Parkinson's Disease

Pathogenesis and Clinical Aspects

Cover image: A case of Parkinson's disease as described and illustrated by William Gowers. See page 112, Chapter 6 for details.

Parkinson's Disease

Pathogenesis and Clinical Aspects

Edited by

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FOREWORD

Parkinson's disease is an increasingly common neurodegenerative condition, which causes not only dysfunction of movement but also a broad range of nonmotor features, including mood disturbance, sleep dysfunction, autonomic dysfunction, cognitive deficits, and dementia, and neuropsychiatric symptoms. For half a century, we have had an effective symptomatic therapy for the cardinal motor features of Parkinson's disease in the form of levodopa, but its long-term utility is limited by the emergence of motor fluctuations and dyskinesia in many patients. A different approach is required to manage the nonmotor symptoms, many of which have a nondopaminergic basis, and these problems can be particularly challenging to treat effectively. Furthermore, our current therapeutic approaches have no impact on the underlying progression of the disease which ultimately leads to significant motor and cognitive disability in many patients. A major conundrum in this condition is understanding its striking clinical variability, which encompasses a spectrum from a benign phenotype with levodopa-responsive symptoms and minimal progression, to a malignant phenotype with rapid progression to severe gait dysfunction, falls and dementia. Understanding the biological basis of heterogeneous forms of the disease is critical to allow development of new therapeutic strategies which are better targeted to different subgroups of patients.

This book integrates the considerable expertise of a range of authors from different disciplines, from clinicians through to basic scientists, in order to present a comprehensive and up-to-date overview of Parkinson's disease. In recent years, we have made significant progress in understanding the pathological and genetic basis of the disease and its heterogeneous forms, and the first section of the book is dedicated to reviewing this. The variable clinical features of the condition and its differential diagnosis are then considered. The final section provides a detailed overview of treatment approaches, including not only pharmacological therapies but also surgical therapies including deep brain stimulation and cell transplantation strategies. The combination of basic biology, clinical knowledge and therapeutics gives this book a very broad appeal. I hope that it will be of value to clinicians and health professionals caring for patients with Parkinson's disease, as well as providing an excellent introduction for junior researchers entering the field.

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PREFACE

The year 2017 marked the 200th anniversary of the publication of James Parkinson's *Essay on the Shaking Palsy*, in which the condition that would later bear Parkinson's name was first described in detail. In this work, Parkinson characterized six cases of patients that had developed a disorder of tremor, slowed movement, and gait disturbance, which he called "paralysis agitans." Since this initial description, it has become clear that Parkinson's disease in fact manifests with a wide variety of neuropsychiatric, cognitive, autonomic, and other nonmotor symptoms, in addition to the characteristic movement disorder that Parkinson described, and that there is probably a spectrum of disease consisting of as yet poorly defined clinical and pathological subtypes. While we know that the neuronal protein α-synuclein is central to Parkinson's disease pathology, and that the movement disorder results largely from loss of dopaminergic neurons of the substantia nigra pars compacta, our understanding of disease mechanisms remains limited, and as such no disease-modifying treatments have been developed.

In this book, we aim to provide an overview and update on several aspects of Parkinson's disease, taking the reader from pathology to patient. We have arranged the book in two sections, with the first five chapters focusing on pathology, and the remaining four chapters tackling clinical aspects and treatment approaches. In Chapter 1, the authors have provided a comprehensive discussion about the causes and mechanisms that underlie neuronal loss in Parkinson's disease, with the following chapters on immunogenetics, *GBA1* mutations, and apoptosis offering detailed overviews of some specific aspects of these problems. Much of what we have learned about Parkinson's disease and its treatment have come from studies in animals, and the models in which these have been conducted, as well as their limitations, are discussed in Chapter 5. The wide range of clinical manifestations of Parkinson's disease are discussed in Chapter 6, as well as the conditions from which Parkinson's disease must be distinguished. Since the introduction of levodopa as a therapy in the 1960s, new developments in Parkinson's disease treatment have been few and far between, but a number of potentially exciting treatment approaches are now on the horizon. The final three chapters provide an overview of the drugs that have been used in the treatment of Parkinson's disease, as well as more recent (deep brain stimulation) and emerging (stem cells) therapeutic developments.

We would like to thank all of the authors for their hard work in contributing toward this book. We hope that the nine chapters presented would provide the reader with useful insight into a broad range of important aspects of Parkinson's disease. We hope that the reader is able to explore the understanding of Parkinson's disease that has been acquired over the years, as well as the ambiguities that remain unsolved.

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Section I

Pathology

1 Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis

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Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder. While a number of non-motor manifestations arise, the typical clinical features involve a movement disorder consisting of bradykinesia, resting tremor, and rigidity, with postural instability occurring at a later stage. The cause of PD is not known, but a number of genetic risk factors have now been characterized, as well as several genes which cause rare familial forms of PD. Environmental influences such as smoking, caffeine consumption, and pesticide exposure have been postulated to alter the risk of PD development, although the role of these remains unclear. The movement disorder arises due to the loss of dopaminergic neurons of the substantia nigra pars compacta, with the pathological hallmark being intracellular aggregates of α-synuclein, in the form of Lewy bodies and Lewy neurites. Several processes have been implicated in PD, including mitochondrial dysfunction, defective protein clearance mechanisms, and neuroinflammation, but the way in which these factors interact remains incompletely understood.

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Keywords: α-synuclein; Lewy body; neurodegeneration; Parkinson's disease; pathogenesis

INTRODUCTION

Parkinson's disease (PD) is a complex progressive neurodegenerative disease characterized by tremor, rigidity, and bradykinesia, with postural instability appearing in some patients as the disease progresses. It was first described by James Parkinson in 1817 and further characterized by Jean-Martin Charcot, and our knowledge of PD is continuing to expand.

PD is the second most common neurodegenerative disease after Alzheimer's disease (AD) (1), with a prevalence of approximately 0.5–1% among those 65–69 years of age, rising to 1–3% among persons 80 years of age and older (2, 3). With an aging population, both the prevalence and incidence of PD are expected to increase by more than 30% by 2030 (4), which will result in both direct and indirect costs on both society and the economy as a whole.

PD is pathologically characterized by the loss of nigrostriatal dopaminergic innervation, although neurodegeneration is not limited to only the nigral dopaminergic neurons but also involves cells located in other regions of the neural network. Such a widespread pathology makes PD a very heterogeneous disorder, and a reliable diagnostic test is not yet available. Currently, diagnosis is based on clinical symptoms with the criteria for a diagnosis requiring the presence of two of the following clinical features: resting tremor, bradykinesia, rigidity and/or postural instability. Clinical criteria, however, can only lead to a diagnosis of probable PD, while a definitive diagnosis requires histopathological assessment, with the identification of α-synuclein-containing Lewy bodies (LBs) or Lewy neurites.

Treatment predominantly focuses on symptomatic relief with drugs aiming to either restore the level of dopamine in the striatum or to act on striatal postsynaptic dopamine receptors. However, as dopamine is not the only neurotransmitter involved in PD, many other drugs are also being used to target specific symptoms, such as depression or dementia. Yet, further investigation on novel therapies to reduce the rate of neurodegeneration or even to replenish the loss of dopaminergic cells remains in the research setting, with some in the early stages of clinical trials. As our understanding of the pathogenesis of PD increases and more is learned about new therapeutic targets, the potential for the development of disease-modifying therapies is promising.

CLINICAL FEATURES

The clinical features historically associated with PD are the triad of motor symptoms, namely, tremor, rigidity, and bradykinesia, with postural instability often appearing as the disease progresses. However, PD is also associated with many non-motor symptoms, and these often precede the motor symptoms by years or even decades.

The pre-motor or prodromal phase of PD may start as early as 12–14 years before diagnosis (5). There is now a great deal of evidence supporting the fact that the disease

may begin in the peripheral autonomic nervous system and/or the olfactory bulb, with the pathology then spreading through the central nervous system affecting the lower brainstem structures before involving the substantia nigra (6). This may thus explain the presence of hyposmia, constipation, and rapid eye movement sleep disorders in PD patients before motor symptoms begin. One study showed that patients with tremor, balance problems, depression, constipation, fatigue and urinary dysfunction at 5 years prior to diagnosis were more likely to develop PD than those without these symptoms (7). Additionally, individuals with constipation or tremor have a higher risk of developing PD over 10 years of follow-up (7).

There is increasing interest in this prodromal state of PD as it may be an ideal time point for therapeutic intervention. Many trials investigating potential therapies include patients with early PD, that is, those within 2 years of diagnosis, but even at this stage, significant dopaminergic neuron loss has already occurred (8)—therefore, it would be optimal for any future disease-modifying treatments to be initiated in the prodromal phase.

Clinical diagnosis of PD is based on the presence of bradykinesia in combination with a resting tremor or rigidity. Early symptoms generally present asymmetrically, with the absence of atypical symptoms (cerebellar signs, early severe autonomic dysfunction, vertical supranuclear palsies, or cortical sensory loss), which would be indicative of an alternative diagnosis (9). An asymmetric onset of symptoms and a good response to levodopa are supportive for a diagnosis of PD and are the two most important features to discriminate PD from other forms of Parkinsonism (9).

As the disease progresses, so does the severity of motor and non-motor symptoms. PD is a very heterogeneous disease and there have been attempts to subclassify the disease further. Although a consensus has yet to be met, one subclassification primarily based on clinical characteristics suggests two subtypes: a tremor dominant PD and a non-tremor dominant PD. A patient with tremor dominant PD predominantly lacks other motor symptoms and in general responds better to dopamine replacement therapy. On the other hand, a patient with a non-tremor dominant PD may have an akinetic-rigid syndrome and a postural instability disorder, as well as an increased incidence of non-motor features. The course of the disease and prognosis differs (10), and it has been postulated that the various subtypes have distinct pathogenesis and etiologies (11).

As the disease progresses, motor symptoms worsen over time, with the onset of further complications associated with long-term levodopa therapy. These include non-motor fluctuations, dyskinesias, and psychosis that are more difficult to manage. In an advanced disease stage, both motor and non-motor symptoms may become resistant to current medications. Postural instability and freezing of gait may lead to falls and fractures, while dementia and hallucinations can develop in some patients, which sometimes warrant care home placement.

Non-motor symptoms are common in early PD but also progress and become more challenging to manage. Early non-motor symptoms include impaired olfactory ability, autonomic dysfunction, pain, fatigue, sleep disorders, and cognitive and psychiatric disturbances. They have a significant impact on the patient's quality of life (12). Autonomic symptoms can be difficult to treat with orthostatic hypotension causing significant problems for patients. Urinary incontinence and constipation are common, and dementia occurs in 83% of patients with PD after 20 years of diagnosis (13). These non-motor symptoms contribute significantly to disability and poor quality of life and also strongly predict admission to care homes (14).

ETIOLOGY

PD is a multifactorial disease, with both genetic and environmental factors playing a role. Age is the biggest risk factor for PD, with the median age of onset being 60 years of age (15). The incidence of the disease rises with age to 93.1 (per 100,000 person-years) in age groups between 70 and 79 years (16, 17). Additionally, there are cross-cultural variations, with higher prevalence reported in Europe, North America, and South America compared with African, Asian and Arabic countries (1).

Cigarette smoking

Cigarette smoking has been extensively studied with respect to PD, with mostly consistent results. Most of the epidemiological reports are case-control studies showing a reduced risk of developing PD, with larger cohort studies also in agreement (18–20). A large meta-analysis including 44 case-control studies and 8 cohort studies from 20 countries showed an inverse correlation between smoking and PD, with a pooled relative risk of 0.39 for current smokers (21). Two other meta-analyses also reported an inverse correlation between smoking and PD, with a pooled odds ratio ranging from 0.23 to 0.70, indicating a protective mechanism against PD (22, 23). They also reported an inverse correlation between the number of pack years, the number of years smoking and the risk of PD, with the risk of developing PD being significantly reduced in heavy or long-term smokers compared with nonsmokers (23).

The reasons underlying this associated reduced risk are not fully understood. Activation of nicotinic acetylcholine receptors on dopaminergic neurons by nicotine or selective agonists has been shown to be neuroprotective in experimental models of PD (24, 25). Nevertheless, nicotine can also stimulate the release of dopamine, which is involved in the reward mechanisms; it is therefore difficult to confirm whether smoking prevents PD or whether PD helps prevent the habitual use of cigarettes. As a result of a reduction in dopamine in patients with PD, patients may be less prone to addictive behaviours, and thus less likely to smoke. This hypothesis is supported by the fact that patients with prodromal PD and PD were able to give up smoking much easier than controls, suggesting this association could be due to the decreased responsiveness to nicotine (26).

Caffeine

Several studies have investigated the effect of caffeine on the development of PD and reported a reduced risk of developing PD among coffee drinkers. Caffeine is an adenosine A_{2A} receptor antagonist, which is believed to be protective in PD (27) and has been shown to be neuroprotective in a mouse model of PD (28). It has been previously reported that there is a 25% risk reduction in developing PD among coffee drinkers (14). Two large prospective epidemiological studies (27, 29), as well as multiple retrospective studies (30), have also shown a reduced risk of developing PD with a relative risk ranging from 0.45 to 0.80 in coffee

drinkers versus non-coffee drinkers. A meta-analysis including eight case-control studies and five cohort studies also showed a significantly reduced risk of developing PD in coffee drinkers (RR 0.69) (21). Regular tea drinkers also have been reported to have a lower risk of developing PD (29).

As with smoking, the causative role of caffeine in preventing PD remains to be established. Furthermore, there were differences noted between studies with respect to gender. In two cohort studies (27, 29), there was a strong inverse correlation between coffee and the development of PD in men, whereas in women this association was weaker. Additionally, in post-menopausal women, the effect of caffeine depended on whether the females were taking hormone replacement therapy including estrogens. As estrogen competitively inhibits caffeine metabolism, interactions between estrogen and caffeine may explain in part why PD risk is dependent on hormone replacement therapy in post-menopausal women (31, 32).

Pesticides, herbicides, and heavy metals

In 1983, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was first discovered to be associated with nigrostriatal degeneration when several people developed typical PD signs after injecting themselves with a drug contaminated with MPTP. MPTP is metabolized into the neurotoxin, MPP+ (1-methyl-4-phenylpyridinium), which is a mitochondrial complex-I inhibitor that selectively damages dopaminergic cells in the substantia nigra (32, 33). The identification of MPTP as a cause of nigral degeneration led to the idea that PD could be caused by an environmental toxin. Since then, several studies have shown an association between pesticides and PD, with one case-control study showing an increased association with professional pesticide exposure in men and late-onset PD (odds ratio [OR] 2.2) (34). Paraquat (a herbicide which is structurally very similar to MPP+) (35) and rotenone (a pesticide) are also selective complex-I inhibitors and induce dopaminergic depletion in animal models of PD (36). The relationship between exposure to these chemicals and the risk of developing PD has been investigated in other epidemiological studies (37). It has also led to the study of surrogate markers, including the association of farming, drinking well water, and living in rural areas with PD risk. Welding and heavy metal exposure (e.g., iron, copper, lead, aluminum, and zinc) have also been investigated, but the relationship between these and PD remains inconclusive.

Genetics

Although PD is generally an idiopathic disorder, there is a minority of cases (10–15%) that report a family history, and about 5% have Mendelian inheritance (38). Furthermore, an individual's risk of PD is partially the product of as-yet poorly defined polygenic risk factors. The genes that have been found to potentially cause PD are assigned a "PARK" name in the order they were identified. To date, 23 PARK genes have been linked to PD. Mutations in the PARK genes demonstrate either autosomal dominant (e.g., *SCNA, LRRK2,* and *VPS32*) or autosomal recessive inheritance (e.g., *PRKN, PINK1,* and *DJ-1*) and are summarized in Table 1. The involvement of some of these genes has not been conclusively confirmed

(PARK5, PARK11, PARK13, PARK18, PARK21, and PARK23), while others are considered risk factors (PARK3, PARK10, PARK12, PARK16, and PARK22) (39).

The numerically most important genetic risk factors predisposing to PD are mutations in *GBA1*, a gene encoding β-glucocerebrosidase—a lysosomal enzyme responsible for the hydrolysis of glucocerebrosides (see Chapter 3) (40). *GBA1* mutations are known to cause Gaucher disease, which is the most common lysosomal storage disorder (41). Other genetic risk factors include the major histocompatibility complex, class II (HLA-DQB1) (42) and the gene encoding the protein tau, *MAPT* (43), among others.

Autosomal dominant PD

The first type of familial PD caused by a point mutation in the α-synuclein gene (*SNCA*) was discovered in 1997 (44). Four additional point mutations, as well as gene duplication or triplication, have now been linked to autosomal dominant PD (45–50). However, these mutations are relatively rare. The most frequent autosomal dominant monogenic PD is caused by mutations in the gene encoding leucine-rich repeat kinase 2 (*LRRK2*). Six *LRRK2* mutations have been confirmed as pathogenic (51), the most common of which is p.G2019S, estimated to account for 1% of sporadic and 4% of familial PD worldwide (51). More recent genetic studies have led to the discovery of additional mutations in other genes responsible for autosomal dominant PD, including *VPS35* (Table 1).

Autosomal recessive PD

Autosomal recessive forms of PD typically present with an earlier onset than classical PD. Three of the PARK-designated genes causing autosomal recessive PD have been linked to mitochondrial homeostasis (*PRKN*, *PINK1,* and *DJ-1*). Specifically, the proteins PINK1 and parkin (encoded by the *PRKN* gene) are both involved in the same mitochondrial quality control pathway, with PINK1 recruiting parkin to dysfunctional mitochondria and thus initiating mitophagy (52). Mutations in *PRKN* are the most common cause of autosomal recessive familial PD, occurring in up to 50% of all early-onset cases (39). Finally, several of the autosomal recessive genes have been linked to atypical parkinsonism with variable features (Table 1), including *ATP13A2* (PARK9), *PLA2G6* (PARK14), *FBX07* (PARK17), and *SYNJ1* (PARK20) (53–56).

NEUROPATHOLOGY OF PARKINSON'S DISEASE

Macroscopically, the brain in idiopathic PD is often unremarkable with mild atrophy of the frontal cortex and ventricular dilation in some cases. The main distinctive morphological change in the PD brain is observed in transverse sections of the brainstem, where almost all cases present with loss of the darkly pigmented area in the substantia nigra pars compacta (SNpc) and locus coeruleus. This pigmentation loss directly correlates with the death of dopaminergic (DA) neuromelanin-containing neurons in the SNpc and noradrenergic neurons in the locus coeruleus (71). Cell death in the SNpc is mostly restricted to a specific

TABLE 1 **PARK-designated genes involved in familial Parkinson's disease**

PARK	Gene	OMIM reference	Inheritance	Description	Clinical deatures
PARK1 PARK4	*SNCA* (44,45,57)	168601	AD	α-synuclein	Ranging from classical PD to early-onset cases with dementia, autonomic dysfunction, and rapid progression
PARK2	*PRKN* (58)	600116	AR	parkin RBR E3 ubiquitin protein ligase	Early-onset PD, slow progression, often features of dystonia
PARK5	*UCHL1* (59)	613643	AD	ubiquitin C-terminal hydrolase L1	Classical PD—only one family, findings not since replicated
PARK6	*PINK1* (60)	605909	AR	PTEN-induced putative kinase 1	Early-onset PD, slow progression
PARK7	*DJ-1* (61)	606324	AR	Parkinsonism-associated deglycase	Early-onset PD, slow progression
PARK8	*LRRK2* (62)	607060	AD	Leucine-rich repeat kinase 2	Classical PD with less frequent dementia and slower progression
PARK9	*ATP13A2* (53)	606693	AR	Cation-transporting ATPase 13A2	Early-onset (adolescence), atypical parkinsonism with dementia, spasticity and supranuclear palsy (Kufor–Rakeb syndrome) (63)
PARK11	*GIGYF2* (64)	607688	AD	GRB10 interacting GYF protein 2	Classical PD
PARK13	*HTRA2* (65)	610297	AR	HtrA serine peptidase 2	Classical PD
PARK14	*PLA2G6* (54)	612593	AR	Calcium-independent phospholipase A2 enzyme	Early onset with atypical features (dystonia parkinsonism)
PARK15	*FBX07* (55)	260300	AR	F-box protein 7	Early onset with atypical features (pallido-pyramidal syndrome)
PARK17	*VPS35* (66)	614203	AD	Vacuolar protein sorting-associated protein 35	Classical PD
PARK18	*EIF4G1* (67)	614251	AD	Eukaryotic translation initiation factor 4 gamma 1	Classical PD
PARK19	*DNAJC6* (56)	615528	AR	HSP40 Auxilin	Early-onset PD, slow progression
PARK20	*SYNJ1* (68)	615530	AR	Synaptojanin 1	Parkinsonism with dystonia and cognitive decline
PARK21	*DNAJC13* (69)	616361	AD	Receptor-mediated endocytosis 8 (RME-8)	Classical PD
PARK23	*VPS13C* (70)	616840	AR	Vacuolar protein sorting-associated protein 13C	Early-onset PD, rapid progression

OMIM: Online Mendelian Inheritance in Man database, AD: autosomal dominant, AR: autosomal recessive. PARK3 PARK10, PARK12, PARK16, and PARK22 are considered risk factors or the genes have not been identified yet and are not included in this table.

group of neuromelanin-containing dopaminergic neurons, namely the A9 neurons, while other neuronal and glial cell types are largely spared (Figure 1).

