression of PD with increasing physical and cognitive burden in advanced stages of the disease. The backbone of PD management is dopamine replacement therapy as either l-dopa (with a dopa-decarboxylase inhibitor) or dopamine agonists. In early disease, either of these can be commenced as monotherapy to treat motor symptoms (although MAOI's can also be used as monotherapy in some early cases). Selection of one or the other is determined by patient and physician preference and discussion, weighing the anticipated benefits and potential side effects of each, not simply the age of the patient. The previous opinion that most patients, particularly younger ones, should be started on a dopamine agonist and l-dopa 'held back' for the future is now largely out-of-date. Motor fluctuations commonly emerge with l-dopa with prolonged use and higher doses, but choice of initial monotherapy does not need to have a major bearing on this (202, 203).

Treatment should aim to maximize current function to ensure continued independence and, where relevant, employment and pursuit of goals and hobbies. It remains prudent not to simply increase doses at each follow-up appointment and symptom severity and impact on daily should be used to guide therapeutic decisions. L-dopa remains the most effective and potent pharmacological therapy against motor symptoms but newer modified release dopamine agonists can lighten tablet burden through once daily dosing or transdermal delivery (201). Side effects are recognized for both classes of drugs and overlap in terms of common side effects such as nausea, vomiting, tiredness

and postural hypotension. Both can contribute to psychotic symptoms in high doses such as hallucinations or delusions. The most feared side effects are motor fluctuations and dyskinesia for l-dopa and impulse control behaviours for dopamine agonists, most of which are dose- and treatment-duration dependent to some extent. Cautious dose increases and vigilance should minimise the emergence and impact of both.

A range of drugs can be used to augment basic dopamine replacement strategies. Monoamine oxidase inhibitors slow the breakdown of l-dopa and there can be used to prolong the effects of single doses throughout the disease course, and reduce motor fluctuations (204). As indicated above, selegiline and rasagiline can be used early in the disease as monotherapy. Although their effect in isolation is fairly mild, anecdotally however in certain patients the effect can be quite profound and sustained for a number of years. Likewise COMT inhibitors such as entacapone and tolcapone can be used to prolong the effect of doses of l-dopa and reduce 'wearing off' (204). Amantadine has dual effects in potentiating l-dopa effects, whilst also possessing an anti-dyskinetic effect (the only oral agent currently available with this activity) (205). Most of the these adjuvant treatments tend to be employed to manage the adverse effects of l-dopa over time such as motor fluctuations and dyskinesia, but increasingly are used to supplement lower doses of l-dopa and dopamine agonists to reduce the need for precipitous increases in these agents (201).

The advanced stages of PD are characterised by a range on non-dopamine-responsive symptoms including cognitive impairment, speech and swallowing deterioration and falls (206). Many encounter the effects of long term treatment with dopaminergic therapy listed above; motor fluctuations and dyskinesia. If these cannot be managed sufficiently through altering l-dopa (increasing/decreasing doses, or fractionating existing dose) and other adjuvant treatment, or addressing contributory factors such as the timing of meals, then many may be considered for advanced PD treatments such as apomorphine, deep brain stimulation and jejunal levodopa, depending of local availability and funding availability. Further information about patient selection, expected benefits and potential adverse effects are shown in Table 2.3. Newer agents that target symptomatic improvement are under investigation/coming to market including IPX066, istradefylline and safinamide. IPX066 is an oral extended release preparation of carbidopa levodopa and phase 3 clinical trials have demonstrated that it reduces 'OFF' time by over an hour compared with standard treatment (207). Istradefyliine is selective adenosine A2A antagonist that reduces 'OFF' time in advanced PD, and is currently under further investigation in phase 3 trials (208, 209). Safinamide has multiple mechanisms of action including MAO-B inhibition and inhibition of dopamine reuptake and glutamate, and two studies have shown improvements in UPDRS scores in the safinamide group compared with placebo (201). Of great interest is the potential benefit from non-pharmacological management including regular exercise with a range of studies suggesting symptomatic improvement (210, 211).

*Place Table 2.3 here.*

## 2.8: Conclusions

PD is a complex disorder that imposes an increasing global burden. The disease can be identified clinically with reasonable accuracy and good symptomatic treatments exist. Major unmet needs include therapies that modify the underlying process and biomarkers that reflect disease activity. These will likely only be achieved through further research into the epidemiology, genetic, pathology and basic disease mechanisms.

Common pathways are being identified for early changes in PD, which should foster biomarker discoveries for initial detection of disease. Small molecule inhibitors are already being developed for LRRK2 and GBA mutations, and clinical trials using antibody therapies for tackling forms of alpha-synuclein are already underway. Finally, further cell based therapy initiatives are being explored (212).

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**Table and Figure legends:**

**Table 2.1**: Mendelian genes associated with Parkinson's disease.

**Table 2.2**:  Comparison of Braak *et al* 2003 (82) and McKeith *et al* 2005 (55) criteria for Lewy body scores.

**Table 2.3:** Advanced treatment options for Parkinson's disease.  Adapted from Worth PJ 2013 (213).

**Figure 2.1:** Alpha-synuclein protein domain structure, point-mutations and post-translational modification sites. Blue arrows depict new mutations. S= serine; Y= tyrosine; K=lysine residues that are modified to form Dopamine adducts. NAC= non-amyloid component. Adapted from Barrett and Greenamyre 2013 (125).

**Figure 2.2:** Alpha-synuclein (phosphorylated at Ser-129) pathology in regions of Lewy body predilection in sporadic (A-E) and *G2019S*-LRRK2 PD (F). Scale bar: 20μm in A-D, 10μm in E and 8μm in F. Black arrows are Lewy bodies and blue dashed arrows are Lewy neurites.

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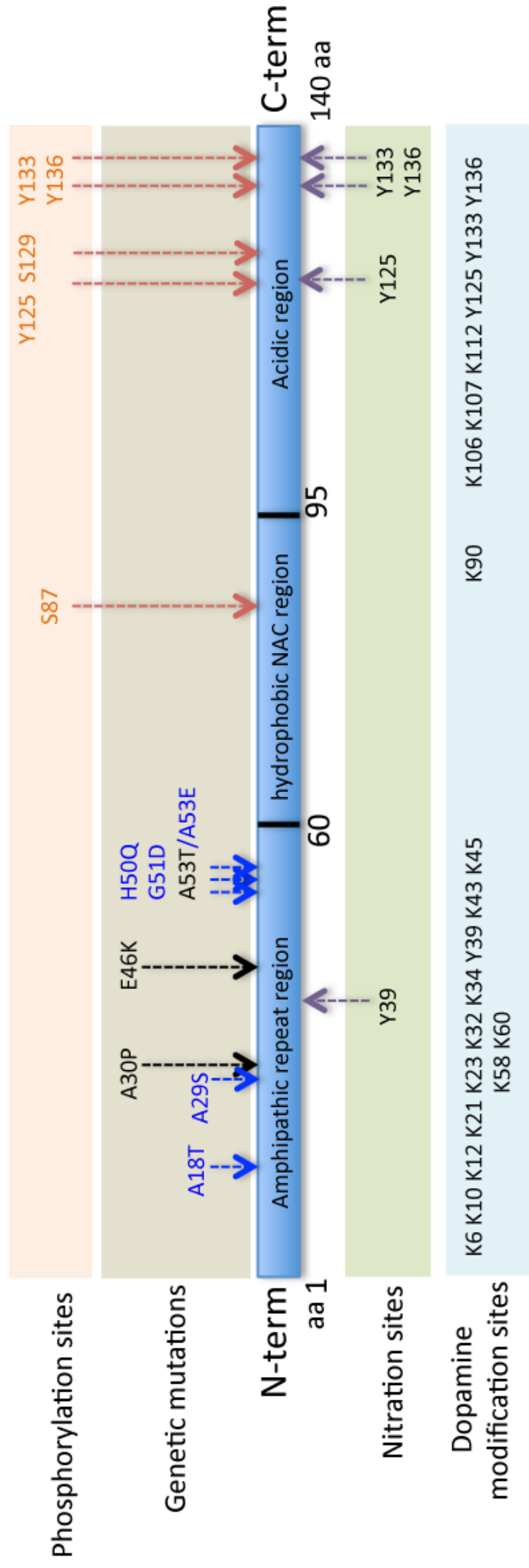(6002-US-005). Annals of neurology. 2008 Mar;63(3):295-302. PubMed PMID: 18306243.