Quantitative morphometric studies in postmortem PD brains have calculated approximately 30% loss of DA neurons in the SNpc by motor symptom onset, adjusting for age (8, 72–75). After the motor symptoms appear, nigral DA neuron loss increases up to 60% or higher and strongly correlates with the severity of motor features and disease duration (8, 76, 77). The result of this remarkable cell loss is the denervation of the nigrostriatal pathway, leading to diminished dopamine levels in the striatum. The reduction of dopaminergic signaling is considered responsible for the appearance of the cardinal motor symptoms in PD. Recent work has shown that nerve cell death in the SNpc is preceded by the loss of axon terminals projecting to the striatum (77). Mechanistically, the early neuron and axon terminal loss observed in PD suggests a substantial preclinical stage that predates the onset of symptoms by several years.

Apart from the SNpc, widespread cell loss can be found in several subcortical nuclei, including the locus coereleus, the nucleus basalis of Meynert, the dorsal

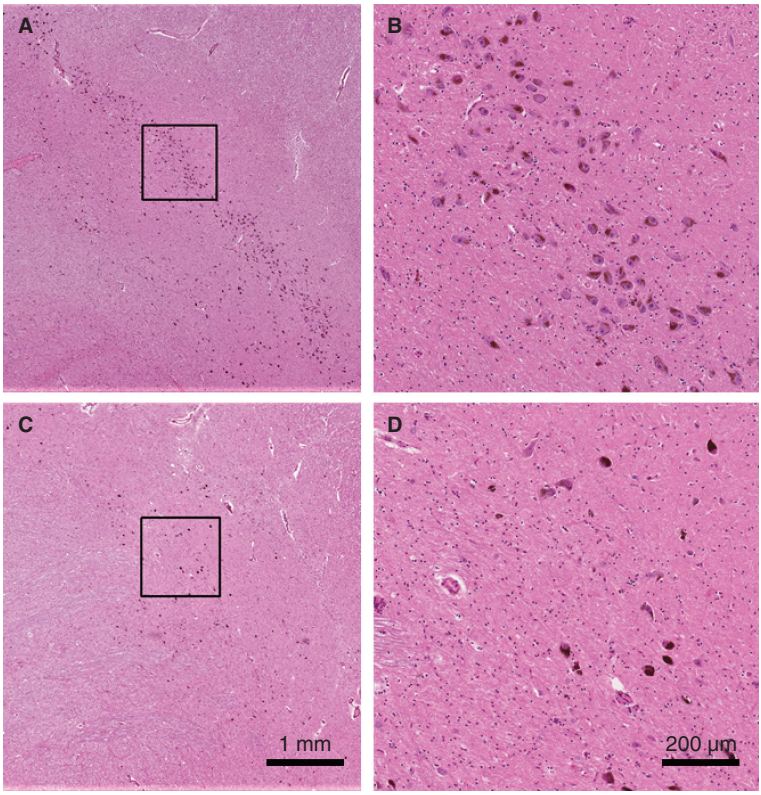

Figure 1 Coronal section at the level of the substantia nigra pars compacta (SNpc) in a control (A and B) and a PD brain (C and D) stained by hematoxylin and eosin. In both sections, the dark brown cells are the neuromelanin-containing dopaminergic (DA) neurons. Dopaminergic cell loss is evident in the SNpc of the PD brain. The squared areas in A and C are magnified in B and D, respectively, to show a closer view of the darkly pigmented DA neurons.

motor nucleus of the vagus nerve, the pedunculopontine nucleus, the raphe nuclei, and also the hypothalamus and the olfactory bulb (76). Multiple non-dopaminergic neurotransmitter systems are affected, such as the cholinergic, adenosinergic, glutamatergic, GABAergic, noradrenergic, serotonergic, and histaminergic (78). Degeneration in those systems is thought to account for some of the non-motor symptoms of PD that do not respond well to dopamine replacement therapies (79). However, the precise pathological mechanisms underlying the non-motor symptoms in PD are still relatively unclear.

Lewy body pathology

Microscopically, the pathological hallmark of PD is the presence of abnormal cytoplasmic deposits within neuronal cell bodies which are immunoreactive for the protein α-synuclein. These pathological protein aggregates are called Lewy bodies (LBs) and are often accompanied by dystrophic neurites (Lewy neurites), which are mostly axonal (80) (Figure 2A–2C).

LBs are intracytoplasmic inclusions consisting of a granular and fibrillar core with a surrounding halo (Figure 2A and 2B). The size of an LB can vary from 5 to 30 μm in diameter, and more than one LB can be found inside a single neuron (81). Two LB types have been described in the literature: classical brainstem

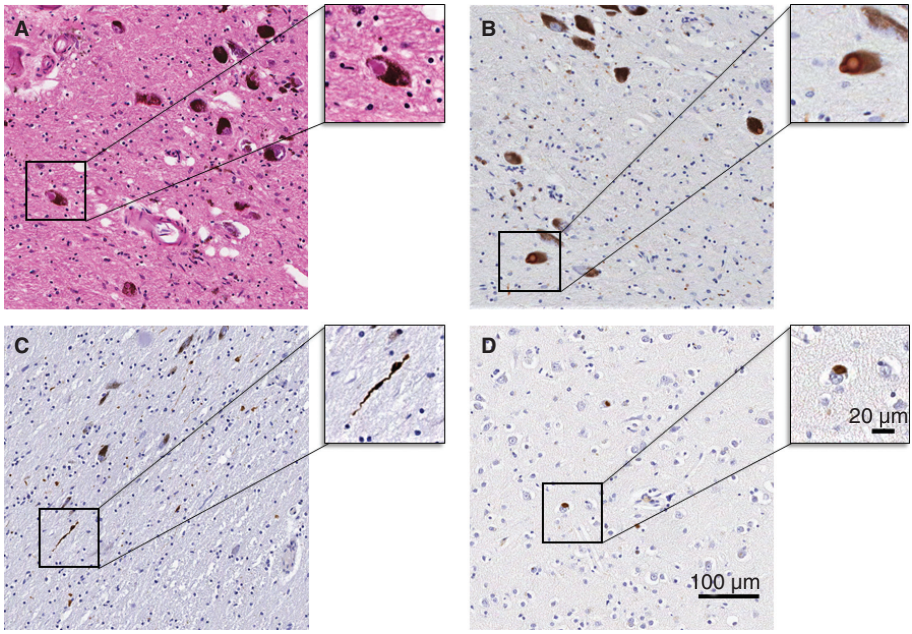

Figure 2 Examples of Lewy-pathology in the SNpc (A–C) and the prefrontal cortex (D) in coronal sections of a PD brain. (A) Typical brainstem Lewy body inside a neuromelanin-containing DA neuron in routine hematoxylin and eosin histological staining. Lewy neurites are not visible in this type of histological preparation. (B) Typical brainstem Lewy body with the characteristic halo, visualized by α-synuclein immunohistochemistry, a much more sensitive method that can also reveal dystrophic Lewy neurites as seen in (C). (D) Cortical Lewy body, less well defined and without a halo.

and cortical LBs (Figure 2B and 2D). Morphologically, the main difference is that cortical LBs have less distinct outlines, are usually smaller, and lack the halo. In the SN, structures that resemble cortical LBs are sometimes called "pale bodies" and are considered LB precursors.

The primary structural component of LBs is filamentous α-synuclein (80), a protein ubiquitously expressed in the brain. In PD and other synucleinopathies, it acquires an amyloid-like filamentous structure and becomes abnormally phosphorylated and aggregated. The halo of an LB is primarily made up of α-synuclein (82). Apart from α-synuclein, the molecular components of an LB include a number of proteins, such as ubiquitin, tau, parkin, heat shock proteins (HSPs), oxidized/nitrated proteins, cytoskeletal proteins (such as neurofilaments, MAPs, and tubulin), proteasomal and lysosomal elements, and others (83).

Braak staging

The main staging system of PD pathology was introduced in 2003 by Braak and colleagues. This was based on the semiquantitative assessment of LB distribution, at postmortem, in a large autopsy series. This work revealed that LB pathology spreads rostrocaudally throughout the brain, in a chronologically predictable sequence (84). At Braak stages 1 and 2, LB lesions are mainly observed in the dorsal motor nucleus (IX/X), the reticular formation, and the anterior olfactory nucleus. At these stages, patients are considered asymptomatic or presymptomatic, although they may present with some early non-motor features, mainly autonomic (e.g., constipation), olfactory, and sleep-related dysfunctions (85, 86). As the disease progresses (Stage 3), the SNpc becomes involved, with LB pathology and neuronal loss being observed in melanized neurons. At this stage, the pathology also extends to the locus coeruleus and the amygdala, subsequently reaching the temporal limbic cortex (transentorhinal region) at Stage 4. During stages 3 and 4, the typical clinical motor features begin to manifest. Finally, during stages 5 and 6, the key feature is the involvement of the entire neocortex and high-order areas, including the prefrontal cortex and primary sensory and motor areas (84, 87). Clinically, this is thought to translate to severe PD with significant gait problems and dementia. The Braak hypothesis was later revised to propose that α-synuclein-associated pathology may in fact be initiated in nasal and intestinal mucosal sites, specifically in the olfactory bulb and the enteric cell plexuses ("dual-hit hypothesis") (88).

Since its introduction in 2003, the Braak staging system has been a subject of controversy. Subsequent studies have shown that a proportion of PD brains do not appear to match this pattern (89, 90), while attempts to correlate Braak staging with clinical dysfunction were also unsuccessful (91). Another criticism of the Braak system is that it is based not on neuronal loss but on the distribution of Lewy-related pathology (92).

α-synuclein and Lewy body distribution outside the brain

Phosphorylated α-synuclein histopathology has also been observed outside the brain. Specifically, it is found in the spinal cord and cervical and thoracic sympathetic ganglia (93). Furthermore, α-synuclein deposition is observed in

several peripheral organs, including the retina, the uterus, the bladder, the skin, parts of the cardiovascular system (predominantly in the aorta and heart ventricles), and the gastrointestinal system, particularly in the submandibular gland, stomach, and the bowels (94, 95). This points to a significant involvement of the peripheral nervous system in PD and raises the question of whether α-synuclein pathology originates in the brain or in the periphery. An epidemiological study from Denmark has revealed that a full truncal vagotomy is associated with a reduced risk of subsequent PD (96), leading to recent interest in the possible role of the gut–brain axis in the pathogenesis of PD (97).

Interaction of α-synuclein with other proteins

Protein misfolding within particular brain areas is a shared feature among many neurodegenerative diseases, such as AD and PD. Therefore, an umbrella term often used for these disorders is "proteinopathy." The type of protein and the characteristic distribution of the pathology is the significant attribute that defines each proteinopathy. Nevertheless, it is now becoming increasingly clear that there is often overlap between the different diseases and an interaction between the pathogenic, misfolded forms of proteins (98). One factor contributing to this phenomenon might be aging, and it is well established that abnormal protein accumulation can occur with age in the absence of neurodegenerative disease (99). Accumulating evidence now shows that within the context of PD there is a clear cross talk between different aggregated forms of proteins with distinct molecular pathways.

One such protein is tau, encoded by the *MAPT* gene. In pathological situations, tau can become abnormally hyperphosphorylated forming intracytoplasmic inclusions, called neurofibrillary tau tangles (NFTs). These aggregates are characteristic of AD, together with amyloid-β plaques. However, abnormal tau protein has been linked to PD as well. Specifically, postmortem studies have revealed a significant increase of tau hyperphosphorylation at Ser262 and Ser396/404 in the striatum of patients with PD and PD dementia (100). Animal studies have further added to this by showing that increased α-synuclein expression can trigger tau hyperphosphorylation both *in vitro* and *in vivo* (101, 102). Furthermore, genome-wide association studies found a strong link between *MAPT* and the risk of PD (43), and subsequent longitudinal work showed that the H1/H1 haplotype of *MAPT* is a strong predictor of early development of PD dementia (103).

Amyloid-β has also been reported to act together with α-synuclein. Cortical deposition of α-synuclein has been associated with amyloid-β plaque formation in a subgroup of PD patients (104). Furthermore, both NFTs and amyloid-β senile plaques are widespread at postmortem in some, though not all, PD patients who develop cognitive dysfunction and dementia (105–108). Current literature seems to suggest that the manifestation of dementia in PD may be due to the convergence of both PD and AD pathology in the cortex, and that a combination of these pathologies is a better correlate of PD dementia (107).

PATHOGENESIS OF PARKINSON'S DISEASE

A number of mechanisms have been implicated in PD pathogenesis, with α -synuclein aggregation central to the development of the disease. Multiple other processes are thought to be involved, with several studies suggesting that abnormal protein clearance, mitochondrial dysfunction, and neuroinflammation play a role in the onset and progression of PD. However, the relationship between these pathways remains unclear.

α-synuclein misfolding and aggregation

Native α-synuclein in the brain is mostly unfolded without a defined tertiary structure (109), although in aqueous solutions it can be present in stable tetramers that resist aggregation (110). Upon interaction with negatively charged lipids, such as the phospholipids that make up cell membranes, α-synuclein folds into α-helical structures through its N-terminal (111). In PD, α-synuclein adopts a β-sheet-rich amyloid-like structure that is prone to aggregate. Indeed, misfolded α-synuclein is found within LBs as 5–10 nm long filaments. Several mechanisms have been proposed for the conformational changes that lead to abnormal α-synuclein aggregation, including serine 129 phosphorylation, ubiquitination, and C-terminal truncation (112, 113). Hence, different species of α-synuclein are found in the PD brain, including unfolded monomers, soluble oligomers, protofibrils, and high molecular weight insoluble fibrils (114).

Recent studies in rodents indicated that the most neurotoxic α-synuclein species is the early oligomeric form, rather than the mature insoluble fibrils (115, 116). The increased toxicity of these oligomers, as opposed to the fibrillary α-synuclein, was validated in cell-based assays (115). The oligomeric species of α-synuclein are capable of "seeding" and accelerating abnormal protein aggregation and Danzer et al. (2011) proposed that this might be the mechanism underlying the spread of α-synuclein pathology in the brain (117).

Mitochondrial dysfunction

Mitochondrial dysfunction is considered a key element in the pathogenesis of both idiopathic and familial PD (118). Early postmortem studies in the SNpc of PD brains reported a deficiency of the mitochondrial complex-I, which is a vital component of the electron transport chain. These data provided one of the first direct links between mitochondrial dysfunction and PD (119). Complex-I deficiency was also found in skeletal muscle and platelets of PD patients compared to healthy subjects (120, 121). Further evidence arose by the discovery that abuse of the substance MPTP caused permanent Parkinsonian symptoms (34), with postmortem examination revealing dopaminergic cell loss (122). Follow-up studies showed that MPTP when oxidized is taken up by DA neurons and leads to complex-I inhibition (123). Other toxins and pesticides that impair mitochondrial complex-I activity, like rotenone and paraquat, also cause a Parkinsonian phenotype and DA cell loss in animals, and potentially in humans (124). Defects in the mitochondrial complex-I may be crucial in driving DA cell death due to energy depletion (118).

Another major clue pointing to the role of mitochondria in PD pathogenesis is that many of the known genes that cause familial PD play a role in

mitochondrial homeostasis. One example is the involvement of PINK1 and parkin (PARK2 and PARK6, respectively), both of which are vital components of the pathway that regulates the removal of dysfunctional mitochondria, a process called mitophagy (52). Loss-of-function mutations in either gene lead to impaired mitochondrial quality control and cause autosomal recessive PD (58, 125).

Finally, α-synuclein by itself is known to interfere with mitochondrial function. For instance, α-synuclein can interact with the mitochondrial membrane and accumulate inside the organelles. This leads to the damage of complex-I activity, ultimately resulting in mitochondrial dysfunction and increased oxidative stress (126, 127). A more recent study reported an interaction between oligomeric (but not monomeric or fibrillar) α-synuclein and the mitochondrial receptor TOM20 (128). This interaction resulted in impairment of the mitochondrial protein import machinery, reduced respiration, and led to excessive production of reactive oxygen species (ROS).

Dysfunctional protein clearance systems

There are two central protein clearance systems within cells responsible for the removal of dysfunctional proteins: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway. The UPS is primarily responsible for breaking down abnormal proteins, and it does so by "tagging" them with ubiquitin and transporting them to the proteasome for degradation. The autophagy-lysosome pathway is divided into three constituents: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Briefly, in macroautophagy, intracellular components, including cytosolic proteins, are engulfed by the autophagosome, which then fuses with the lysosome, leading to the breakdown of its contents. On the other hand, in microautophagy, the lysosome alone engulfs and destroys cytoplasmic components. CMA is a more selective process, whereby molecular chaperones target specific proteins and transport them to the lysosome for degradation (129). Monomeric α-synuclein is generally cleared by both the UPS and the autophagy-lysosome pathway (130), and damage in either of their machineries is implicated in the pathogenesis of PD by contributing to the accumulation of defective proteins, in particular soluble misfolded α-synuclein (131, 132).

Ubiquitin-proteasome system

Proteasomal abnormalities are a shared feature among many proteinopathies, that is, neurodegenerative diseases characterized by abnormal protein accumulation (133). Evidence of such abnormalities in PD was first provided by postmortem studies in the SNpc, where the catalytic activity of the UPS was found substantially reduced compared to healthy brains (134). The same findings were later reported in peripheral blood mononuclear cells of PD but not in healthy individuals (135). Apart from diminished activity, a lower expression of different proteasomal components has also been identified in the SNpc of PD brains. Specifically, the 20S proteasome α-subunit (136) and other molecules involved in the normal function of the UPS, like PA700 and PA28 (proteasome activators), are reduced (137). Additional evidence is provided from genetic studies and the discovery that two of the PARK genes linked to monogenic PD encode proteins

involved in UPS function, namely, parkin (PARK2; E3 ubiquitin ligase) (58, 138) and UCH-L1 (PARK5; Ubiquitin C-terminal hydrolase) (59).

Following on from findings in human PD, altered proteasome activity was observed in different disease models. Marmosets injected with the toxin MPTP had diminished enzyme activity in the UPS, in addition to decreased levels of the 26S subunit components (139). In a second set of experiments, the same group showed that pharmacological inhibition of the proteasome in wild-type rats leads to dopaminergic cell death (140). Similarly, Bedford and colleagues using transgenic mice with proteasomal defects (knockout for 26S proteasome regulatory subunit 4) showed dopaminergic cell degeneration and observed LB-like inclusions in the brain, which however lacked the dense core of classical human LBs, and it is unclear whether they contained aggregated α-synuclein (141). Nevertheless, all these studies show that dysfunction of protein turnover can result in neuronal cell death, thus providing a potential pathogenic mechanism for PD.

Autophagy- lysosome system

Similar to findings in the UPS system, numerous lysosomal and autophagy-related components are malfunctioning or differentially expressed in PD. In nigral neurons of PD brains, the levels of the autophagosome marker LC3-II were increased, suggesting an accumulation of autophagic vacuoles (142, 143). In contrast, vital proteins of lysosomal membranes (LAMP1 and LAMP2A), and several molecular chaperones from the heat-shock protein family (such as hsc70 and hsp35) were found to be decreased at postmortem examination (144, 145). Furthermore, of particular note is the discovery of a point mutation in the gene of the lysosomal protein ATP13A2 (PARK9), leading to an autosomal recessive atypical Parkinsonian syndrome, referred to as Kufor–Rakeb syndrome (63). Point mutations in two more PARK genes impair the function of either parkin (PARK2) (58) or PINK1 (PARK6) (60), both of which are involved in the autophagic turnover of mitochondria (52). Additionally, the emergence of *GBA1* mutations, which result in dysfunction of the lysosome-autophagy system, as a strong genetic risk factor for PD adds weight to the idea that this system is important in the development of PD (see Chapter 3). These studies lend support to the hypothesis that malfunction in the autophagy-lysosome pathway may be contributing to the pathogenesis of PD.

Neuroinflammation

Postmortem brain studies have described microglial and complement activation, T-lymphocyte infiltration, and increased concentration of pro-inflammatory cytokines in the SNpc and striatum of PD patients compared to healthy individuals (146–149). Furthermore, positron emission tomography (PET) neuroimaging with the [^{11}C]-PK11195 radioligand has demonstrated increased microglial activation early on in PD in the brainstem, basal ganglia, and frontotemporal cortices, with added involvement of the parietal and occipital cortices in patients with PD dementia, compared to healthy subjects (150, 151).

While initially thought to be a secondary phenomenon, there is now evidence that inflammatory responses can by themselves contribute to disease pathogenesis. It has been demonstrated in early studies with rodent models of PD (6-hydroxydopamine and MPTP) that inhibition of microglial activation with

minocycline pre- and post-neurotoxic insult led to a significant attenuation of DA cell death in the SNpc, suggesting that microglia-induced inflammatory processes may be contributing to the degeneration of these cells (152, 153). There is also a plethora of evidence suggesting that α-synuclein can directly trigger microglial activation and initiate inflammatory processes. For instance, in primary cultures, α-synuclein mediates a dose-dependent activation of microglia (154).