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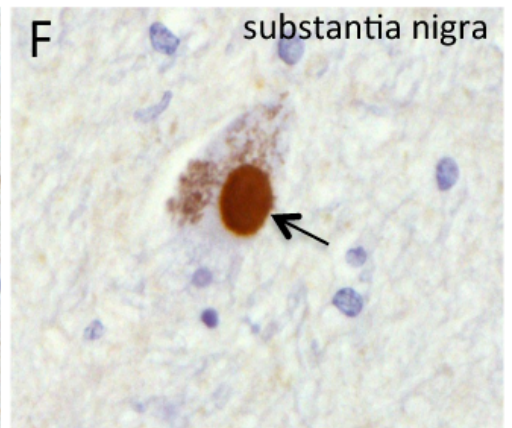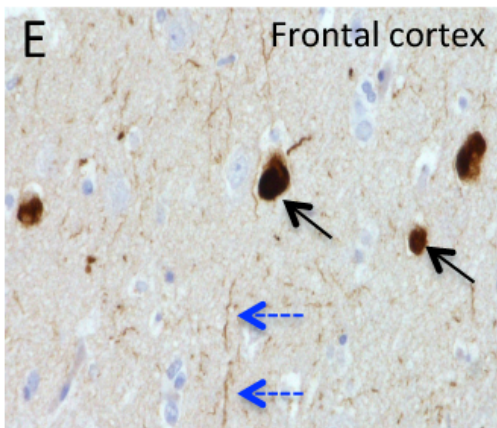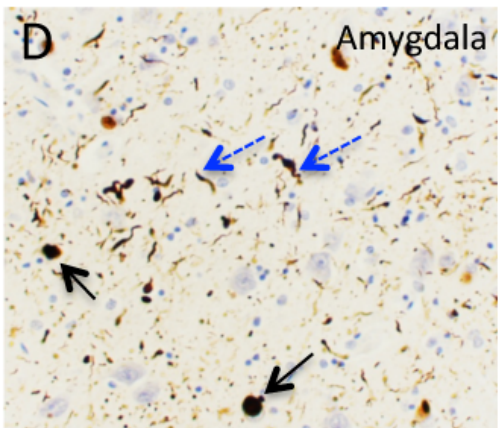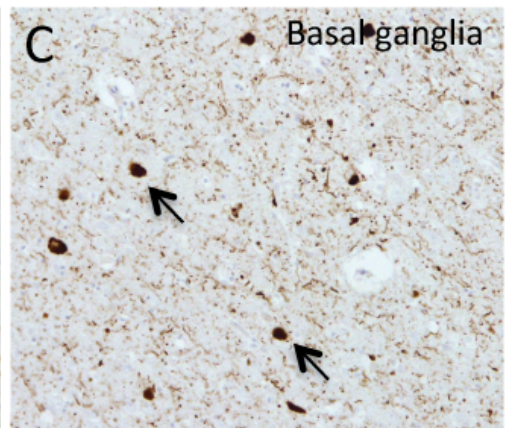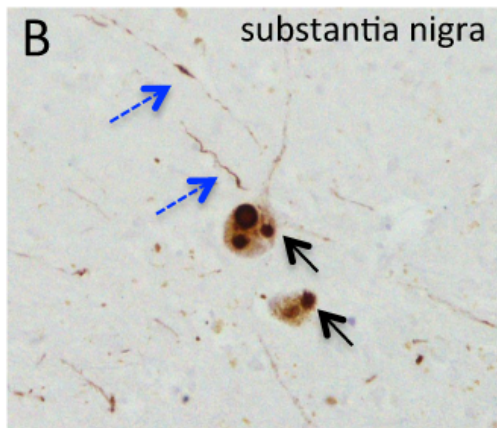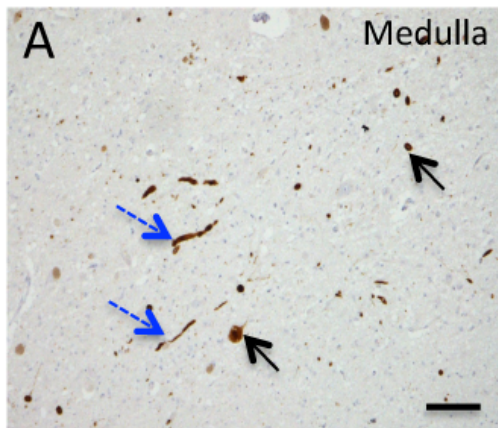
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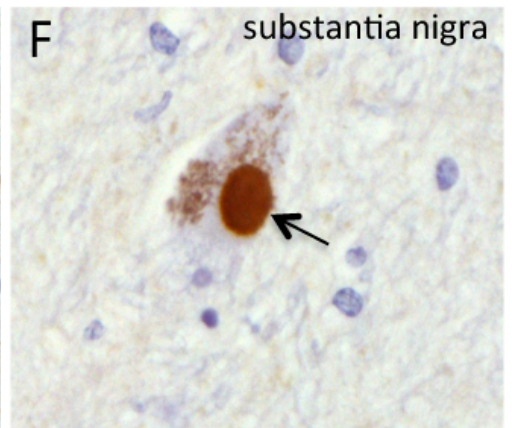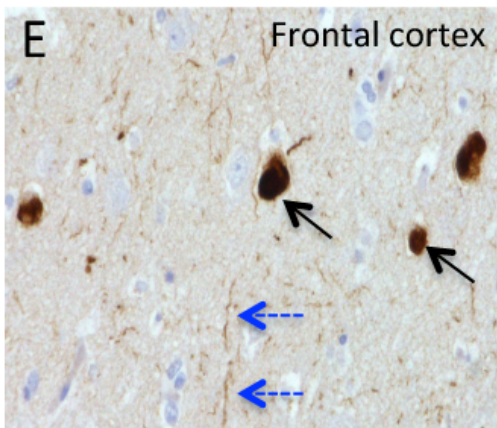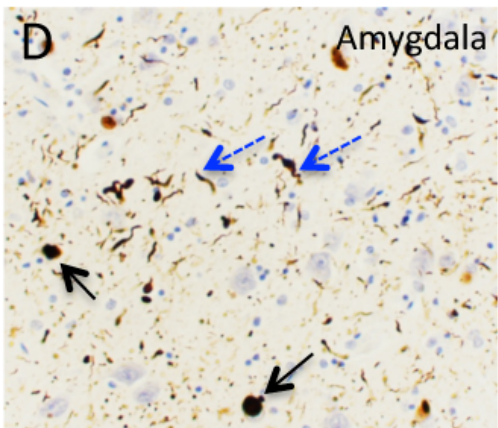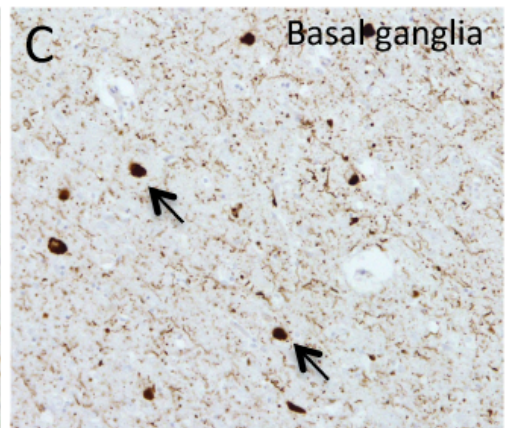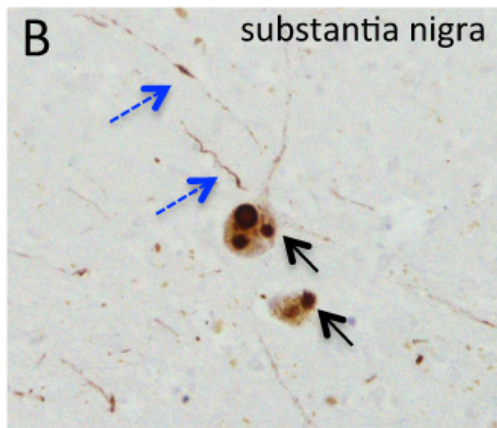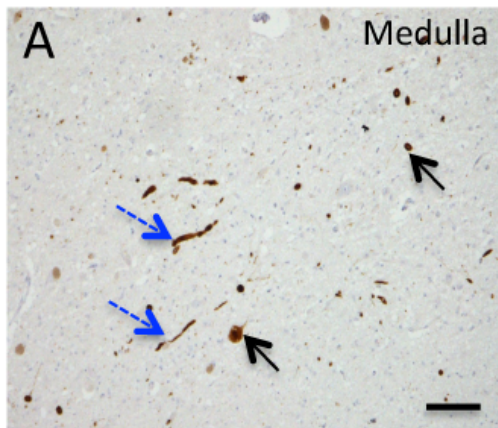
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Phosphorylation sites: Y125 S129 Y133 Y136, S87