Genetic clues suggesting that immune activation might contribute etiologically in PD come from the identification of a strong association between the human leucocyte antigen (HLA) class II region (a key molecule of the immune system) and the risk of developing PD (155)—a finding that was later confirmed in genome-wide association studies (42). Additionally, extensive epidemiological studies suggest a decreased PD risk with regular use of the nonsteroidal anti-inflammatory drug ibuprofen (156). Finally, recent data showed that in PD patients at diagnosis a more 'pro-inflammatory' immune marker profile in the serum is associated with a faster motor symptom progression and more impaired cognitive function (157).

Regardless of whether neuroinflammatory responses are a direct trigger of neurodegeneration in PD or are activated as a response to neuronal damage, it is now becoming clear that the engagement of the immune system can initiate a vicious cycle, thereby exacerbating neuronal dysfunction. Hence, manipulation of the immune system remains a promising topic for disease-modifying therapies.

CONCLUSION

PD is a complex neurodegenerative condition, for which the etiology and pathogenic mechanisms remain incompletely understood. While a small proportion of PD patients have a monogenic cause for their disease, the majority of cases probably are not associated with a specific genetic abnormality. Instead, it is likely that the risk of PD is in part, determined by a combination of polygenic susceptibility factors. Environmental influences may also contribute to PD risk, although the relationship between the development of the disease and factors such as smoking, caffeine, and pesticide exposure continues to be poorly understood. Pathologically, the movement disorder occurs due to loss of dopaminergic neurons in the SNpc, with a number of other brain regions also being involved. The histopathological hallmark of PD are LBs, which predominantly contain aggregated α-synuclein, but it is not clear how these may result in neurodegeneration. Understanding these pathogenic processes can allow for the identification of novel therapeutic targets, and, hopefully, the development of disease-modifying treatments in the future.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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2 Immunogenetics of Parkinson's Disease

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Abstract: Inflammation is a key feature of Parkinson's disease (PD). In postmortem PD brains, microglial activation and enhanced major histocompatibility class II (MHCII) expression are seen concomitant to the accumulation of alpha-synuclein (α-synuclein) and loss of dopaminergic cells in the substantia nigra. Recent findings showed that α-synuclein epitopes can be presented and recognized by T-cells. PD is not a single disorder; rather, it encompasses a range of clinical, epidemiological, and genetic subtypes. Around 10% of the cases have a monogenic origin, and several of the disease-causing mutations are linked to inflammatory processes. The remaining 90% of the cases are complex, where environmental and genetic risk factors synergize to induce PD pathology. To date, 41 genetic loci have been identified in genome-wide association studies as associated with PD risk, and among these, two are within the HLA region, coding for immune genes including MHCII. Thus, genetic and immune findings indicate that the immune system has a role in the etiology of PD. Experimentally, inflammatory stimuli can cause selective nigral cell loss in preclinical models of PD, and MHCII is required to elicit α-synuclein-induced pathology in mice. In this chapter, we focus on immunogenetics, that is, the relation between genetic risk factors and immune processes in PD.

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INTRODUCTION

Parkinson's disease (PD) is an increasingly prevalent and progressively disabling neurodegenerative disease that encompasses a range of clinical, epidemiological, and genetic subtypes (1). The high inter-individual variation in onset, progression, and symptoms is in part due to a complex interplay between genes and environment. According to the latest criteria by the International Parkinson and Movement Disorders Society, PD diagnosis should be based on the presence of general bradykinesia in combination with either rest tremor, rigidity or both (2). Neuropathologically, PD is characterized by loss of nigral dopaminergic neurons that innervate the striatum and pathological accumulation of α-synuclein in Lewy bodies and Lewy neurites (3). In addition to the neurodegenerative phenotype, local neuroinflammation is a hallmark of PD and includes activation of microglia and astrocytes as well as an upregulation of major histocompatibility class II (MHCII) molecules. The inflammatory activation in PD is not only confined to the brain but also involves the peripheral immune system. One example is the increased expression of inflammatory molecules both in the central (4) and peripheral nervous systems (5). At a cellular level, there is an increased infiltration of immune cells into the brain parenchyma and an altered peripheral leukocyte profile in PD (6). The finding that α-synuclein epitopes can be recognized by T-lymphocytes (7) further strengthens the notion that PD is an inflammatory disease, with both innate and adaptive immune responses. Although these findings strongly link inflammation to PD, they do not answer whether inflammation is a cause or consequence of the disease. However, the recent advances in genetic analyses of familial and idiopathic PD strongly support inflammatory processes to play a critical role in disease etiology.

ETIOLOGY OF PARKINSON'S DISEASE

Genetic studies of familial PD have led to the identification of disease-causing mutations in single genes, that is, monogenic forms of PD. Mutations that have been causatively linked to PD are located to the genes encoding α-synuclein (SNCA), leucine-rich repeat kinase 2 (LRRK2), vacuolar protein sorting-associated protein 35 (VPS-35), parkin (PARK2), PTEN-induced putative kinase 1 (PINK1), DJ1 (PARK7), and glucocerebrosidase (GBA) (8) (Figure 1). Although mutations in these genes are rare and only account for <10% of all PD cases (9), they have identified key molecular players and processes in PD etiology. This can be illustrated by SNCA, which is both neuropathologically and genetically linked to PD. Lewy bodies and Lewy neurites containing α-synuclein accumulations are present in both familial and idiopathic PD, and in addition to SNCA mutations and copy number variations (CNVs) linked to dominantly inherited monogenic PD (10), common genetic variants in SNCA are associated with increased risk of developing idiopathic PD (11).

In 90% of PD patients, there is no monogenic inheritance pattern, and the disease is determined as idiopathic. Idiopathic PD is sometimes referred to as sporadic but has a multifactorial etiology, where environmental and genetic factors interact, synergize, and together determine an individual's susceptibility to disease. The genetics of idiopathic PD is therefore complex, similar to many other common conditions like Alzheimer's disease, diabetes, and different forms of cancer. In the quest to understand the etiology of idiopathic PD, efforts have been made to identify genetic variants associated with disease risk. These variants include single nucleotide polymorphisms (SNP; the change of a single base pair) and structural variants (microsatellites, minisatellites, insertions, deletions) that, depending on their frequency in the population, are defined as polymorphisms (>1%) or mutations (<1%). Genome-wide association studies (GWAS) are based on the genetic association analysis of SNPs covering the entire genome. Due to the large number of SNPs examined, the analysis is unbiased, but requires large sample sizes. Meta-analyses of several different GWAS have identified 41 PD risk loci (12, 13), each representing common genetic variants conferring an increased risk of developing PD (Figure 1).

In 1983, exposure to the heroin side product 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was identified as the causative agent for severe irreversible parkinsonism in humans and primates (14). MPTP crosses the blood–brain barrier and is converted to MPP+, which accumulates in dopaminergic neurons through dopamine transporters. MPP+ inhibits complex 1 of the electron transport chain, leading to impaired mitochondria and loss of nigral dopaminergic neurons. Although MPTP is not present as an environmental hazard, it pointed out the potential of the molecule to induce parkinsonism and increased interest in environmental risk factors for PD. Exposure to pesticides such as rotenone, paraquat, organophosphates, and pyrethroids has been associated with increased risk of PD in several case-control studies (15). The mechanisms behind the risk increments are not completely understood but, like MPTP, rotenone and paraquat are thought to induce dopaminergic degeneration through oxidative stress and damage. Rotenone acts on complex 1 of the respiratory chain, while paraquat triggers a redox cycle that generates toxic superoxide free radicals.

Environmental and genetic factors are now considered to act in a synergistic manner and modify the risk for idiopathic PD. One such example is genetic variations in glutathione transferase genes which modify the risk conferred by paraquat exposure (16). The risk of PD has also been reported to be increased by head trauma (17) and recurrent CNS infections (18), while moderate amounts of nicotine, caffeine consumption, and the use of non-steroid anti-inflammatory drugs have been reported to reduce the risk for PD (19). Thus, genetic and environmental factors act together to modify PD risk.

IMMUNOGENETICS OF MONOGENIC PD

Advances in the genomics field have generated unprecedented opportunities to define the genetic basis of complex diseases. By applying a genetic strategy,

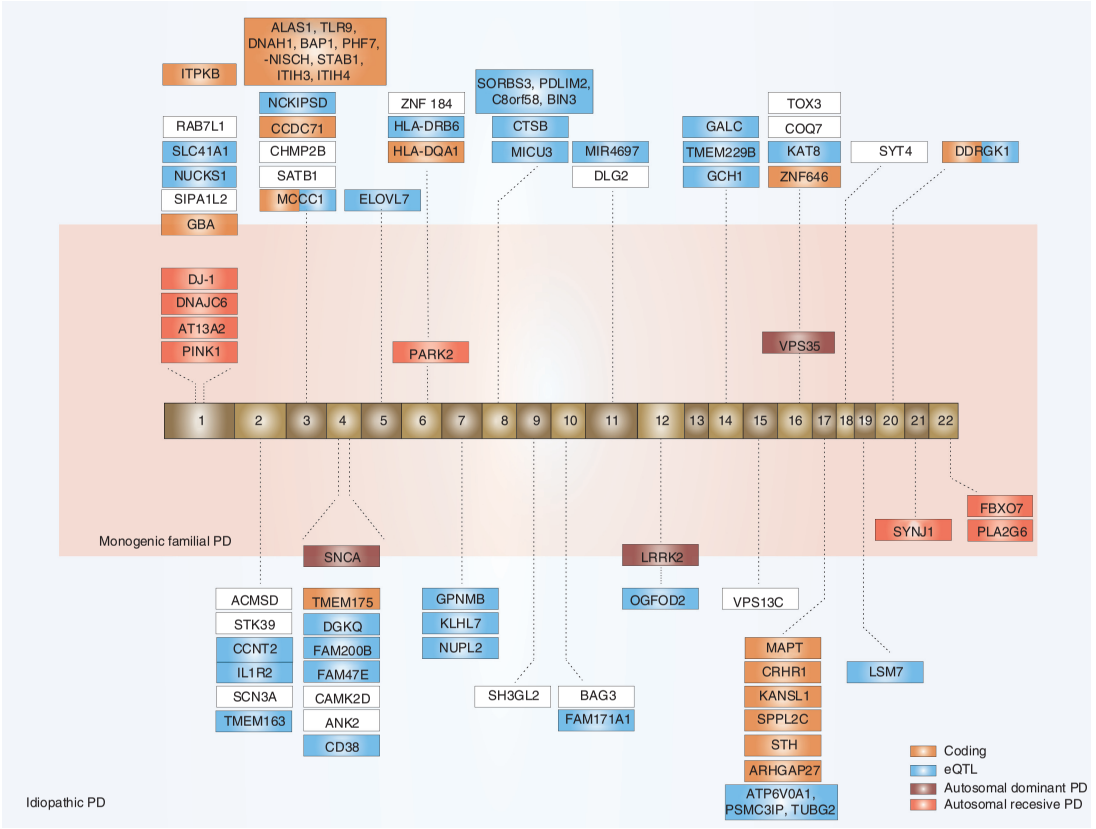

Figure 1 **Insights into the genetics of Parkinson's disease (PD).** The largest meta-analyses until now have identified 41 PD-risk loci (12, 13). Candidate genes are annotated for each region that has been significantly associated with PD. For some of the regions, there is more than one candidate gene.

one can discriminate between causes and consequences of a disease. Furthermore, in situations where a specific gene variant affects the response to therapy, knowledge about an individual's genotype can inform clinical decisions. Immunogenetics specifically studies the relationship between genetics and the immune system, that is, how genetic variants contribute to the inter-individual variation in immune responses.

Autosomal dominant forms

Interestingly, many of the genetic variants linked to monogenic PD also play a critical role in modulating inflammatory responses. Mutations in LRRK2 account for 1–2% of all PD cases (20, 21), but the prevalence varies substantially depending on the population studied. The penetrance is not complete, meaning environmental factors and/or other genes can modulate the disease-causing effect of LRRK2 mutations. LRRK2 encodes a large protein with multiple functions and has a moderate homology to the receptor-interacting protein kinases, a family of kinases with a known role in immunity. Expression of LRRK2 in microglia is induced by pro-inflammatory stimuli and affects microglial activation (22). LRRK2 is also expressed in many other tissues (23), including peripheral immune cells, where its expression is increased by inflammatory mediators such as interferon-γ (IFNγ) and lipopolysaccharide (LPS) (24, 25). Variants at the LRRK2 locus have been reported to confer increased risk of Crohn's disease (26) and leprosy (27). In idiopathic PD patients, the expression of LRRK2 in B-lymphocytes, T-lymphocytes, and monocytes is increased compared to controls and is positively correlated with cytokine expression in T-lymphocytes (28). LRRK2 is thus strongly linked to immune processes in the CNS and periphery and is a promising therapeutic target for both monogenic and idiopathic PD.

Mutations in the SNCA gene and the presence of the encoded protein, α-synuclein, in Lewy bodies were described in 1997 (29, 30), revealing the functional link between α-synuclein and PD. α-synuclein is a nuclear and presynaptic protein, and its overexpression and aggregation within neuron somas and neurites precedes neurodegeneration of dopaminergic cells. Several animal models have been developed that overexpress human α-synuclein in dopaminergic neurons (31), leading to α-synuclein accumulation, dopaminergic neurodegeneration, and microglial activation (32–34). Human macrophages upregulate α-synuclein after LPS stimulation (35), while microglia from mice lacking α-synuclein present a highly activated phenotype in terms of cytokine profile and morphology (36, 37). α-synuclein is a ligand for toll-like receptor 2 (TLR2) on microglia (38), linking α-synuclein to the innate immune system. TLR2 is also present on T-lymphocytes, B-lymphocytes, monocytes, and macrophages, cells that are part of the adaptive immune system. Recently, it was reported that α-synuclein epitopes can be presented on MHC molecules and activate both helper and cytotoxic T-lymphocytes (7). α-synuclein can thus elicit both innate and adaptive immune responses.

Autosomal recessive forms

Parkin, PINK1, and DJ-1 are linked to autosomal juvenile recessive parkinsonism. These three genes are involved in mitochondrial function and oxidative stress

and are also coupled to immune responses. Although loss-of-function mutations in the parkin gene (encoding an E3 ubiquitin ligase) cause early loss of dopaminergic neurons in patients, parkin-deficient mice do not display nigrostriatal pathway degeneration unless they are challenged with low dose of LPS (39). The need of an inflammatory stimulus suggests that the loss of parkin function increases the vulnerability of nigral dopaminergic neurons to inflammation-related degeneration or vice versa. Gene expression profiling in PINK1-deficient mice showed that loss of PINK1 altered the expression of immunomodulatory genes in the striatum (40). In addition, systemic LPS treatment induced higher levels of the pro-inflammatory cytokines interleukin (IL)-1β, IL-12, and tumor necrosis factor α (TNFα) in brain homogenates from PINK1-deficient mice compared to wild-type mice. DJ-1 is implicated in mitochondrial function as a regulator of oxidative stress rather than mitophagy (41). In the human brain, DJ-1 is mostly expressed by astrocytes (42), and astrocytes from DJ-1-deficient mice display an augmented response to LPS and produce more inflammatory cytokines such as IL-6, possibly via increased activation of MAPK p38 and JNK (43). Loss-of-function of parkin, PINK1, and DJ-1 thus seem to increase the sensitivity of dopaminergic neurons to degeneration through oxidative stress and pro-inflammatory immune responses.

IMMUNOGENETICS OF IDIOPATHIC PD

The availability of high-throughput technologies has allowed genotyping of hundreds of thousands to millions of SNPs in the human genome in a cost and time-efficient manner. These technological advancements allow large-scale GWAS, which identify associations between genetic variants and a particular trait or disease. In case-control studies, SNP allele frequencies are compared between patients and controls (44). To date, meta-analyses of GWAS have identified 41 risk loci associated with PD (12, 13). One of the challenges of association studies is the identification of the casual variants, which are most likely genetic variants in linkage disequilibrium (LD) with genotyped SNPs. The consequence of LD, that genetic variants located on the same chromosome have a distance-dependent likelihood of a recombination event during meiosis, is that closely located variants often are inherited together. In addition, associated SNPs can be attributed to different candidate genes and biological function depending on the genetic map used and the availability of gene expression data. Most PD-associated variants confer relatively small risk increments, and the majority are found in non-coding regions regulating gene expression. Such variants are also known as expression quantitative trait loci (eQTLs) and can regulate the expression of multiple genes. Allele-dependent expression of immune-related genes has been reported for eQTLs near or in SNCA, LRRK2, HLA-DQB1, and MAPT (45), with antigen presentation being the most enriched regulated process. Below, we discuss the immune functions of HLA (Figure 2) and other risk loci identified for idiopathic PD (Figure 3) in two GWAS meta-analyses (12, 13).

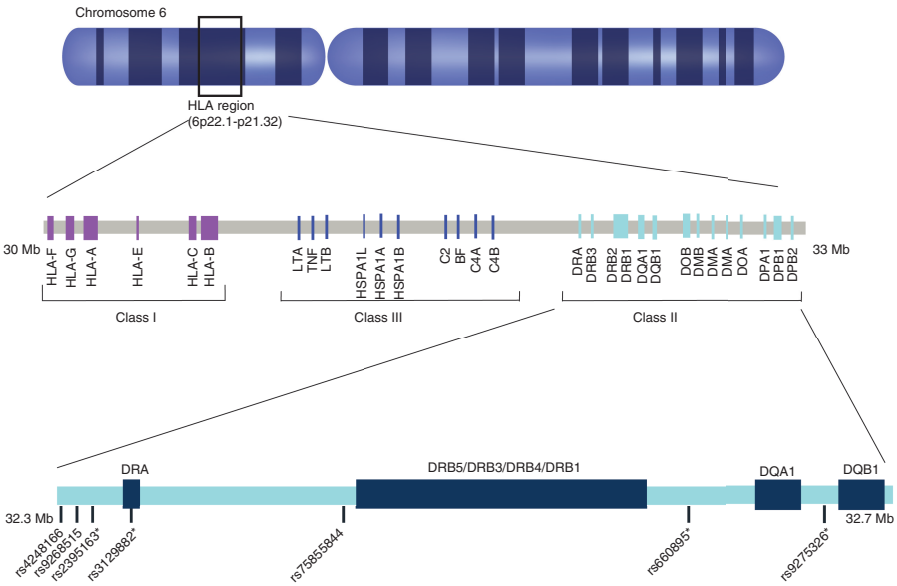

Figure 2 Single nucleotide polymorphisms (SNPs) in the human leukocyte antigen (HLA) locus associated with increased risk for Parkinson's disease (PD). Map of HLA class I, II, and III regions indicating alleles and SNPs associated with PD. An asterisk (*) denotes that the SNP is acting as an expression quantitative trait locus (eQTL). (Adapted from Ref. 85).

Antigen presentation

From the 41 risk loci identified by GWAS, two are within the human leukocyte antigen (HLA) region. HLA is one of the most polymorphic regions in the human genome and presents a complex combination of alleles in high LD (Figure 2). HLA class I and class II genes encode MHCI and MHCII molecules that present antigens to CD8+ and CD4+ T-lymphocytes, respectively, and thereby regulate adaptive immune responses. Different HLA alleles encode MHC molecules with different antigen-binding affinity and are associated with numerous disorders, including autoimmune diabetes and rheumatoid arthritis (46). A combined GWAS of PD with type 1 diabetes, Crohn's disease, ulcerative colitis, rheumatoid arthritis, celiac disease, psoriasis, and multiple sclerosis identified 17 loci shared between PD and these autoimmune disorders (47). Most of the PD risk alleles, including HLA-DQB1, HLA-DRB5, MAPT, and LRRK2, also increased the risk for the autoimmune disorders. Others, including BOLA2, SETD1A, CXCR4, IL12A, and GAK, had opposite effects. The identification of common genetic pathways for PD and autoimmune disorders further strengthens the importance of immunogenetics and immune therapy in PD.

Several studies have found association between SNPs and alleles in the HLA class II region and PD. These are summarized in Tables 1 and 2 and outlined in Figure 2. Using a GWAS approach, Hamza et al. reported a non-coding variant in HLA-DRA (rs3129882) associated with late-onset PD (48). This variant has

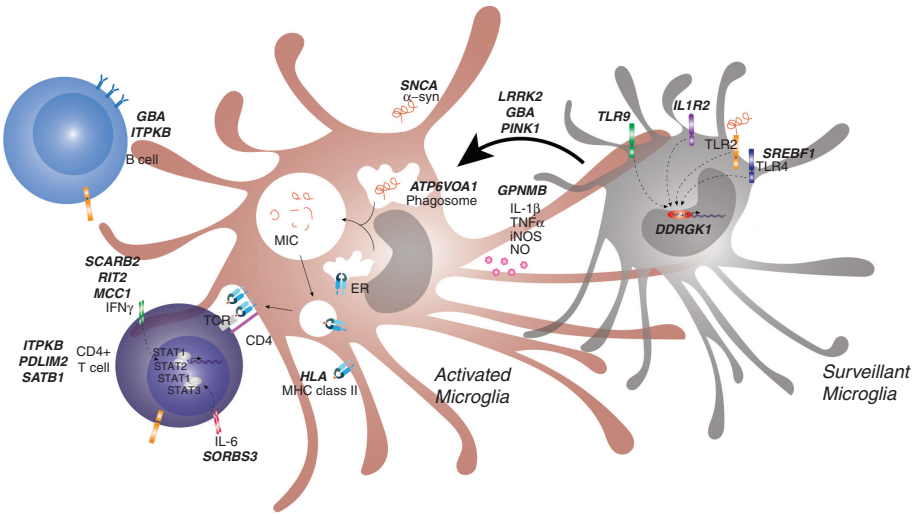

Figure 3 Insights into the immunogenetics of Parkinson's disease (PD). Schematic illustration of the link between genetic risk factors and immune mechanisms underlying PD development. Genes (indicated in bold italics) represent nominated risk genes for idiopathic and/or monogenic PD.