Genetic mutations: A18T, A29S, A30P, E46K, H50Q, G51D, A53T/A53E

N-term · C-term · aa 1 · 140 aa · 60 · 95

Amphipathic repeat region · hydrophobic NAC region · Acidic region

Nitration sites: Y39, Y125, Y133, Y136

Dopamine modification sites: K6 K10 K12 K21 K23 K32 K34 Y39 K43 K45 K58 K60, K90, K106 K107 K112 Y125 Y133 Y136

A  Medulla

B  substantia nigra

C  Basal ganglia

D  Amygdala

E  Frontal cortex

F  substantia nigra

| Mendelian causes[a] | | | | | |
|---|---|---|---|---|---|
| Gene | Locus | Inheritance | Protein | Pathology | Mechanism |
| PARK1 & 4 (SNCA) | 4q21-23 | AD | Synuclein | Lewy pathology | Protein aggregation, oxidative stress inflammation, macroautophagy, chaperone mediated autophagy |
| PARK2 | 6q25-27 | AR | Parkin | Lewy pathology rare | Mitophagy, UPS |
| PARK6 (PINK1) | 1p35-36 | AR | PINK1 | Lewy pathology* | Mitochondrial dysfunction/mitophagy |
| PARK7 (DJ1) | 1p36 | AR | DJ-1 | NK | Oxidation regulation, mitophagy |
| PARK8 (LRRK2) | 12q12 | AD | Dardarin | LP, tauopathy | Altered kinase and GTPase activity, inflammation, autophagy |
| PARK17 (VPS35) | 16q11.2 | AD | VPS35 | NK | Lysosomal degradation |

[a] Mendelian causes of Parkinson's disease does not include early-onset parkinsonism with additional features, such as such as PARK9 (Kufor-Rakeb), PARK14 (PLA2G6), and PARK15 (FBX07). NK = NOT KNOWN; *only one case

**Table: 2.1: Mendelian genes associated with Parkinson's disease.**

| Anatomical distribution of Lewy bodies | Braak staging | McKeith criteria for Lewy bodies |
|---|---|---|
| Peripheral nervous system, olfactory system<br>Medulla oblongata - dorsal motor nucleus of vagal and glossopharyngeal nerves | 1 | Brain-stem predominant |
| Pons - locus coeruleus, magnocellular areas, reticular formation posterior raphe nucleus | 2 | Brain-stem predominant |
| Substantia nigra, basal forebrain (magnocellular nuclei including nucleus basalis of Meynert), amygdala | 3 | Brain-stem predominant |
| Pathology stage 3 plus<br>Temporal mesocortex (Transentorinhal cortex) and allocortex (CA2 plexus) | 4 | Transitional/ Limbic subtype |
| Pathology of stage 4 plus<br>Sensory association areas of the neocortex and prefrontal neocortex | 5 | Diffuse neocortical |
| Pathology stage 5 plus<br>Advanced neocortex (primary cortical areas) | 6 | Diffuse neocortical |

**Table 2.2:** Comparison of Braak *et al* 2003 (82) and McKeith *et al* 2005 (55) criteria for Lewy body scores. Adapted from Jellinger 2012 (69).

| Treatment | Mechanism/target | Considerations | Advantages | Potential adverse effects |
|---|---|---|---|---|
| Apomorphine | D1/D2 dopamine receptor agonist.<br><br>Subcutaneous bolus injections can be used to treat unpredictable and/or severe 'OFF' periods.<br><br>Subcutaneous continuous infusion can be used to manage motor symptoms in advanced disease. | For patients requiring advanced treatment including those with motor fluctuations and dyskinesia, refractory to changes in oral medication.<br><br>Suitable for patients that can set up the pen (intermittent form) or pump (continuous), and inject himself or herself daily, or that have a relative or carer that can perform these tasks. | Improvement in 'ON' time.<br><br>L-dopa medication can often be reduced, improving dyskinesia.<br><br>Most minimally invasive of the advanced treatments.<br><br>Can be used in situations where DBS would be unsuitable.<br><br>Cheapest initiation costs of the advanced treatment. | Nodule formation<br><br>Related to dopamine agonist effect:<br>• Nausea, vomiting<br>• Hallucinations<br>• Somnolence<br>• Impulsivity<br><br>Over-use of intermittent boluses<br><br>Haemolytic anaemia |
| Deep brain stimulation | Subthalamic nucleus – most available evidence.<br><br>Globus pallidus interna – may be better if prominent dyskinesia.<br><br>Ventral intermediate nucleus of the thalamus – best for intractable tremor<br><br>Parapontinue nucleus – may improve gait and balance | Used for motor fluctuations, dyskinesia and tremor.<br><br>Appropriate patient and target selection is crucial.<br><br>L-dopa unresponsive symptoms are unlikely to be improved by DBS. | Trials show improvement in motor function and quality of life.<br><br>Ability to tailor treatment according to symptoms: selection of target for stimulation and settings for stimulation after implanting.<br><br>Lower costs after year 2 following implantation compared with other therapies. | Hardware or surgical:<br>• Bleeding<br>• Infection<br>• Seizures<br>• Lead fracture or migration<br><br>Stimulation:<br>• Dysarthria<br>• Depression<br>• Weight gain |
| Jejunal dopamine | Carbidopa levodopa gel delivered directly to intestine via percutaneous gastrojejunostomy.<br><br>Address fluctuations and dykinesia prompted by altered gut motility. | Not available in all countries.<br><br>In some places, attempts to use apomorphine or DBS should have been considered or made before patients are eligible for jejunal dopamine treatment. | Trials show improvement in motor function and quality of life.<br><br>Probably the most expensive of the advanced therapies but this depends on many factors. | Tube-related:<br>• Displacement<br>• Kinking or twisting<br>• Site infection<br><br>Drug-related:<br>• Neuropathy |

**Table 2.3. Advanced treatment options for Parkinson's disease.**
Adapted from Worth PJ 2013 (213).