TABLE 1 Key HLA Haplotypes Associated with PD

HLA allele	Association with PD	Meta-analysis *p*-value	Meta-analysis Odds ratio (OR)	Reference
B*07:02	Risk	3×10^{-4}	1.23	(85)
B*40:01	Protective	2×10^{-3}	0.76	(85)
C*03:04	Protective	8×10^{-6}	0.72	(85)
C*07:02	Risk	2×10^{-4}	1.23	(85)
DRB1*04:04	Protective	4×10^{-5}	0.65	(85)
DRB1*15:01	Risk	6×10^{-5}	1.26	(85)
DRB4*01	Protective	4×10^{-5}	0.83	(85)
DRB5*01	Risk	5×10^{-5}	1.25	(85)
DQA1*01:02	Risk	1×10^{-3}	1.17	(85)
DQA1*03:01	Protective	1×10^{-6}	0.77	(85)
DQB1*03:02	Protective	7×10^{-6}	0.74	(85)
DQB1*06:02	Risk	$4 \times 10^{-5*}$	1.26*	(86)

Class I and class II HLA alleles associated with PD together with meta-analysis data for *p*-values and odds ratios. HLA, human leukocyte antigen; PD, Parkinson's disease.

been reported to be a *cis*-acting eQTL that correlates significantly with expression levels of HLA-DRA, DRB5, and DQA2 (49, 50). Studies following the GWAS approach, conducted in a Dutch population by the International Parkinson Disease Genomics Consortium, confirmed the association of the HLA class II region (rs4248166 and chr6:32588205, respectively) with PD (51, 52). Another study reported the presence of three HLA class II variants (not in LD) to be significantly associated with PD risk (53). Taken together, several studies confirm the association of the HLA class II region with PD risk and suggest associated variants

TABLE 2 SNPs in the HLA Region Associated with PD

SNP	Allele/ gene	Tissue	*p*-value	Effect size	Data base/ original article
rs3129882	DRA4		9×10^{-11}	1.30*	(48)
	DRB6	Brain	[6.4×10^{-7}; 2.4×10^{-7}]	[0.52; 0.58]	Gtex
		Whole blood	2.7×10^{-17}	0.45	Gtex
	DRB5	Hypothalamus	1.6×10^{-5}	–0.44	Gtex
		Whole blood	6.6×10^{-9}	–0.25	Gtex
	DQA2	Whole blood	3.3×10^{-5}	0.27	Gtex
	C4A	Whole blood	3.7×10^{-5}	–0.24	Gtex
	DQB1-AS1	Whole blood	4.4×10^{-5}	–0.16	Gtex
	DRB9	Whole blood	1.5×10^{-5}	0.22	Gtex
rs660895	DRB1-DQA1		8×10^{-7}	0.80*	(87)
	DQA1	Whole blood	1.3×10^{-6}	–0.18	Gtex
	DQA2	Brain	[6.0×10^{-17}; 1.6×10^{-9}]	[0.83; 1.1]	Gtex
		Substantia nigra	6.2×10^{-9}	0.95	Gtex
	DQB1	Brain	[6.8×10^{-6}; 9.7×10^{-6}]	[–0.55; –0.65]	Gtex
		Whole blood	3.3×10^{-12}	–0.41	Gtex
	DQB1-AS1	Whole blood	2.2×10^{-6}	–0.24	Gtex
	DQB2	Whole blood	7.9×10^{-13}	0.52	Gtex
	DRB1	Cortex	5.5×10^{-6}	0.58	Gtex
		Whole blood	5.3×10^{-14}	–0.21	Gtex
	DRB6	Brain	[7.8×10^{-7}; 5.4×10^{-6}]	[0.59; 0.58]	Gtex
		Whole blood	5.3×10^{-14}	–0.21	Gtex
	LY6G5B	Whole blood	1.4×10^{-5}	–0.12	Gtex

Table continued on following page

TABLE 2 **SNPs in the HLA Region Associated with PD** (Continued)

SNP	Allele/ gene	Tissue	*p*-value	Effect size	Data base/ original article
rs2395163	DRA/ BTNL2		3×10^{-11}	0.81*	(88)
	DAQ1	Whole blood	7.8×10^{-6}	–.017	Gtex
	DQA2	Brain	$[1.2 \times 10^{-9}; 1.2 \times 10^{-6}]$	[0.73; 0.83]	Gtex
		Whole blood	8.4×10^{-31}	0.89	Gtex
	DQB1	Whole blood	7.5×10^{-7}	–0.30	Gtex
	DQB2	Whole blood	1.0×10^{-8}	0.43	Gtex
	DRB1	Brain	$[5.4 \times 10^{-7}; 3.8 \times 10^{-5}]$	[–0.45; –0.46]	Gtex
		Whole blood	1.7×10^{-16}	–0.23	Gtex
	DRB6	Brain	$[3.6 \times 10^{-5}; 5.9 \times 10^{-6}]$	[0.55; 0.57]	Gtex
		Whole blood	1.2×10^{-10}	0.46	Gtex
rs9275326	DQB1		1.19×10^{-12}	0.826*	(13)
	DQA1	Whole blood	3.9×10^{-5}	–0.20	Gtex
	DQB1	Whole blood	1.4×10^{-7}	–0.41	Gtex
	DQA2	Brain	$[2.5 \times 10^{-9}; 4.1 \times 10^{-7}]$	[1.0; 1.1]	Gtex
		Whole blood	1.9×10^{-20}	0.96	Gtex
	DRB1	Whole blood	6.1×10^{-8}	–0.20	Gtex
	DRB6	Brain	$[1.1 \times 10^{-7}; 2.1 \times 10^{-5}]$	[0.79; –0.94]	Gtex
		Whole blood	1.4×10^{-6}	0.45	Gtex
	TAP2	Whole blood	67×10^{-8}	–0.28	Gtex
rs9268515			4×10^{-4}	1.25*	(53)
rs4248166	DRA/ BTNL2		0.07	1.08*	(51)
rs75855844	DRAB5		4×10^{-4}	1.25*	(13)

Summarized eQTL data from publically available databases (Gtex) for whole blood and brain regions. An asterisk (*) denotes values from meta-analyses. p-values were generated for each variant-gene pair by testing the alternative hypothesis that the slope of a linear regression model between genotype and expression deviates from 0. The effect size of the eQTLs is defined as the slope of the linear regression and is computed as the effect of the alternative allele relative to the reference allele in the human genome reference GRCh37/hg19 (i.e., the eQTL effect allele is the alternative allele). HLA, human leukocyte antigen; PD, Parkinson's disease; SNP, single nucleotide polymorphism.

to be eQTLs, that is, regulating gene transcription. This could provide a functional link to the increased expression of MHCII molecules observed in PD brains and affect the interaction between antigen-presenting cells and lymphocytes.

T- and B-lymphocyte development

From the 41 PD-risk loci identified by GWAS (Figure 1), ITPKB, PDLMI2, SATB1, and BST1 are involved in T- or B-lymphocyte development. Inositol 1,4,5-trisphosphate 3-kinase B (ITPKB) controls positive selection of T-lymphocytes and modulates Erk activity, an important kinase that regulates extracellular signal response and plays a crucial role in the production of pro-inflammatory cytokines and chemokines. Studies in mice have shown that nonsense mutations in ITPKB attenuate Erk signaling in T-lymphocytes (54) and that ITPKB-deficiency leads to defects in B-lymphocyte survival, developmental alterations of B-lymphocytes, and antigen unresponsiveness *in vivo* (55). PDLIM2 has been reported to inhibit T-helper 17 (TH17) cell development through signal transducer and activator of transcription 3 (STAT3). PDLIM2 deficiency in mice resulted in the accumulation of STAT3 in the nucleus and enhanced the extent of TH17 cell differentiation, known to have a pathogenic role in inflammatory diseases (56). SATB1 encodes for special AT-rich binding protein 1, a T-lymphocyte-enriched transcription factor and chromatin organizer essential for controlling a large number of genes participating in T-lymphocyte development and activation (57). Moreover, it has been observed that mouse SATB1 coordinates the expression of Th2 cytokine genes (58). BST1 encodes for the leukocyte surface protein CD157 that is upregulated in bone marrow cells from patients diagnosed with rheumatoid arthritis (59) and may facilitate pre-B-lymphocyte growth.

NF-κB and IFNγ-signaling

Four loci reported to be associated with PD relate to the transcription factor NF-κB that regulates a number of immune genes in response to different stimuli. These loci include MCCC1 and DDRGK1 (12) (Figure 1) as well as RIT2 and SCARB2 reported in an earlier GWAS meta-analysis (13). MCCC1 knockdown strongly inhibits induction of IFNs and inflammatory cytokines in response to viral infection (60). It has also been observed that expression patterns of RIT2 and IFNγ are positively correlated in PD brains, indicating that RIT2 may modulate IFNγ signaling (61). Depletion of DDRGK1 dramatically inhibits the expression of NF-κB target genes, suggesting that DDRGK1 plays an important role in regulating the NF-κB signaling pathway through interaction with IκBα (62). SCARB2 is a known receptor for GBA and for enterovirus 71 (EV1) and is highly expressed in human plasmacytoid dendritic cells where it has been reported to regulate the production of type I IFN through TLR9 and IFN regulatory factor 7 (63).

Regulation of inflammation through metabolic pathways

Biallelic mutations in GBA cause Gaucher's disease, and carriage of one mutated GBA allele substantially increases the risk for PD (64). Although GBA mutations are the single largest risk factor for idiopathic PD, the mechanisms behind

the risk increment are not fully understood. There are several immune-related effects of GBA deficiency, including multisystem inflammation, B-lymphocyte hyperproliferation (65), increased levels of pro-inflammatory cytokines (66), microglial activation and astrogliosis (67). Less is known about the role of GPNMB, SREBF1, and ACMSD in conferring increased risk for PD. GPNMB encodes for glycoprotein nonmetastatic melanoma B and is highly expressed in microglia after LPS treatment. Inhibition by GPNMB siRNA dramatically suppressed the expressions of TNF-α, IL-1β, and inducible nitric oxide synthase (iNOS) in activated mouse BV2 cells, indicating a role in microglial activation and pro-inflammatory cytokine release (68). SREBF1 may regulate innate immune responses through its actions on lipid metabolism since it contributes to resolution of pro-inflammatory TLR4 signaling by reprogramming fatty acid metabolism (69). ACMSD has a well-described biological function in the kynurenine pathway, where it regulates and limits the formation of quinolinic acid. Quinolinic acid is an NMDA receptor agonist with excitotoxic properties that can also modulate inflammatory responses. ACMSD could therefore reduce inflammation-induced neurodegeneration (70).

Innate immune response

PD risk-loci linked to innate immune responses include TLR9, IL1R2, and ATP6V0A1. TLR9 is part of the toll-like receptor family and can recognize mitochondrial DNA as an endogenous danger-associated molecular pattern (DAMP) and activate an inflammatory cascade (71). IL-1 receptor type 2 (IL1R2) acts as a decoy receptor for IL1 by competing with IL1R1 for ligands and co-receptors. IL1R2 has been implicated in arthritis, endometriosis, organ transplantation, and Alzheimer's disease (72). *In vitro*, the expression of IL-1R2 is suppressed by pro-inflammatory agents like LPS and IFN-γ (73). The ATP6V0A1 gene is expressed in microglia and their precursors and is involved in the acidification of intracellular compartments and the phagosomal fusion, a process that is crucial for phagocytosis (74). In addition to these, many gene variants conferring increased risk for PD act, in some way, on the complement system. These include *SNCA*, *MAPT*, *GBA*, *STK39*, *LRRK2*, *HLA*, *GPNM8*, *GCH1*, *DDRGK1*, *SCARB2*, *FGF20,* and *SREBF1* (75).

ENVIRONMENTAL FACTORS AFFECTING IMMUNOGENETICS IN PARKINSON'S DISEASE

As mentioned above, the incomplete penetrance of monogenic forms of PD and the complex genetic structure of idiopathic PD suggest the presence of environmental components that modify disease risk. The link between inflammation and PD genetic risk factors described above is strong, but how immunogenetics interacts with environmental factors is a research field still in development. For example, a SNP (rs*3129882*) in *HLA-DRA* associated with increased MHCII molecule expression has been reported to significantly increase the risk of PD in synergy with environmental exposure to pyrethroid (76). The use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been suggested to be neuroprotective,

with ibuprofen being significantly associated with a reduced risk for PD (77, 78). Any interaction between the effect of NSAIDs and genetic risk factors for PD is, however, not known.

The gastrointestinal tract has an extensive immune and neuronal network and is in direct contact with the external environment. According to the Braak observations (79), the enteric nervous system is affected by α-synuclein pathology before the substantia nigra. It has been proposed that PD pathology actually starts in the gut and propagates through the vagus nerve to reach the substantia nigra; however, this hypothesis remains under debate (80). Several of the genes linked to familial PD or associated with idiopathic PD are also linked to the gastrointestinal tract. As mentioned above, GWAS have identified variants at the LRRK2 locus which are also known to be associated with Crohn's disease (26), and FGF20 has been associated with colitis and has demonstrated therapeutic activity in experimental models of intestinal inflammation (81). The overlapping susceptibility between inflammatory bowel disease and PD suggests that inflammatory processes in the intestines may promote PD pathology. Patients with PD have also been shown to have an altered gut microbiota pattern compared with controls (82, 83), and there is emerging evidence that the microbiota can influence the development of PD. In α-synuclein-overexpressing mice, microbiota were required for α-synuclein pathology, microglial activation, and motor deficits to occur (84). In addition, transplantation with microbiota from PD patients, but not from control subjects, worsened the physical impairment in the α-synuclein-overexpressing mice. These findings suggest the microbiome not only as a risk factor for PD but also as a potential therapeutic target. How genetic factors contribute to the microbiome and its impact on PD risk remains to be determined.

CONCLUSION

Many of the identified gene mutations linked to monogenic PD and common variants associated with idiopathic PD are involved in immune pathways. There is thus increasing evidence that inflammation has a causative role rather than being a consequence of neurodegeneration in PD. The involved pathways include both innate and adaptive immune responses in the CNS and in the periphery. If the risk for PD is, in part, mediated through immune mechanisms, these are obvious targets for therapeutic intervention. The field of immunogenetics in PD is therefore likely to unravel more of the etiology underlying PD, as well as identifying potential targets for novel treatments.

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3 Pathological Mechanisms and Clinical Aspects of *GBA1* Mutation-Associated Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder, characterized by a motor syndrome consisting of bradykinesia, rigidity, resting tremor, and postural instability. Mutation in the *GBA1* gene, which encodes the lysosomal enzyme glucocerebrosidase, has recently emerged as the most common genetic abnormality associated with PD. Approximately 5% of PD patients carry a *GBA1* mutation, in comparison to <1% of the healthy population.

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Heterozygous or homozygous *GBA1* mutations increase the risk of PD 20–30 fold. The pathogenic mechanisms of *GBA1* mutation-associated PD are not fully understood. Several studies suggest loss of enzyme activity underlies the pathogenicity of *GBA1* mutations, while others suggest that a gain of function due to enzyme misfolding is important. Lysosomal-autophagic and mitochondrial dysfunction, as well as endoplasmic reticulum stress and alpha-synuclein accumulation, have all been demonstrated in association with *GBA1* mutation. The clinical features of *GBA1* mutation-associated PD are similar to that of sporadic disease but with an earlier age of onset and a more rapid cognitive and motor decline. Given this impact of clinical course and its relatively high frequency, understanding the pathogenic mechanisms of *GBA1* mutations will allow for the development of targeted, potentially disease-modifying treatments that will have implications for many patients with PD.

Keywords: α-synuclein; Gaucher disease; *GBA1*; Glucocerebrosidase; Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder, characterized clinically by a typical motor syndrome consisting of bradykinesia, rigidity, resting tremor, and postural instability (1). The motor disorder in part results from the loss of striatal dopamine, as a consequence of degeneration of the dopaminergic neurons of the substantia nigra (1). Most individuals also develop neuropsychiatric manifestations (such as anxiety, depression, and hallucinations), sleep disturbances, autonomic dysfunction, and cognitive decline and dementia, due to involvement of extra-nigral brain regions including the cortex and multiple brainstem sites (2).

The natural history of PD may follow a more benign motor-predominant course in some patients, while in others the disabling non-motor features predominate. The underlying basis of the clinical heterogeneity is poorly understood, but it is becoming clear that this is, at least in part, due to genetic factors (1, 3, 4). One of these genetic risk factors is mutation in the *GBA1* gene, which has emerged numerically as the most important genetic abnormality associated with PD (5, 6), being found in about 5% of patients with the so-called sporadic PD. In this chapter, we discuss the epidemiology, pathogenic mechanisms, clinical features, and implications for treatment of *GBA1* mutation-associated PD.

THE GBA1 GENE AND GLUCOCEREBROSIDASE

GBA1 encodes the lysosomal enzyme glucocerebrosidase (GCase). The gene consists of a 7.6-kb sequence, with 11 exons and 10 introns, and is sited on the long arm of chromosome 1 (7). *GBA1* gives rise to at least two mRNA sequences, resulting from alternate polyadenylation sites, and the resultant amino acid peptides are processed to form the 496 amino acid mature protein (6); 16 kb downstream of

the *GBA1* gene lies a 5.7-kb sequence, which is almost identical to the *GBA1* gene (6, 7). This pseudogene has complicated genetic sequencing and the identification of novel pathogenic mutations.

GCase is responsible for the hydrolysis of glucosylceramide (also termed glucocerebroside) glycosphingolipids to ceramide and glucose, in the lysosome (8). It consists of three domains, with the catalytic site residing in the third domain, which has the structure of a triose phosphate isomerase barrel (9). The enzyme is synthesized in the rough endoplasmic reticulum (ER) and traverses the Golgi apparatus via a phosphatidylinositol-4-kinase (PI4K)-dependent pathway to the lysosome. Transit is mediated by a specific transporter, lysosomal membrane protein-2 (LIMP-2) (10, 11). This differs from the majority of other lysosomal proteins, where delivery to the lysosome is dependent on mannose-6-phosphate receptors (12). GCase and LIMP-2 are bound in the ER, and they pass through the Golgi apparatus in complex. On delivery to the lysosome, the low pH results in the dissociation of LIMP-2 from GCase (Figure 1) (11). Enzyme activity is dependent on the substrate-presenting co-factor saposin C, which is cleaved from prosaposin (13).

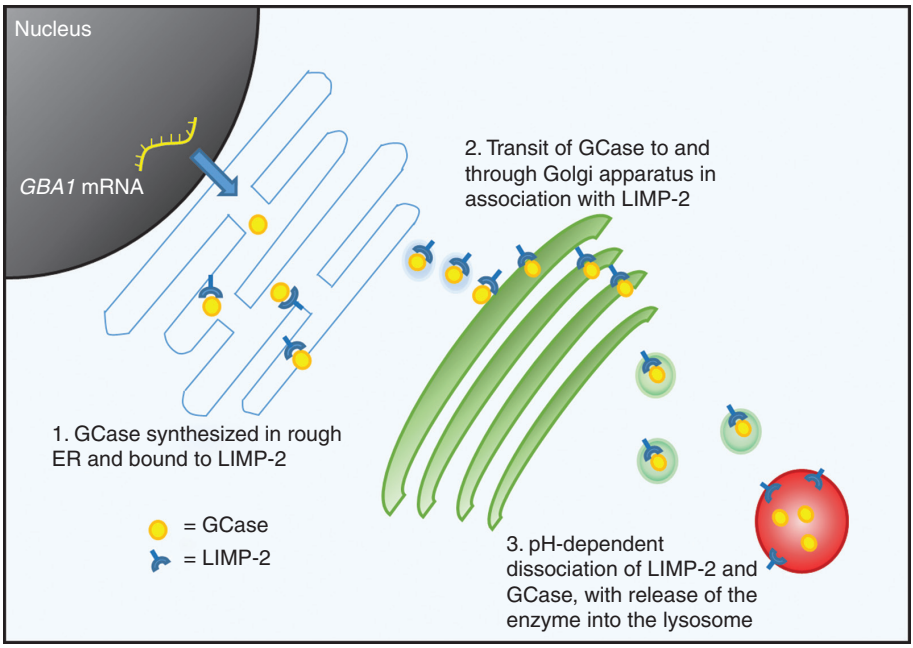

Figure 1 Synthesis and processing of GCase. GCase is synthesized in the rough ER where it becomes associated with LIMP-2. These two proteins transit in complex to the Golgi apparatus for processing. The GCase–LIMP-2 complex then migrates to the lysosome, where GCase is released into the lysosomal compartment, facilitated by pH-dependent dissociation from LIMP-2. ER, endoplasmic reticulum; GCase, glucocerebrosidase; LIMP-2, lysosomal integral membrane protein-2; mRNA, messenger ribonucleic acid.

GBA1 MUTATIONS AND GAUCHER DISEASE

Over 300 mutations in the *GBA1* gene have been identified, which include point mutations, insertions, deletions, splice-site mutations, and recombinations (14, 15). Bi-allelic mutations result in negligible GCase enzyme activity and the autosomal recessive lysosomal storage disorder, Gaucher disease (GD), in which glucosylceramide accumulates in various organs and cells (particularly macrophages) (14). GD is classified based on the presence or absence of neurodegenerative features and the rate of clinical progression (16). Type 1 (non-neuronopathic) GD is characterized by hepatosplenomegaly, anemia and thrombocytopenia, and bone lesions. Types 2 and 3 (neuronopathic) GD are also associated with progressive neurological decline, which is rapid in type 3 GD (6, 14).

ASSOCIATION BETWEEN *GBA1* MUTATION AND PARKINSON'S DISEASE

At the end of the 20th century, reports of an association between PD and GD began to emerge. A proportion of patients with type 1 GD were observed to develop a parkinsonian syndrome in adult life (17–19), and it was subsequently noted that first-degree relatives of GD patients (who were obligate or confirmed carriers of a single *GBA1* mutation) also carried an increased risk of developing PD (20, 21). PD occurs in about 10% of patients with type 1 GD before the age of 80 years, compared to about 3–4% in the normal population (22).

A number of studies have since reported on the prevalence of *GBA1* mutation in the PD population, with estimates ranging up to 21% (23). The prevalence of *GBA1* mutations in PD is likely to have been overestimated in postmortem studies, in which cohorts may be more likely to include "atypical" or early-onset PD. It is generally accepted that about 5% of PD patients carry a *GBA1* mutation (24–28). In PD patients of Ashkenazi Jewish origin, the carrier frequency of *GBA1* mutation is much higher at 15–20% (5, 27). In contrast, <1% of the general healthy population carries a mutation (3% in the healthy Ashkenazi Jewish population) (27). This makes it numerically the most important known genetic risk factor for PD (6). Additionally, *GBA1* mutations have been associated with dementia with Lewy bodies, providing further support that there is a pathogenic link between this gene and α-synucleinopathies (29–31). It is, however, important to understand that *GBA1* mutations do not cause a Mendelian form of PD, but are considered a genetic risk factor, increasing the risk 20–30 fold (6, 15, 27, 32). In absolute terms, the penetrance of PD in association with this mutation is relatively low, with about 30% of carriers developing the disease before the age of 80 (6).

PATHOGENIC MECHANISMS

The hallmark of PD pathology is the presence of Lewy bodies and Lewy neurites which consist predominantly of α-synuclein (33, 34). Abnormalities in a number

of intracellular processes have now been implicated in *GBA1* mutation-associated PD, of which the one with greatest evidence base is dysfunction of the lysosome-autophagy system—a system known to be important in the clearance of α-synuclein (35–38).

Loss of function or gain of function?

A growing number of studies have linked loss of GCase activity to PD pathology (35, 39–42). Some studies have found that loss of enzyme function results in accumulation of sphingolipid substrates, which potentially play a pathogenic role, as discussed below (35, 39, 42). The loss-of-function hypothesis offers therapeutic targets for putative disease-modifying agents, which may act by augmenting GCase activity or removing substrates through other pathways.

GCase activity is reduced in several brain regions of individuals with sporadic and *GBA1* mutation-associated PD, and indeed in association with normal aging (43). Chemical suppression of GCase activity using conduritol-β-epoxide increases α-synuclein levels in wild-type mice and in neuroblastoma cells (39, 40). Higher levels of α-synuclein have also been observed in the olfactory bulb (44) and substantia nigra (40) in GD mice. Additionally, *GBA1* knockout in wild-type mice results in aberrations in the lysosome-autophagy system and mitochondrial function, suggesting that loss of enzyme activity can negatively impact on other systems relevant to PD pathogenesis (45). Conversely, enhancing GCase activity can reduce α-synuclein pathology in animal models (46, 47). While these studies have correlated reduced GCase activity with PD-relevant pathology, the mechanism by which this happens is yet to be clearly demonstrated. One possibility is that reduction in GCase activity results in accumulation of its sphingolipid substrates (42–44), which in turn stabilize the pathogenic species of α-synuclein, contributing to its accumulation and aggregation (35, 48).

However, only a minority of GD patients, in whom GCase activity is negligible, develop PD (22). If the increased risk of PD in heterozygous *GBA1* mutation carriers relates to a loss of enzyme function, then one would expect a much higher incidence of PD within the GD population. Additionally, *GBA1* mutations which do not significantly reduce enzyme activity have been associated with PD (49). It appears, therefore, that the pathogenic mechanisms of *GBA1* mutation-associated PD are more complex than a simple reduction of enzyme activity and accumulation of substrates. Additionally, there are *GBA1* mutations that do not cause GD, but that increase the risk of PD, further suggesting that the pathogenesis of *GBA1* mutation-associated PD differs from that of GD (49).

The majority of pathogenic mutations in the *GBA1* gene are missense mutations that result in misfolding of the enzyme, and thus, an alternative hypothesis is that the pathogenicity of these mutations is conveyed by the gain of a toxic function of the misfolded enzyme. Misfolded GCase may be retained in the ER, leading to activation of the unfolded protein response (UPR) and ER-associated protein degradation (ERAD—see below). Prolonged activation of these systems and ongoing retention of misfolded enzyme may ultimately lead to activation of apoptotic pathways and neuronal loss. However, there are null mutations associated with PD risk, and as discussed above, knocking down *GBA1* or inhibition of GCase activity in animal and cell models can recapitulate relevant α-synuclein

and PD pathology (35, 39–42). Gain of function therefore does not fully account for the increased risk of PD.

The mechanisms by which *GBA1* mutations predispose to PD pathology are multifaceted, with several elements potentially being important. A further degree of complexity is added by the fact that different *GBA1* mutations increase the risk of PD to different extents, and (at least in GD) there is a degree of genotype–phenotype correlation. For example, the common N370S mutation is rarely associated with neuronopathic GD, while homozygous L444P mutations result in type 3 GD, and some known mutations carry a much greater risk of PD development (e.g., L444P and D409H) than others (e.g., N370S) (5, 50). It is therefore conceivable that different pathogenic mechanisms are more or less pronounced in carriers of different mutations. The remainder of this section will outline what is currently known about the intracellular pathogenic mechanisms of *GBA1* mutation-associated PD.

Lysosome-autophagy dysfunction

Autophagy is an umbrella term for some of the cell's major mechanisms of protein clearance and organelle turnover (51). Macroautophagy and chaperone-mediated autophagy are important in the degradation of α-synuclein, and therefore, it is feasible that dysfunction of these systems can potentiate PD pathology. In chaperone-mediated autophagy, soluble proteins are directed to lysosomes by chaperone proteins for degradation by lysosomal enzymes (52, 53). In contrast, macroautophagy involves the formation of vacuolar structures called autophagosomes from cystosolic membrane fragments (phagophore). These autophagosomes take up proteins and other cellular waste before fusing with lysosomes to form an autolysosome. The contents are then degraded by the lysosomal compartment and recycled (Figure 2) (51, 53).

Dysfunction of the lysosome-autophagy system (in particular, macroautophagy) appears to be central to the pathogenic role of *GBA1* mutations, with a growing number of studies reporting autophagy dysfunction in association with *GBA1* mutation (35, 36, 42, 54). For example, increased numbers of lysosomes have been reported in neuroblastoma cells carrying *GBA1* mutations (54) and induced-pluripotent stem cell (iPSC)-derived dopaminergic neurons from homozygous or heterozygous *GBA1* mutation carriers (36, 42). Higher autophagosome numbers have also been reported in these studies (42, 54). Additionally, increased numbers of autophagosomes and lysosomes have been detected in neurons from wild-type mice treated with conduritol-β-epoxide, suggesting that these changes can occur due to reduced GCase enzyme activity (39).

While the lysosome-autophagy system is clearly implicated in PD associated with *GBA1* mutations, the specific nature of the dysfunction is not understood. The possible sites of perturbation in macroautophagy could be at the level of:

i. biosynthesis of autophagosomes and/or lysosomes
ii. uptake of the targets of degradation into autophagosomes
iii. fusion of autophagosomes with lysosomes
iv. enzymatic degradation of the contents of the autolysosome (Figure 2).

The aforementioned observations of increased numbers of lysosomes and autophagosomes in *in vitro* and *in vivo* models of *GBA1* mutation-associated disease

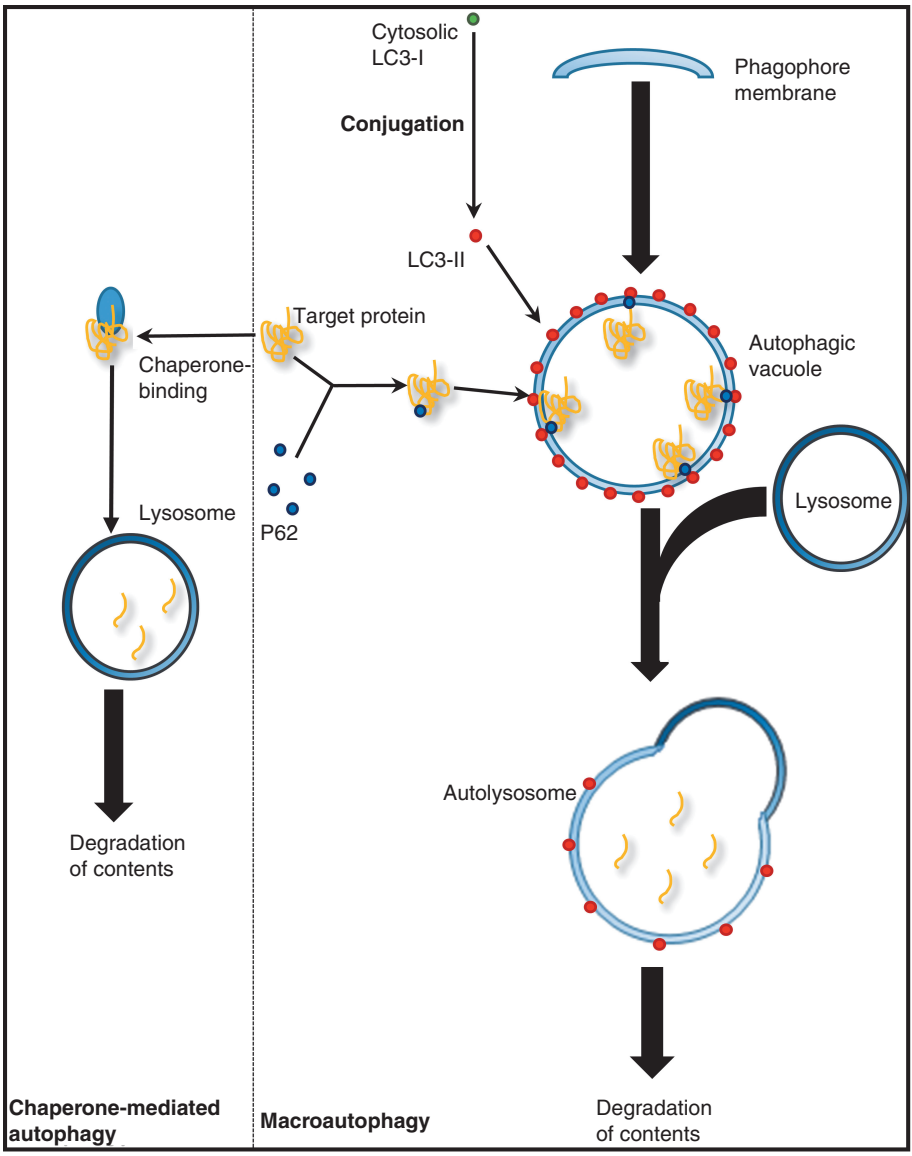

Figure 2 Macroautophagy and chaperone-mediated autophagy pathways. During macroautophagy, phagophore membrane fragments form autophagosomes. During this process, the protein LC-3 is conjugated. Proteins are targeted to the autophagosome, which involves the protein P62. The autophagosome fuses with the lysosome to form an autolysosome, and the contents are degraded by lysosomal enzymes. In chaperone-mediated autophagy, proteins for removal bind to protein chaperones, which help to target them directly to the lysosome for enzymatic degradation. Together, these mechanisms constitute important pathways for α-synuclein clearance.

suggest that the site of dysfunction is not in the generation of these structures, but that it lies in the later stages of the pathway (36, 39, 42, 54). One possibility is that the increased number of these structures is due to increased activation of macroautophagy, perhaps as a compensatory response to other intracellular stresses. Alternatively, it could reflect an impairment in the fusion step of macroautophagy, which is supported by the observation that iPSC-derived neurons from *GBA1* mutation carriers have reduced co-localization of autophagosomes and lysosomes compared to isogenic controls (42).

Other studies, however, suggest that the problem lies in the enzymatic function within the lysosome. Mazzulli and colleagues reported reduced lysosomal proteolysis in primary mouse cortical neurons with sh-RNA-mediated knock-down of *GBA1* (35). Reduced lysosomal proteolysis may also be inferred by the observation that lysosomes in iPSC-derived dopaminergic neurons from *GBA1* mutation carriers are enlarged, with increased electron dense material, suggesting that their contents are not cleared appropriately (36). Therefore, while alterations in the function of the lysosome-autophagy system associated *GBA1*-mutation appear to occur, especially in the later stages of the macroautophagy pathway, the precise site of the dysfunction has yet to be conclusively shown.

Accepting that there is a problem with the lysosome-autophagy system in *GBA1* mutation carriers, a question remains as to whether this is relevant to the increased risk of PD or whether it is an incidental observation associated with the mutation (taking into account the fact that only a minority of GD patients develop PD). α-synuclein clearance is dependent on multiple pathways, but both macroautophagy and chaperone-mediated autophagy (both dependent on lysosomal hydrolysis) have been shown to be fundamentally important in its degradation (38, 55). In particular, a growing number of *in vitro* and *in vivo* studies have identified increased levels of α-synuclein in association with lysosome-autophagy dysfunction in *GBA1* mutation or GCase suppression (35, 39–41, 44, 54, 56–58). Therefore, for example, *GBA1* knock-down in mouse cortical neurons has been shown to lead to reduced lysosomal proteolysis associated with reduced clearance of soluble and insoluble α-synuclein (35). Lysosomal dysfunction has also been shown to increase exosomal release of α-synuclein from the cell (59, 60), which may in turn then be taken up by adjacent neurons, resulting in aggregation of endogenous α-synuclein in surrounding cells, all of which could account for the more accelerated clinical course seen in GBA-PD.

Taking into account the above discussion, it is clear that lysosome-autophagy system dysfunction in *GBA1* is complex. A bidirectional loop has been proposed in which reduced GCase activity leads to perturbation in lysosomal function and accumulation of glycosphingolipid substrates, and accumulation of pathogenic oligomers of α-synuclein (35). The GCase substrate glucosylceramide has been shown *in vitro* to lead to stabilization of pathogenic high molecular weight recombinant α-synuclein oligomers, before accelerated aggregation (35, 48). The bidirectional loop is completed by the observation that α-synuclein appears to impede transit and processing of GCase between the ER and the lysosome, resulting in a further reduction in lysosomal GCase activity (35). Further evidence for this comes from the observation that overexpression of α-synuclein in cortical neurons results in an increase in the ER form of the enzyme and a reduction in the post-ER, glycosylated form of the enzyme (35), which fits with the

finding of reduced GCase activity in some PD patients with wild-type *GBA1* in some studies (39).

Mitochondrial dysfunction and the role of oxidative stress

Mitochondrial dysfunction is clearly important in PD pathogenesis, illustrated by the fact that mitochondrial toxins such as rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induce death of dopaminergic neurons (61–63) and the fact that some hereditary forms of PD occur due to mutations in genes related to mitochondrial function and health (e.g., *PINK-1* and *DJ-1*) (1). Few studies have commented on mitochondrial dysfunction in *GBA1* mutation-associated disease, but there is some evidence that it plays a role (41, 45, 57, 64).

Suppression of GCase activity using conduritol-β-epoxide in neuroblastoma cells (41) and wild-type neurons in culture (57) results in a reduction in ATP production and oxygen consumption, demonstrating that mitochondrial dysfunction can occur as a result of reduced GCase enzyme activity. Additionally, a progressive reduction in mitochondrial membrane potential has been observed *in vitro* with prolonged GCase suppression, further supporting this idea (41). Furthermore, mitochondrial morphology and function are abnormal in *GBA1* knockout mice, with the degree of abnormality greatest in homozygous compared to heterozygous knockout animals (45). In addition, reactive oxygen species are found in higher levels in the fibroblasts of PD patients carrying *GBA1* mutations in comparison with those with wild-type *GBA1* (65). These cells also had increased levels of the antioxidant, NQ01—a potential compensatory mechanism (65). Finally, protein aggregates including α-synuclein have been noted to be localized to mitochondria in the hypomorphic prosaposin murine model of GD (57). While all these studies support a potential role for mitochondrial dysfunction in *GBA1* mutation-associated PD, evidence is sparse in this area, and it is not clear whether these aberrations are directly related to abnormalities in GCase and the *GBA1* gene, or alternatively are incidental markers of neurodegeneration.

It is possible that perturbations in mitochondrial function arise downstream of *GBA1* mutation-induced α-synuclein accumulation and aggregation. However, it has recently been suggested that oxidative stress is an earlier component of the pathogenic process in PD (66). Mitochondrial oxidant stress has recently been shown to be associated with *DJ-1* knockout or dysfunction in dopaminergic neurons generated from patient-derived iPSCs, and after prolonged culture, this results in reduced GCase enzyme activity and impaired lysosomal proteolysis (66). While this process occurs in cells from patients with sporadic PD, it was accelerated in neurons derived from patients with homozygous *DJ-1* mutations, suggesting that mitochondrial dysfunction is an important aspect of PD pathogenesis, potentially through inducing lysosomal dysfunction (66). Furthermore, mitochondrial antioxidants reduce the levels of soluble α-synuclein, suggesting that oxidant stress contributes to α-synuclein accumulation (66). This important study suggests that a sequential pathogenic pathway exists in PD, in which mitochondrial oxidant stress leads to reduced GCase activity and lysosomal function, which potentially leads to α-synuclein accumulation. *GBA1* mutation may feed into this by lowering the threshold for, or altogether bypassing the need for, mitochondrial oxidant stress.

ER-stress

As is discussed above, GCase is produced in the rough ER before it is processed in the Golgi apparatus and transported to the lysosome (10, 11). If proteins are not folded correctly in the ER, the misfolded protein may be retained for re-folding, mediated by ER chaperones (67, 68). Failure to correct the protein structure results in removal of the offending proteins via ERAD and the ubiquitin-proteasome system (67–69). *GBA1* mutations, which result in structural change and misfolding of the protein, may result in activation of these systems (70, 71). Once their capacity to remove the misfolded protein is exceeded, activation of the UPR ensues (36, 72). If ER-stress is prolonged and the UPR fails to restore normal function, the cell may be directed to an apoptotic cell death fate. In support of this, increased levels of chaperone markers of ER-stress have been found in iPSC-derived neurons from PD patients carrying *GBA1* mutations compared to controls, along with increased levels of markers of the UPR (36).

Interaction with α-synuclein

An increasing number of studies have identified α-synuclein accumulation in the setting of *GBA1* mutation (36, 46, 56). This may occur due to reduced clearance, secondary to dysfunction of the lysosome-autophagy system as discussed above (35, 36, 66). However, GCase also appears to interact directly with α-synuclein, and the significance of this is not known (73, 74). In one postmortem study, GCase was identified in 32–90% of Lewy bodies of PD patients with *GBA1* mutation, and in 10% of those with wild-type *GBA1* (75). It is possible that this co-localization is a manifestation of the cell's attempt to clear these aggregates, but alternatively it may be that mutant GCase stabilizes α-synuclein species or serves as a platform for fibrillization to occur. As well as these postmortem findings, recombinant GCase and α-synuclein have also been shown to interact *in vitro* (73, 74). At lysosomal pH, membrane-bound α-synuclein forms a complex with GCase, resulting in reduced enzymatic function (47, 73, 74). This interaction potentially explains the alterations in GCase activity that has been reported in PD with wild-type *GBA1* (50, 76).

CLINICAL ASPECTS OF *GBA1* MUTATION-ASSOCIATED PD

The characteristic motor features of PD include bradykinesia, rigidity, and resting tremor. Onset is usually asymmetric, and the disease progresses slowly over years. Other motor manifestations include postural instability, hypomimia, dysphonia, and altered posture. There are often additional non-motor features, such as anosmia, neuropsychiatric symptoms including depression and anxiety, sleep disturbance, autonomic dysfunction, and cognitive decline and dementia (1). At an individual level, PD patients carrying *GBA1* mutations are clinically indistinguishable from those with sporadic PD. However, at a population level, *GBA1* mutations are associated with differences in the age of onset, and incidence of cognitive involvement, in comparison with PD patients with wild-type *GBA1* (24, 77–81).

In this section, the clinical and imaging aspects of *GBA1* mutation-associated PD will be discussed.

Clinical features

Age of onset is earlier in *GBA1* mutation-associated PD, with multiple studies suggesting that motor manifestations occur between 2 and 10 years earlier than in sporadic PD patients (77–83). Additionally, *GBA1* mutations are twice as common in those with PD of onset before age 50 than in later-onset cases (81, 82).

The clinical syndrome in PD associated with *GBA1* mutations is similar to that seen in sporadic PD. However, motor progression to Hoehn and Yahr stage 3 (bilateral disease with development of postural instability) is accelerated in patients with *GBA1* mutation, with an associated increase in mortality (24, 77, 84). One study reported that *GBA1* mutation carriers are more likely to present with tremor compared to patients with sporadic PD and that they have an increased risk of development of levodopa-induced dyskinesia (82). However, it should be noted that this group of patients were on significantly higher doses of levodopa compared to the sporadic PD patients which may explain the increased incidence of dyskinesia (82). Non-motor features, including REM-sleep behavior disorder, depression, anxiety, and hallucinations, are also said to be more prevalent in PD patients with *GBA1* mutations compared to those without (81, 85–88). Depression and REM-sleep behavior disorder are also more common in individuals with heterozygous *GBA1* mutations without a diagnosis of PD, compared to individuals with no mutation, which may reflect a prodromal phase of PD (85). Olfactory dysfunction is considered to be a prodromal non-motor feature of PD, and hyposmia has been identified at a greater incidence in individuals with heterozygous and homozygous *GBA1* mutations compared to those with wild-type *GBA1* (85, 86, 89). The greater burden of non-motor symptoms in *GBA1* mutation carriers has been associated with lower quality-of-life scores compared to individuals with wild-type *GBA1* (87). While autonomic features including constipation, bladder and erectile dysfunction, and orthostatic hypotension occur in *GBA1* mutation-associated PD, they do not appear to be any more prevalent than in sporadic PD (86, 88, 89).

The most significant difference in the clinical features of *GBA1* mutation-associated PD compared to sporadic PD is the increased incidence and rate of cognitive decline (24, 77, 84, 86, 88). The prevalence of dementia in individuals with sporadic PD who are over 65 years old is estimated to be between 24 and 31% (90). One retrospective longitudinal study found that 56% of *GBA1* mutation carriers had dementia at age 70, compared with 15% of sporadic PD patients (84). Carriers of *GBA1* mutations had a threefold increased risk for dementia and approximately twofold increased risk of mortality, which was not entirely attributable to the dementia (84). This is consistent with findings from other studies (77, 86, 88, 91). Cognitive decline occurs more frequently in *GBA1* mutation-associated PD compared to sporadic PD, while the rate of progression is accelerated. In a community incident cohort of PD patients, those with *GBA1* mutations had a median time to diagnosis of dementia of approximately four years, compared to eight years for those without *GBA1* mutations (24).

Neuroimaging findings

Imaging studies, using dopamine transporter imaging scans, magnetic resonance imaging (MRI) scans, transcranial sonography, and positron emission tomography (PET) scans, have all been used to look for differences between sporadic PD and PD associated with *GBA1* mutation.

Dopamine transporter PET imaging (DaTSCAN) using the dopamine transporter ligands 18F-fluoropropylcarbomethoxyiodophenylnortropane (18F-FP-CIT) and 123-ioflupane (123I-FP-CIT) have shown a greater degree of dopaminergic neuronal loss in PD associated with *GBA1* mutation than in patients with wild-type *GBA1* (84, 92–94). Asymmetry of radio ligand uptake is more pronounced in PD associated with *GBA1* mutations than in some of the genetic forms of PD associated with a Mendelian inheritance pattern (*SNCA*, *PINK1*, or *Parkin* mutations) (92). However, a larger study in which F18-fluorodopa PET imaging was used to investigate patients with *GBA1* mutation did not find any significant difference in dopamine synthesis, when compared with sporadic PD patients (95). Magnetic resonance diffusion tensor imaging (DTI) demonstrates reduced fractional anisotropy in a number of white matter tracts (corpus callous, anterior limb of internal capsule bilaterally, right anterior external capsule, and olfactory tracts) in PD patients with *GBA1* mutations compared to those without mutations (96, 97). Although some subtle differences in the imaging features of *GBA1* mutation-associated PD and sporadic PD have been described, the utility of these imaging techniques is largely limited to research at present. However, as refined treatments are developed, imaging findings may be useful in guiding genetic testing in PD, particularly when seeking mutations in the *GBA1* gene, as sequencing is complicated by the presence of a pseudogene in close proximity to the gene (as is discussed above).

THERAPEUTIC PROSPECTS AND IMPLICATIONS

Current treatment of *GBA1* mutation-associated PD is the same as for sporadic PD. There are no specific treatments that modify the disease course in PD, with or without *GBA1* mutation, and the current treatment involves the use of dopaminergic agents to improve motor symptoms. Understanding the mechanisms by which *GBA1* mutations predispose to PD pathogenesis may offer novel therapeutic avenues and a greater prospect of identifying disease-modifying agents for this group of patients. Furthermore, given that some patients with sporadic PD also show a significant reduction in GCase activity (see above), it may also be that therapies targeting GCase dysfunction and its associated pathology are useful in other groups of PD patients as well (43).

As is discussed above, many studies suggest that reduced GCase enzyme activity is important in the pathogenesis of *GBA1* mutation-associated PD (35, 39–42). One approach, therefore, may be to augment enzyme activity, as is done in the treatment of non-neuronopathic GD (98). Enzyme replacement therapy and small molecule substrate reduction therapies are useful for the peripheral manifestations of GD, but do not cross the blood–brain barrier, therefore are unlikely to provide any meaningful improvement in the course of PD (99).

A number of small molecule chaperones have recently been investigated for their ability to restore GCase function by binding to the misfolded enzyme that is retained in the ER and facilitating its correct re-folding, thus increasing lysosomal GCase levels and reducing ER stress. One of these compounds, isofagamine, yields an improvement in motor function, reduction in microglial activation, and a reduction in α-synuclein aggregation in α-synuclein over-expressing mice (100). This demonstrates that these compounds have the potential to alter central nervous system pathology. Another chaperone, ambroxol hydrochloride, is currently being investigated as a potential treatment for *GBA1* mutation-associated PD. Ambroxol has been used as an expectorant since the 1980s and has been shown to increase lysosomal GCase levels and activity in several *in vitro* models (65, 71, 101, 102). Binding of ambroxol to GCase is pH-dependent, being strongest at the neutral pH of the ER, and allowing dissociation to occur in the acidic pH of the lysosome (103). Additionally, it seems that this increase in GCase activity enhances α-synuclein clearance (65,102). However, ambroxol does not appear to cross the blood–brain barrier, at least at the doses that are used clinically, and it remains to be seen whether this approach can be useful in *GBA1* mutation-associated PD or GD (104).

As well as investigating GCase enhancement as a therapeutic option for *GBA1* mutation-associated PD, it may be possible to slow disease progression by targeting the downstream mechanisms by which *GBA1* mutations increase PD risk, such as lysosome-autophagy system dysfunction. Rapamycin, an immunosuppressant agent, is a well-established inducer of macroautophagy (105), which has been shown to reduce α-synuclein accumulation in a *GBA1* mutation model *in vitro* (56). While rapamycin is unlikely to be used as a treatment for PD in view of its adverse effect profile, other drugs that are able to correct the lysosome-autophagy defect may be useful in *GBA1* mutation-associated PD. This may involve repurposing of drugs that are already established as treatments for other conditions, which are found to have a beneficial effect on autophagy. Potential examples of these agents include the tricyclic antidepressant nortriptyline, the disaccharide trehalose, and the antihypertensive rilmenidine (106–113).

Gene therapy, in which the *GBA1* gene is introduced using a viral vector, has also been investigated in rodent models (46, 114). Memory impairment in a GD mouse model was reversed using this approach (114). However, given the widespread nature of pathology in *GBA1* mutation-associated PD, reflected by the increased incidence and degree of cognitive impairment, it will be challenging to achieve effective delivery of the gene to the relevant population of cells, and much more work is required before this approach is considered suitable for the clinic.

CONCLUSION

As we enter the era of genomic sequencing, our understanding of genetic risk factors for conditions such as PD is becoming increasingly important. There are currently no disease-modifying therapies available for PD, and in view of the heterogeneous pathogenic mechanisms present in PD patients, it is likely that different treatment approaches will be necessary for different groups.

Establishing the processes by which genetic susceptibility factors increase the risk of PD pathology provides the opportunity to develop specific therapies that target the relevant pathways in individual patients. *GBA1* is now recognized as one of the most important genetic risk factors for PD, both numerically and clinically, with it leading to a greater degree of cognitive impairment and other non-motor features, all of which lead to a reduction in the quality of life in this population. Identification of drugs that are able to attenuate *GBA1* mutation-induced pathology can therefore provide significant clinical benefit, and investigations toward this are under way.

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4 Apoptosis and its Role in Parkinson's Disease

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Abstract: Parkinson's disease is one of the most common neurodegenerative diseases in the elderly. The motor symptoms occur predominantly due to substantial dopamine depletion, caused by degeneration of the dopaminergic neurons in substantia nigra pars compacta. Apoptosis has been implicated as the main mechanism of neuronal death in Parkinson's disease. Apoptosis is mediated by a number of initiator and executioner caspases, and occurs via the intrinsic or extrinsic pathways. Activation of initiator caspase-9 mediates the intrinsic pathway—also called the mitochondria-mediated pathway. Alternatively, activation of initiator caspase-8 mediates the extrinsic apoptotic pathway—the cell death receptor–mediated pathway. Both initiator caspases converge onto a common pathway of executioner caspases, involving caspase-3 and caspase-6. Activation of the executioner caspases leads to the morphological features characteristic of apoptosis, such as DNA cleavage and its subsequent fragmentation. Proapoptotic factors, such as Bax, have been implicated in neuronal cell death in Parkinson's disease, and there is evidence that both the intrinsic and extrinsic apoptotic pathways may play a role. This chapter provides an overview of apoptosis and its significance in Parkinson's disease.

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INTRODUCTION

Neuronal death occurs during normal development and in response to a myriad of pathological factors, such as traumatic injury (1), ischemia (2), infectious agents (3), or genetic aberrations (4). The major mechanisms by which neurons may die are apoptosis and necrosis. Apoptosis is the predominant mode of neuronal death in many neurodegenerative diseases (5, 6), including Parkinson's disease (7). Whilst the pathogenic processes of Parkinson's disease are not completely understood, convergent mechanisms result in neuronal death through apoptosis, making apoptotic pathways interesting potential therapeutic targets. Apoptotic cell death has been observed in cell culture and animal models of Parkinson's disease, and also in nigrostriatal regions of the brains of patients with Parkinson's disease at postmortem (8–10). This chapter provides an overview of apoptosis and its role in Parkinson's disease.

APOPTOSIS

Apoptosis—the major pathway for programmed cell death—can be initiated by a number of broad classes of death stimuli, including abnormal intracellular calcium concentrations (excitotoxicity) (11), afferent or efferent trophic factor deprivation (12), activation of death receptors (13), and stress (12). Neuronal apoptosis is common during development and maturation, and is essential for shaping of the nervous system and development of appropriate circuitry (14). Apoptosis consists of a sequence of events, which are energy dependent. It is characterized by specific morphological and biochemical changes, including shrinkage of the cell, the chromatin becoming condensed, nuclear DNA fragmentation, and formation of apoptotic bodies, which contain nuclear material. During this process, the cell membrane retains its integrity. Apoptotic bodies are eventually removed by phagocytosis, importantly without a consequent inflammatory response (15, 16). Biochemically, apoptosis is characterized by increased rates of protein degradation (17, 18) and increased caspase activity (19). The biochemical components of the apoptosis pathways were first described in genetic studies on the nematode, *Caenorhabditis elegans* (20, 21), with subsequent studies identifying the mammalian homologues (22–24). These apoptotic biochemical components are a group of molecules called the B-cell lymphoma (Bcl-2) family, apoptotic peptidase activating factor (Apaf-1), and caspases (25).

Caspases

Caspases constitute a family of at least 14 cysteine proteases that regulate apoptosis (26). Caspases are present in normal cells as inactive zymogens, which are activated in response to apoptotic stimuli. In general, a single peptide precursor is

cleaved, via one or two chronological proteolytic steps, into an active enzyme, which consists of large and small subunits (27). Caspases can be subdivided into three functional categories: (i) inflammatory caspases-1, -4, -5, -11, -12, -13, and -14, are involved in immune responses to microbial pathogens by mediating the proteolytic activation of inflammatory cytokines (28, 29); (ii) apoptotic initiator caspases-2, -8, -9, and -10, have long pro-domains containing a caspase activation and recruitment domain (e.g., caspase -2 and -9), or a death effector domain (e.g., caspase -8 and -10); and (iii) apoptotic executioner caspases-3, -6, and -7, have short pro-domains. Initiator caspases, which are involved in the initiation of apoptosis, are able to carry out auto-cleavage and the cleavage and activation of common downstream executioner caspases (30). Executioner caspases do not have the ability to perform auto-cleavage, so their activation is dependent on this cleavage step. Once activated, the executioner caspases carry out the downstream events of apoptosis by cleaving a number of cellular substrates (30).

Caspases mediate several intracellular events that are important in apoptosis. These include:

(i) Disabling homeostatic and repair processes, such as DNA repair (31)
(ii) Cessation of cell cycle progression (31)
(iii) Signal amplification and inactivation of apoptosis inhibitors, through cleavage of pro- and antiapoptotic proteins (32)
(iv) Facilitation of nuclear and cytoskeletal disassembly (31)
(v) Marking dying cells for engulfment and disposal (31).

In addition, caspases have been shown to cleave Ca^{2+}-AMPA glutamate receptors, thereby preventing Ca^{2+}-mediated excitotoxicity and subsequent necrosis of neurons (33). Though some studies have suggested that caspases may play a role in necrotic death in some circumstances (34), in general they divert the cell to an apoptotic, rather than necrotic, fate (33, 35).

Apoptotic pathways

Caspase activation can be triggered by two well-characterized apoptotic pathways: the mitochondria-mediated (intrinsic) pathway (Figure 1), and the cell surface death receptor (extrinsic) pathway (Figure 2) (36). The intrinsic apoptotic pathway is mediated by members of the Bcl-2 family and the permeability transition pore (PT-pore) (Figure 1) (37). Bcl-2 is a family of proteins that possess either proapoptotic (e.g., Bax) or antiapoptotic (e.g., Bcl-2) properties. Members of this family exist on the cytoplasmic surface of mitochondria as well as many other organelles (38), and act as regulators of the PT-pore (39, 40). Opening of the PT-pore at contact sites between the inner and outer mitochondrial membranes results in depolarized mitochondria, loss of small molecular weight substances from the matrix, and ruptured outer mitochondrial membrane as a result of osmotic mitochondrial enlargement (41). The proapoptotic Bcl-2 family proteins induce outer mitochondrial membrane permeabilization, leading to release of cytochrome c, which normally exists in the mitochondrial intermembranous space (42). When released in the cytosol, it is bound by a protein called Apaf-1 in an ATP-dependent fashion, resulting in the formation of a multimeric Apaf-1/cytochrome c complex. The formation of the Apaf-1/cytochrome c complex is considered the commitment event that makes caspase activation irreversible,

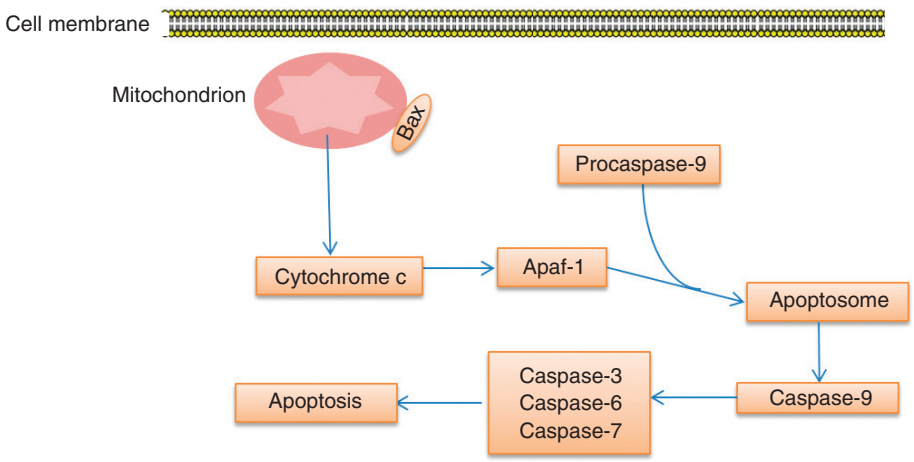

Figure 1 The intrinsic apoptotic pathway. In response to apoptotic stimuli, proapoptotic proteins, such as Bax, induce the permeabilization of the outer mitochondrial membrane, leading to release of cytochrome c from the mitochondrial intermembranous space. Cytochrome c is then bound to Apaf-1, resulting in the formation of a multimeric Apaf-1/cytochrome c complex that recruits procaspase-9 forming the apoptosome. Consequently, procaspase-9 is activated through proteolysis and subsequently dissociated from this complex. Once activated, caspase-9 activates executioner caspases-3, -6, and/or -7, which mediate proteolytic events that eventually lead to apoptosis.

as this complex recruits procaspsase-9, resulting in formation of the apoptosome (43). Procaspase-9 is then activated through proteolysis (42). Once activated, caspase-9 dissociates from this complex and subsequently activates executioner caspases, -3, -6, and/or -7 (43). The construction of an Apaf-1/cytochrome c complex sets a relatively high threshold for caspase activation, preventing inadvertent commitment to apoptotic death due to leakage of cytochrome c from the mitochondria (43).

The extrinsic apoptotic pathway is dependent on the activation of cell surface death receptors (Figure 2). These constitute a group of trans-membrane proteins that belong to the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily. These receptors possess extracellular domains which include a highly conserved cysteine-rich repeat. Structurally associated molecules belonging to the TNF superfamily are the activating ligands for these death receptors (e.g., FAS ligand) (44). Binding of activating ligands to the receptors results in receptor trimerisation and recruitment of specific intracellular receptor-associated proteins, such as procaspase-8. Procaspase-8 is then immediately cleaved into the active form (caspase-8) that comprises two catalytic subunits which are able to activate downstream executioner caspases (45).

The downstream steps in the apoptotic pathways are then mediated by the executioner caspases, which cleave a large number of specific substrates (46). For instance, caspase-3 and caspase-7 inhibit DNA repair by cleaving the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which normally participates in DNA repair (47). Caspase-3 also degrades DNA-dependent protein kinase

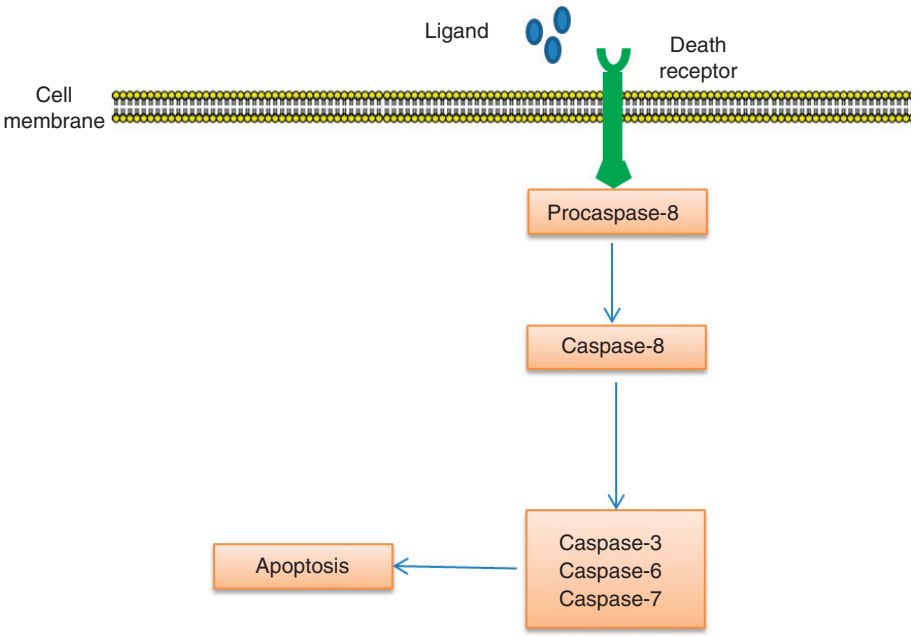

Figure 2 The extrinsic apoptotic pathway. Specific death signal ligands bind to death receptors, resulting in receptor trimerisation, and subsequent recruitment of specific intracellular receptor-associated proteins, such as procaspase-8. Procaspase-8 is then immediately cleaved into the active form, which is able to activate downstream executioner caspases-3, 6, and/or 7 that mediate proteolytic events of cellular proteins and structures eventually leading to apoptosis.

(DNA-PK), leading to reduced DNA repair capacity of the cell and subsequent promotion of the characteristic DNA cleavage that occurs in apoptosis (48). Furthermore, caspase-3 digests cytoskeletal proteins, such as actin and fodrin inducing cell shrinkage and membrane blebbing (49). Caspase-3 also leads to chromatin condensation and nuclear fragmentation through proteolytic activation of protein kinase C delta (50). Caspase- 6 cleaves lamins, the main structural proteins of the nuclear envelope, resulting in nuclear shrinkage and the ultimate formation of apoptotic bodies (51). Morphological features of apoptosis include chromatin condensation, which starts peripherally along the nuclear membrane forming a ring-like structure, internucleosomal fragmentation of double-stranded DNA, and nuclear fragmentation (52). In addition, other morphological characteristics of apoptosis are membrane blebbing (53), cell shrinkage (54), and formation of apoptotic bodies, which are tightly packed with cytoplasmic organelles and nuclear fragments, and are ultimately engulfed by neighboring cells without provoking inflammation (55). The chief molecular components of apoptosis in neurons are the same as those in other nonneuronal cell types (56).

APOPTOSIS IN PARKINSON'S DISEASE

Apoptosis is the main mechanism of neuronal loss in Parkinson's disease, as evidenced by the identification of DNA fragmentation and apoptotic chromatin changes in dopaminergic neurons of Parkinson's disease patients in postmortem studies (10). In addition, the role of apoptosis in the pathogenesis of Parkinson's disease was confirmed in postmortem and *in vitro* studies that illustrated elevated activity of caspase-3 and increased expression of active caspase-3 in substantia nigra pars compacta (57–59). Furthermore, dopaminergic neuronal death is inhibited by overexpression of anti-apoptotic proteins, such as Bcl-2, in cell models of Parkinson's disease (60). Caspase inhibitors have also been shown to rescue neurons from death in cell models of Parkinson's disease, adding further support to the notion that apoptosis is the main mechanism of neuronal death in Parkinson's disease (61). Elevated levels of proapoptotic proteins, such as Bax, have also been seen in postmortem brain tissue from Parkinson's disease patients (62).

Whilst there is some suggestion that the extrinsic apoptotic pathway may be active in Parkinson's disease, its role remains unclear. The predominant mechanism of neuronal death is thought to be the intrinsic apoptotic pathway. Mitochondria-mediated apoptosis has been extensively studied in Parkinson's disease. It involves a sequence of events including increased generation of reactive oxygen species, cytochrome c release and ATP depletion, as well as caspase-9 and caspase 3 activation (63). It remains unclear as to how the multiple pathogenic processes of PD such as alpha-synuclein (α-synuclein) aggregation and mitochondrial dysfunction, for example, interact with one another to converge toward apoptotic cell death. In the remainder of this section, some of the possible triggers of apoptosis in Parkinson's disease are discussed. These include the interaction of α-synuclein with the mitochondrial membrane, the presence of nuclear DNA mutations, accumulation of mitochondrial DNA deletions, and mitochondrial dysfunction through other mechanisms (64).

α-synuclein is abundantly expressed in the central nervous system, particularly presynaptically (65). It is prone to fibrillar aggregation forming a major component of the Lewy bodies that are the pathological hallmark of Parkinson's disease (65). α-synuclein aggregates and inclusions are formed in Parkinson's disease brains, and rodents and cells treated with mitochondrial toxins (66–68). Accumulation of wild-type α-synuclein in dopaminergic neurons leads to decreased activity of mitochondrial complex I and increased reactive oxygen species generation—an effect which is more pronounced by the expression of the aggregation-prone mutant A53T α-synuclein (69). α-synuclein has also been shown to localize to the mitochondrial membrane in SHSY cells overexpressing A53T mutant or wild-type α-synuclein, and in isolated rat brain mitochondria (70), and this interaction has been suggested to lead to oxidative stress and the release of cytochrome c into the cytosol, in *in vitro* systems. Subsequent to its release into the cytoplasm, cytochrome c interacts with pro-survival, antiapoptotic proteins, triggering mitochondria-mediated apoptosis (70, 71).

Indeed, mitochondrial dysfunction may be an early occurrence in humans and in animal models of Parkinson's disease (72–74). A defect in the activity of mitochondrial complex I has been observed in substantia nigra of Parkinson's disease

patients (75). Dopamine metabolism leads to the generation of reactive oxygen species, which may lower the threshold for apoptotic cell death (76–78). Dopamine is enzymatically metabolized by monoamine oxidase (MAO), leading to the production of H_2O_2, which subsequently yields reactive oxygen species (76–78). Degradation products of dopamine undergo autoxidation, leading to increased reactive oxygen species generation (76–78). Hence, nigral dopaminergic neurons are particularly susceptible to dysfunction of mitochondrial complex I (79), which is believed to be one of the principal sources of reactive oxygen species in Parkinson's disease. Reactive oxygen species production may therefore represent a potential important mechanism contributing to dopaminergic neuronal death through apoptosis (80). Defects in the activity of mitochondrial complex I are proposed to increase the susceptibility of dopaminergic neurons for degeneration, through lowering of the threshold for activation of the intrinsic apoptotic pathway (62, 81–83).

A number of mitochondrial toxins result in selective degeneration of dopaminergic nigral neurons through apoptosis, lending support to the idea that these neurons are particularly susceptible to mitochondrial dysfunction. These include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and 6-hydroxydopamine (6-OHDA), which inhibit mitochondrial complex I causing mitochondrial dysfunction and generation of reactive oxygen species (8, 84, 85).

Dopamine itself is suggested to inhibit mitochondrial complex I, resulting in mitochondrial dysfunction (86). It undergoes autoxidation causing the excessive production of toxic metabolites that lead to oxidative stress and mitochondrial swelling and subsequent opening of the mitochondrial transition pore, which results in the release of anti- and proapoptotic factors (87, 88). Hence, cytochrome c is released into the cytosol, where it induces the intrinsic apoptotic pathway (87, 89, 90). It is also associated with significant increase in p53 phosphorylation, which is suggested to induce apoptosis (91, 92). Addition of antioxidants inhibits the activation of caspase-9 and caspase-3 and prevents apoptosis in response to dopamine exposure, supporting the fact that reactive oxygen species are important in dopamine-induced apoptosis (87, 90). Furthermore, overexpression of the antiapoptotic factor Bcl2 can partially attenuate dopamine-induced apoptosis (93).

MPTP is a neurotoxin that is selective to dopaminergic neurons of the substantia nigra pars compacta (94). MPTP is a lipophilic substance that actively crosses the blood–brain barrier to enter the central nervous system, where it is transformed to its active metabolite called MPP+ (1-methyl-4-phenylpyridinium) (95). This conversion is carried out by MAO that is present in the glial cells (95). Following its reuptake by dopamine transporter, MPP+ builds up in the mitochondria of dopaminergic neurons inhibiting the mitochondrial complex I, leading to ATP depletion and increased generation of reactive oxygen species (96, 97). As a consequence, nigrostriatal dopaminergic neurons die via apoptotic pathways involving caspases (98). MPTP-induced apoptosis is characterized by reactive oxygen species generation, cytochrome c release, p53 expression, cleavage of caspase-3, and DNA fragmentation, as well as by other morphological features characteristic for apoptosis (59, 99). MPTP-induced apoptosis is attenuated by overexpressed Bcl-2 levels (100, 101).

Similarly, rotenone inhibits mitochondrial complex I, resulting in the overproduction of reactive oxygen species and oxidative stress (102). Consequently, depletion in

ATP levels occurs resulting in selective nigrostriatal dopaminergic degeneration via mitochondria-mediated caspase-dependent apoptosis (102).

6-OHDA inhibits mitochondrial complex I, induces Bax, and causes activation of caspase-3 and caspase-9 (103). 6-OHDA-induced dopaminergic neuronal degeneration is attenuated by caspase inhibitors (104). 6-OHDA also induces apoptosis that occurs via a mitochondria-dependent pathway (85). Whilst these nigral toxin models are not necessarily truly reflective of the pathogenic mechanisms that are occurring in patients, they offer insight into the susceptibility of nigral neurons to mitochondria-mediated apoptosis.

A number of inherited forms of Parkinson's disease occur due to mutations in genes related to mitochondrial health and function. These include mutations in *Parkin*, *LRRK2*, *PINK1,* and *DJ-1,* for example (105). Whilst these mutations are rare within the Parkinson's disease population, they offer some supportive evidence to the fact that nigral neurons are susceptible to mitochondrial damage and mitochondria-mediated apoptosis, and that these processes may be relevant in idiopathic Parkinson's disease.

Parkin deficiency results in mitochondrial dysfunction in mice (106). Parkin has many roles that are potentially relevant in Parkinson's disease pathogenesis. For example, it can promote mitochondrial biogenesis, mtDNA replication, and transcription of mitochondrial genes (107). Thus, *Parkin* is vital for mitochondrial respiration and function (107). In addition, *Parkin* acts as an E3 ubiquitin protein ligase that targets particular substrates for degradation via the ubiquitin-proteasome system, including the glycoslyated form of α-synuclein (108). The loss of *Parkin* activity is thought to contribute to the buildup of toxic protein aggregates causing Parkinson's disease (108). Interestingly, *Parkin* acts downstream of one of the other aforementioned genes—*PINK1*—a mitochondrial kinase in which mutations can cause an autosomal recessive familial form of early onset Parkinson's disease. This is demonstrated by the fact that *Parkin* overexpression can compensate for mutations in *PINK1* (109, 110). Whilst the mechanisms by which these mutations precipitate Parkinson's disease pathology are unclear, there is some evidence that the *PINK1-Parkin* pathway may play a role in susceptibility to mitochondria-mediated apoptosis. For example, upregulation of wild-type *PINK1* reduces cytochrome c release and caspase activation (111, 112).

Mutations in *DJ1*, which is present in the mitochondrial matrix and intermembranous space, can cause early onset Parkinson's disease (113). Lack of *DJ1* increases susceptibility to free radical-associated injury (114), whilst overexpression of wild-type *DJ1* can be protective (115). Mutations in *DJ1* result in increased oxidative stress. In addition, mutant DJ-1 binds very tightly to mitochondrial Bcl-XL, which is an antiapoptotic protein, resulting in dissociating Bax from Bcl-XL and its subsequent enrichment in the outer mitochondrial membrane, leading to the dopaminergic neuronal degeneration via mitochondria-mediated apoptosis (116).

In vitro studies have suggested a toxic gain of function brought about by *LRRK2* mutations that cause Parkinson's disease (117). *LRRK2* mutation can lead to defective mitochondrial morphology and dynamics and increase generation of reactive oxygen species in cells (118). *LRRK2* mutations have been suggested to cause dopaminergic neuronal death by mitochondria-mediated apoptosis subsequent to mitochondrial dysfunction. Apoptosis can be induced *in vitro* by the

overexpression of mutant *LRRK2* with cell death being prevented by caspase inhibitors and genetic ablation of Apaf1 (61).

Mitochondrial DNA deletions have been observed in nigrostriatal dopaminergic neurons in aging and Parkinson's disease, possibly increasing their susceptibility to mitochondria-mediated apoptosis (119, 120). Mechanisms underlying mitochondrial DNA deletions are unknown with the possible involvement of oxidative stress (121). Combination of mitochondrial DNA depletion and deletion (without any alteration in the overall mitochondrial mass) results in reduced mitochondrial function and integrity, which increases the risk of cytochrome c release and apoptosis (122, 123). In addition, a rare form of inherited PD may occur due to mutations in the nuclear gene encoding DNA polymerase G (*POLG*), which plays an important role in the expression of a number of the genes encoded in mitochondrial DNA (124, 125).

THERAPEUTIC IMPLICATIONS

Given that the end-point of the Parkinson's disease pathogenic pathway is apoptotic neuronal death, treatments that target the molecular and biochemical events that allow progression of apoptosis may protect against the loss of dopaminergic neurons. As has been discussed, apoptosis is dependent on caspase activation (126). Thus, caspase inhibition has been considered as a novel therapeutic approach in neurodegenerative diseases occurring via apoptosis (126). Indeed, caspase inhibition prevents cell death of dopaminergic substantia nigra pars compacta neurons induced by MPTP or its active metabolite MPP+ *in vitro* and *in vivo* (127). However, although the dopaminergic neurons could be rescued, the nigrostriatal terminals were disrupted, suggesting that this approach may simply allow for the survival of dysfunctional neurons, suggesting that inhibition of apoptosis alone may in fact be detrimental (127). However, concomitant administration of glial cell line-derived neurotrophic factor (GDNF) circumvented this problem, allowing for restoration of striatal dopamine concentrations (127). It may therefore be feasible that caspase inhibition in combination with specific growth factors could play a role in future treatment of Parkinson's disease.

Interfering with events in the induction phase of apoptosis upstream to activation of caspases was regarded as strategy to prevent death of dopaminergic neurons and restore their function (128–132). For instance, Bax is upregulated in dopaminergic neurons subsequent to MPTP treatment (128). In addition, genetic deletion of Bax prevented dopaminergic neurodegeneration in the MPTP mouse model of nigrostriatal degeneration (128). Furthermore, Bax inhibition could decrease the loss of the nigral dopaminergic neurons that was caused by intrastriatal administration of 6-OHDA, suggesting Bax-inhibiting peptides as possible therapeutic avenue for Parkinson's disease (129).

The propargylamine derivative CGP 3466 (dibenzo[b,f]oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine) has been shown to possess neuro-rescuing and anti-apoptotic characteristics (130). CGP3466B suppresses neuronal apoptosis by upregulating protein-L-isoaspartate (D-aspartate) O-methyltransferase (PCMT1), which is an enzyme that repairs damaged L-isoaspartyl residues in intracellular proteins. Upregulated PCMT1 leads to overexpression of the antiapoptotic Bcl-2

and underexpression of the proapoptotic Bax and active-caspase3, and thus inhibiting mitochondria-dependent apoptosis (133). Concomitantly, it prevents dopaminergic cell death both *in vitro* and in rodent models of Parkinson's disease, and it consequently inhibits the development of MPTP- and 6-OHDA-induced motor symptoms (131, 132). Consequently, CGP 3466 may be promising in inhibiting dopaminergic neuron degeneration and the consequent progression of the neurodegenerative process in patients with Parkinson's disease (131, 132). Thus, treatments that interfere with the apoptotic pathways may represent promising therapeutic strategies in the protection against the loss of dopaminergic neurons and the subsequent pathogenesis of Parkinson's disease in the patients.

Having discussed these approaches, it must be acknowledged that there are concerns regarding the targeting of apoptosis in neurodegenerative disease. As has been discussed in this chapter, apoptosis in PD is thought to be triggered by a number of intracellular pathologies, with mitochondrial dysfunction being particularly important. Inhibition of apoptosis, therefore, may prevent the programmed removal of dysfunctional, nonviable neurons, which may ultimately lead to necrosis and a potential inflammatory response. In cell culture models of Parkinson's disease, treatment with caspase inhibitors did indeed trigger a switch from neuronal apoptosis to necrosis (134). In addition, although genetic deletion of Bax inhibited dopaminergic neuronal death in response to 6-OHDA in transgenic mice, it could not improve behavioral deficits that were associated with Parkinson's disease, and the surviving dopaminergic neurons displayed marked neuronal atrophy (135). Furthermore, systemic administration of an antiapoptotic compound may allow for the prolonged survival and accumulation of dysfunctional and potentially neoplastic cells in many tissues, which would clearly be detrimental. Thus, although apoptosis is the final step in the pathogenic pathway in PD, it remains to be seen whether or not inhibition of apoptosis in Parkinson's disease can be effective and safe, and cautious evaluation is necessary.

CONCLUSION

Apoptotic cell death is suggested to be involved in the pathogenesis of Parkinson's disease based on *in vitro*, *in vivo*, and human postmortem studies. Elucidation of the triggers of the apoptotic process in Parkinson's disease can lead to a better understanding of the sequence of events that result in programmed cell death in Parkinson's disease. Consequently, it would be possible to identify the potential factors that can be targeted therapeutically to stop or slow the progression of the disease and to recognize the individuals who are susceptible to developing Parkinson's disease at early and preclinical stages.

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5 Animal Models of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a heterogenous disease with a varying age of onset, symptoms, and rate of progression. This heterogeneity requires the use of a variety of animal models to study different aspects of the disease. Neurotoxin-based approaches include exposure of rodents or non-human primates to 6-OHDA, MPTP, and agrochemicals such as the pesticide rotenone, the herbicide paraquat, and the fungicide maneb. Acute exposure to neurotoxins induces motor deficits and rapid nigro-striatal dopaminergic cell death by disrupting mitochondrial function and/or increasing oxidative stress, while chronic administration of neurotoxins induces progressive models which can include alpha-synuclein (α-synuclein) aggregates. Genetic-based approaches to model Parkinson's disease include transgenic models and viral vector-mediated models based on genes linked to monogenic Parkinson's disease, including SNCA, LRRK2, UCH-L1, PRKN, PINK1, and and DJ-1, as well as manipulation of dopaminergic transcription factors. SNCA mutations, overexpression, and introduction of α-synuclein preformed fibrils induce toxic protein aggregates and variable nigro-striatal neurodegeneration and motor deficits, depending on the specific model. Species, genetic background of a

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strain, and environment affect the display of symptoms and neurodegenerative hallmarks of animal models. These models can be combined to study the interplay between genetics and environment and untangle the heterogeneity and mechanisms underlying Parkinson's disease. In this chapter, we discuss the strengths and limitations of mouse, rat, and non-human primate models of Parkinson's disease.

Keywords: Animal models; Genetic models; Neurotoxic models; Parkinson's disease; Non-human primates; Mouse, Rat

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized by loss of nigro-striatal dopaminergic neurons and aggregation of the α-synuclein-rich inclusions called Lewy bodies and Lewy neurites. The characteristic symptoms of PD are progressive motor deficits, including tremor, bradykinesia, akinesia, rigidity, postural instability, and gait difficulties. Non-motor symptoms, including depression, anxiety, sleep disturbance, cognitive decline, and anosmia are also prevalent in PD patients, and often occur prior to the onset of motor symptoms (1).

Although around 10% of all PD cases have a monogenic origin, the majority are idiopathic, with multiple contributing environmental and genetic risk factors. Similar to the etiology, the clinical phenotype of PD is heterogeneous. While motor and non-motor symptoms are clinically detectable, the brain pathology in humans can only be confirmed by examining post-mortem tissues (2). Thus, there is a great need for experimental models to deepen our understanding of this multifaceted disease and expand the currently limited treatment options. The heterogeneity of both PD etiology and pathology requires a diverse range of models that can replicate different aspects of PD in animals (2).

Two main approaches are used to model PD in experimental animals: neurotoxins and genetics. Neurotoxins can model dopaminergic neurodegeneration arising from environmental factors that have been implicated in PD. They generally induce a strong and rapid cell loss in the substantia nigra pars compacta (SNpc), elicit motor symptoms and behavioral changes, but lack the formation of Lewy bodies (3). By contrast, genetic-based models not only demonstrate variable cell loss and motor symptoms but can also exhibit α-synuclein pathology, depending on the specific model. Genetic mutations or changes in gene expression can be modeled using transgenic animals or be induced by viral transfection. Both neurotoxins and genetic approaches are applied in different animal species to model PD. This chapter provides an overview of the currently available animal models of PD.

SPECIES-SPECIFIC CHARACTERISTICS

There are three animal groups that are commonly used for modeling human diseases, and PD is no exception: rodents, non-human primates (NHP), and

non-mammalian species. Each group has its own advantages and limitations that determine the suitability for a given experiment. Understanding species-specific differences therefore facilitates experimental design as well as interpretation of behavioral observations and pathophysiology.

Rodents

Rodents are extensively studied across biomedical fields because they are convenient to care for in laboratory conditions and have associated robust experimental protocols, including different forms of drug administration, generation of transgenic strains, and behavioral assessments. The majority of 23,000 animal studies of PD published since 1990 involve rodents (Figure 1). An advantage of rodent PD models is that nigro-striatal dopaminergic degeneration correlates to motor deficits in mice and rats. These can be observed and measured with a series of behavioral tests most of which involve measuring movement, grip, or strength of the front paws. Behavioral tests in rodents include the open field test for a general assessment of locomotor activity, the stepping test to measure akinesia, and the pole test to measure bradykinesia (4, 5). Strength is measured by grip strength and grip coordination tests (5). It is difficult to directly measure rigidity in rodents, but the performance on the rotarod test accounts for multiple factors such as balance, strength, and coordination (5).

Rodents with unilateral lesions will display asymmetric motor behavior where deficits in contralateral limb use can be measured and compared to the ipsilateral limb as an internal control. Drug-induced circling behavior is a more dramatic measure of a unilateral lesion (5, 6). In these tests, administration of amphetamine stimulates the release of dopamine in the intact contralateral side, resulting in ipsilateral rotation, while apomorphine causes contralateral rotations due to dopamine hypersensitivity of the lesioned side. Tremor and posture are often described qualitatively, and tremor monitors can be used to record shaking frequency (5). Gait can be assessed with paw print, but this is rarely done (5). Many of these motor tests can also be used to assess dyskinesia.

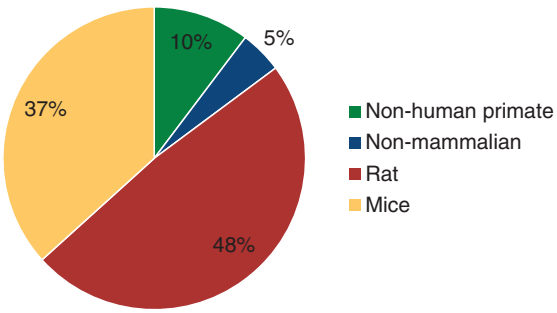

Figure 1 Proportion of animal models used in 23,000 research articles on Parkinson's disease published from January 1990 to June 2018. Numbers of original article publications were obtained from following searches on Web of Science: "Parkinson AND (mice OR mouse)"; "Parkinson AND rat"; "Parkinson AND (primate OR monkey)"; "Parkinson AND (non-mammalian OR drosophila OR elegans)."

To study non-motor symptoms in rodents, the preferred method is using a model such as a partial nigro-striatal lesion, which does not cause concurrent motor deficits that may affect the test results. Sleeping, drinking, and eating patterns are monitored to assess sleep disturbance and weight loss (7). To model neuropsychiatric symptoms, a panel of complementary tests can be used, where the tail suspension test or the forced swim test is used to model depression or behavioral despair (7). Excessive grooming is a stereotypy signaling anxiety or compulsive behavior, and reduction in mouse species-specific nest building behavior can be used to model motivation and goal-oriented tasks (5).

Non-human primates

Studies of NHP give valuable insights into PD pathology owing to their anatomic and genetic similarity to humans (8). Compared to rodents, NHP are larger, have a longer life span, require more demanding care, incur higher costs, and involve more complex ethical considerations. Based on publications, only 10% of animal studies on PD are performed in NHP (Figure 1) and these are often reserved for preclinical evaluation of therapies (8). NHP models are currently based on neurotoxic or viral vector-mediated PD pathology, which generally induces parkinsonian symptoms and behaviors that are similar to those in humans. Therefore, a Unified Parkinson's Disease Rating Scale (UPDRS)-like measure can be used to assess the severity of the phenotype (9). However, unlike the clinical scale used for PD patients, these assessments are not standardized worldwide.

The NHP used most commonly in PD studies include macaques (10) followed by common marmosets. These species are convenient to use because of their smaller size, high reproductive efficiency, and relative ease of care and housing in laboratory conditions (11). Squirrel monkeys, African green monkeys, and baboons have also been used to model PD (10). PD models in the old world species such as macaques exhibit Levodopa-induced dyskinesia, similar to human chorea and dystonia, which are better distinguished than in new world monkeys such as marmosets (10). Other examples of NHP species-specific behavior include marmosets' jumping in the tower test to assess akinesia, and the righting reflex in the hourglass test to assess axial rigidity (12). Pre-diagnostic non-motor symptoms affecting sleep or social behavior have been preferentially studied in macaques since they are diurnal and better replicate human sleeping patterns, in contrast to rodents which have higher nocturnal activity (13). Social behavior changes, such as increased aggressiveness, can be assessed by monitoring facial expressions in female macaques (14).

In addition to behavioral assessments, NHP can be used for neuroimaging studies (15). These are especially valuable during preclinical drug trials, because these can be compared to patients during clinical phases (16). Thus, NHP models have many advantages but are demanding in terms of resources.

Non-mammalian species

Non-mammalian models, including drosophila and *Caenorhabditis (C.) elegans*, have been used in a small proportion of experimental PD studies over the past decades (Figure 1). These models, however, have several advantages such as easily generated genetic manipulations, a rapid reproductive cycle, low costs of maintenance, and well-defined neuropathology and behavior.

A variety of transgenic drosophila and *C. elegans* models have been generated (17, 18). Due to the low cost and rapid completion of experiments, these models can be used for large screens for drug discovery. Furthermore, *C. elegans* models have the advantage of a fully mapped connectome possessing only 302 neurons, out of which only 8 are dopaminergic. Drosophila's larger connectome containing 135,000 neurons is also currently being mapped. Therefore, these models can be used to study fundamental principles governing cellular, genetic, and network changes resulting from dopaminergic loss. Since studies in non-mammalian species are, so far, used in a small fraction of PD models and can be challenging to translate to humans, this chapter will focus on the most commonly used neurotoxic and genetic PD models in rodents and NHP.

NEUROTOXIC MODELS

Several PD models are based on degeneration of dopaminergic neurons induced by local or systemic administration of neurotoxins. The first to be discovered was 6-hydroxydopamine (6-OHDA) (19), initially used in the periphery to degenerate sympathetic nerves (20), and later in the brain to model PD. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered through cases of chemically induced parkinsonism after failed synthesis of the opioid drug 1-methyl-4-phenyl-4-propionpiperidine (MPPP) (19). Reports of an increased risk of PD in populations exposed to pesticides for agricultural purposes (21) have resulted in additional neurotoxin-based PD models and studies on environmental risk factors of PD (Table 1).

MPTP

MPTP is a commonly used neurotoxin for modeling PD and can be administered acutely or chronically by different routes (22). It easily crosses the blood–brain barrier (BBB) within minutes due to its lipophilic nature. Glial cells take up MPTP, where it is converted first to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) by monoamine oxidase-B (MAO-B) followed by spontaneous oxidation into the toxic agent 1-methyl-4-phenylpyridinium (MPP+) (23). Once released back into the parenchyma through the organic cation transporter 3, MPP+ is transported selectively into dopaminergic cells through the dopamine transporter (DAT) and accumulates in the cytoplasm and in vesicular monoamine transporter (VMAT+) vesicles (24). MPP+ blocks mitochondrial complex I, which reduces ATP production, increases oxidative stress, and eventually causes cell death and neuroinflammation (23). This mechanism of action has contributed to the study of dopaminergic neurodegeneration, mitochondrial dysfunction, oxidative stress, and neuroinflammation in PD.

MPTP has been used to model PD in mice and NHP. However, rats are resistant to moderate doses of MPTP, while higher doses increase the mortality rates (26). The reliability and accessibility of the mouse MPTP model makes it a popular choice in PD research. The extent of the lesion depends on the dosage, route of administration, and the mouse strain used (33). Large lesions are useful to model late stages of PD for preclinical studies in drug discovery and development of other treatment strategies, such as deep brain stimulation (DBS), cell transplantation,

TABLE 1 **Summary of characteristics, uses, and limitations of neurotoxic models**

PD model	Characteristics	Uses	Limitations
MPTP Neurotoxin: Inhibition of complex I (23)	Acute dose: (10, 22) - No α-synuclein aggregates - Rapid and strong dopaminergic neurodegeneration - Strong motor deficit Subacute/chronic dose: (10, 25) - Progressive model - α-synuclein aggregates - No dopaminergic neurodegeneration - No motor deficit	- Common preclinical model - Systemic injection (bilateral parkinsonism) - Used in mice and NHP (24)	- Rats are resistant (26) - Functional recovery in mice and NHP (5, 10)
6-OHDA Neurotoxin: Inhibition of complex I and oxidative stress (19)	Injection site-dependent lesion: (23) - No α-synuclein aggregates - Rapid and strong dopaminergic neurodegeneration - Strong asymmetric motor deficits	- Common preclinical model - Intracranial injection into SNpc, striatum, or medial forebrain bundle - Hemiparkinsonism (19)	- High mortality with bilateral injection (24)
Rotenone Pesticide: Inhibition of complex I	- α-synuclein aggregates - Moderate dopaminergic neurodegeneration - Some motor deficits (27)	- Model environmental risks - Evaluate risk of pesticides	- Toxicity in humans remains controversial (28)
Paraquat and maneb Herbicide and fungicide: Oxidative stress (29)	- α-synuclein aggregates - Some dopaminergic neurodegeneration - Some motor deficit (30, 31)	- Model environmental risks - Evaluate risk and interaction of pesticides	- Toxicity in humans remains controversial (28) - High doses of paraquat cause pulmonary fibrosis (32)

NHP: non-human primates, SNpc: substantia nigra pars compacta.

and gene therapy (24). Most commonly, mice are administered an acute dose of MPTP by intraperitoneal (i.p.) injection which causes a loss of dopaminergic neurons, specifically in the SNpc rather than nearby regions such as the ventral tegmental area (VTA) (3). The neurodegeneration occurs within hours and stabilizes within 7 days. Four injections of an acute dose (up to 20 mg/kg) with 2-hour intervals cause 90% of striatal dopamine depletion and 70% loss of dopaminergic neurons in the SNpc but no α-synuclein aggregates (22).

MPTP-induced dopaminergic degeneration in mice correlates with motor deficits. However, these deficits can recover within a few days post-acute injection, which creates limitations on the duration of behavioral studies (5). The extent of

degenerative effects of MPTP and functional recovery seems to be partially determined by the mouse strain used, that is, the genetic background (34). The MPTP model has also been used in studies on gut microbiota dysbiosis in PD. Fecal microbiota transplants from MPTP-treated mice reduced striatal dopamine levels and induced motor impairments in non-wild-type mice, while transplants of normal microbiota alleviated PD-like symptoms in MPTP-treated mice (35).

As an alternative to the acute mouse model, repeated daily i.p. injections of subacute doses result in delayed nigro-striatal neurodegeneration and progressive build-up of α-synuclein inclusions (25, 36). The progressive nature of prolonged MPTP exposure in mice resembles PD, including neuroinflammation, α-synuclein inclusion pathology, and molecular mechanism preceding cell death. By contrast, these models do not show apparent motor deficits.

MPTP is the gold standard for PD models in NHP (24). Most commonly, bilateral parkinsonism is induced through multiple low-dose systemic injections (10), but hemiparkinsonism can also be induced by intracarotid infusion (37). Usually, 1–2 mg/kg of MPTP is administered systemically over days to months. Symptoms develop over several months, and are then stable for another few months (10). A chronic MPTP model in NHP also exists, comparable to the subacute MPTP mouse models. This is induced in NHP by daily doses of MPTP of 0.2 mg/kg over 2–3 weeks and is used to model the progressive pre-symptomatic dopaminergic cell loss seen in early stages of PD (10). Just like with the MPTP mouse model, NHP recover function a few months post-injection (10). However, another round of MPTP exposure can be given to prolong the experiment.

Thus, in NHP, MPTP can cause dose-dependent dopaminergic degeneration in the SNpc and putamen as well as α-synuclein aggregates that closely mimic PD pathology, and the extent of the lesion determines the severity of motor deficits (3). The neuropathology and symptoms are, however, more pronounced in PD patients (10). The presence of tremor correlates with the dopamine depletion in the basal ganglia and spontaneous firing, as seen in PD patients. Also, similar to PD patients, administration of L-dopa initially rescues motor deficit in NHP, but prolonged use elicits dyskinesia. Bilateral MPTP parkinsonism also displays some extra-nigral pathology, such as anosmia (38) and sleep disturbance (39). Thus, the resemblance of MPTP models in NHP to PD in humans makes them suitable for preclinical research of therapeutic strategies that have previously been validated in rodents.

6-OHDA

6-OHDA is an analogue of dopamine and norepinephrine. It is produced endogenously through the hydroxylation of dopamine metabolites and has been found in the human caudate nucleus (6, 24). 6-OHDA does not cross the BBB and needs to be administered directly into the brain to induce neurodegeneration. It enters dopaminergic and noradrenergic neurons through monoamine transporters; therefore, the injected solution must contain selective noradrenergic reuptake inhibitors (e.g., desipramine) to selectively target dopaminergic neurons (6). 6-OHDA induces cell death through oxidative stress by inhibiting mitochondrial complex I and by producing reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (19). This reaction is catalyzed by iron, and toxicity can be prevented by iron chelating agents, such as

vitamin E and MAO-B inhibitors (selegiline) (19). The specifics of 6-OHDA neurotoxicity remain poorly understood, but many antioxidant agents have neuroprotective effects.

Intracerebral 6-OHDA injections in rodents and NHP allow the targeting of the SNpc, striatum, or medial forebrain bundle (23), and the generation of unilateral, hemiparkinsonian models (19). Bilateral injections often cause adipsia, aphagia, seizures, and high mortality (24). In rats, SNpc injections of 6-OHDA result in large dopaminergic degeneration within 24 hours and 90% striatal dopamine loss within a couple of days (6, 23). However, injected in the striatum, 6-OHDA causes a retrograde degeneration of nigro-striatal neurons over a span of 1–3 weeks (23). Unilateral injections lead to asymmetric motor deficits and rotational behavior, also used to assess L-dopa-induced dyskinesia (6, 23). The unilateral models allow the use of the non-lesioned ipsilateral limbs as an internal control.

NHP require serial intracerebral 6-OHDA injections, which can result in post-surgical complications (15). In marmosets, nine striatal injections of 8μg 6-OHDA induced rotational behavior indicative of unilateral nigro-striatal lesion, but symptoms recovered after 10 weeks. Doubling the number of injections delayed functional recovery to 17 weeks post-surgery. 6-OHDA NHP models do not display α-synuclein inclusions but have been used to study hyperactivity of the subthalamic nucleus, dyskinesia, anhedonia, and depressive behaviors (41). The 6-OHDA NHP models are also used to develop treatments, for example, DBS, neuroprotective agents, transplantation, and gene therapies (42, 43).

Pesticides and herbicides

The most recent and most debated neurotoxins used for modeling PD are pesticides and herbicides (44). Correlations between agro-chemical exposure in populations and increased risk for PD have been difficult to conclude due to a lack of details on exposure to particular agents (28). Emphasis has been made on rotenone, paraquat, and maneb as possible environmental causes of PD. So far, these pesticides and herbicides have been mostly used in rodents to try to model PD.

Both rotenone and paraquat are thought to cause dopaminergic degeneration by inducing oxidative stress. Rotenone probably acts through complex I, while paraquat exerts its toxicity through cellular redox cycling (29), despite being structurally similar to MPP+. It is still unclear how paraquat penetrates the BBB despite its high polarity (PQ2+). It seems that penetration is slow and inefficient as PET scans on macaques showed that the BBB prevents paraquat from penetrating the brain (45), and low levels of paraquat are seen after systemic exposure to rats (46). PQ2+ is not transported by DAT (47) but is converted to a monocation radical PQ+ through redox cycling. PQ+ seems to be the active agent penetrating the dopaminergic neurons through DAT and exerting cytotoxicity, as shown in cultured cells and mice (48). Despite the unclear mechanisms of action, rotenone and paraquat models are used to induce parkinsonian pathology and to study inhibition of inflammatory and oxidative stress pathways in adult rodents.

The rotenone rat model has gained some reliability as a pesticide model of PD due to its ability to cause dopaminergic degeneration, α-synuclein inclusions, and motor deficits (27). Daily i.p. injections of 3 mg/kg of rotenone for 6–10 days are sufficient to induce 45% loss of dopaminergic neurons in the SNpc, striatal

dopamine depletion, and motor symptoms such as bradykinesia, postural instability, and rigidity (49). Rotenone can also induce non-motor symptoms such as sleep disturbances in rats (50).

Chronic paraquat administration in mice induces some dopaminergic cell loss in SNpc but does not cause dopamine depletion in the striatum or clear motor deficits (51). High doses of paraquat are not suitable for modeling PD, since they cause pulmonary fibrosis which could have secondary effects on behavior (32).

Pesticide models can increase the understanding of how environmental factors can affect PD risk. The primary research focus is on the mechanisms of action and the interaction with other risk factors, for example, genetic risk, age, and environmental exposure to other agro-chemicals. The combination of the herbicide paraquat and the fungicide maneb can elicit selective dopaminergic cell loss, dopamine depletion in the striatum, aggregation of α-synuclein, as well as PD-like motor and non-motor symptoms in rodents. Repeated administration of 10 mg/kg paraquat and 30 mg/kg maneb to mice twice a week over the span of 6 weeks exacerbate parkinsonian pathology compared to each compound alone (30). The response to this combined exposure, however, varies between individuals from the outbred Sprague Dawley rat strain as a result of peripheral effects and/or genetics (31).

GENETIC MODELS

Genetics plays an important role in PD pathogenesis (see chapters 6 and 7). Disease-causing mutations have been identified through linkage analyses in familial PD, while genetic risk factors for idiopathic PD have been identified through association analyses in patients and controls (52). Monogenic PD can be inherited through autosomal dominant or autosomal recessive mutations with variable penetrance. However, *de novo* mutations of these genes can also be seen in patients with no family history of PD.

SNCA was the first gene to be linked to familial PD and together with the finding that the encoded protein α-synuclein is aggregated in Lewy bodies, this led to a breakthrough in PD research (53, 54). Since then, a lot of research has focused on α-synuclein models and pathology. The identification of additional monogenic PD mutations has been used to study the effects of mutated proteins and to develop new reliable animal models (Table 2). Most genetic models have only been effective at reproducing some of the PD hallmarks (Table 3). Development of new genetic tools, however, allows for the generation of new genetic-based models. One such example is viral vectors that can be used to introduce wild-type (WT) or mutated genes for targeted expression of a disease-associated protein.

α-synuclein and transgenic models

Aggregated α-synuclein protein is a main component of Lewy bodies in PD patients. The encoding gene, SNCA, was the first to be linked to familial PD, and was named PARK1 (Table 2). Three autosomal dominant SNCA point mutations that are fully penetrant have been identified: A53T, A30P, and E46K (62, 73).

TABLE 2 Summary of familial PD mutations that have been replicated in animal models

Locus	Gene	Mutation	Protein	Inheritance
PARK1	SNCA	A30P, A53T, E46K	α-synuclein	Dominant
PARK2	PRKN	Various mutations, exonic deletions, duplication and triplication	Parkin	Recessive
PARK4	SNCA	Duplication and triplication	α-synuclein	Dominant
PARK5	UCH-L1	I93M and S18Y	UCH-L1	Dominant
PARK6	PINK1	G309D, exonic deletions	PINK1	Recessive
PARK7	DJ-1	Homozygous exon deletion, L166P	DJ-1	Recessive
PARK8	LRRK2	G2019S, R1441C/G, and others	Dardarin	Dominant

SNCA: α-synuclein, UCH-L1: ubiquitin carboxyl-terminal hydrolase isozyme L1, PINK1: serine/threonine-protein kinase PINK1, DJ-1: protein/nucleic acid deglycase DJ-1, LRRK2: leucine-rich repeat serine/threonine-protein kinase 2.

TABLE 3 Characteristics, uses, and limitations of genetic models for Parkinson's disease

PD model	Characteristics	Uses	Limitations
SNCA transgenic rodents Point mutations (A53T, A30P, E46K) and overexpression of α-synuclein (ASO)	Mice (55) and rats (56) - Widespread α-synuclein aggregation - No dopaminergic neurodegeneration - Some motor deficits	- Models familial mutations of SNCA - Study α-synuclein function/ propagation and synucleiopathies	- May affect development (57) - No transgenic NHP
Viral transfection of α-synuclein Adeno-associated virus and lentivirus vectors	Mice, rats, and NHP: (58) - α-synuclein aggregates - Moderate dopaminergic neurodegeneration - Moderate motor deficits	- Administered into SNpc - Study α-synuclein function, propagation, and synucleiopathies - Potential preclinical use	- Potential vector toxicity and interference of viral vector
α-synuclein preformed fibrils Seeding of α-synuclein aggregates	Mice, rats (59), and NHP (60): - Widespread α-synuclein aggregation - Mild dopaminergic neurodegeneration - Lack of motor deficits in rats and NHP, some in mice	- Administered to the striatum (59) - Study α-synuclein function/ propagation and synucleiopathies - Can be combined with α-synuclein viral vector (61)	- Slow/weak onset of pathology (59)

TABLE 3 **Characteristics, uses, and limitations of genetic models for Parkinson's disease** (Continued)

PD model	Characteristics	Uses	Limitations
LRRK2 (WT, G2019S, R1441C) Transgenic and viral vector-mediated	Mice and rats: (3, 62) - Affects inclusion formation - Little dopaminergic neurodegeneration - Most lack motor deficits	- Study the role of LRRK2, its interaction with α-synuclein, and toxicity of mutant LRRK2	- Large gene size prevents the use of AAV vectors (58) - No NHP models
UCH-L1 I93M mutation	Mice: (63) - No α-synuclein aggregates - Dopaminergic neurodegeneration - Mild motor deficits	- Study role of UCH-L1 - Used in combination with α-synuclein and MPTP to study interaction	- Relevance to pathogenesis of PD is debated (23)
Parkin, PINK, DJ-1 Transgenic KO, silencing, or overexpression using viral vectors	Rodents: (62) - Affects aggregation in α-synuclein models - Most give no dopaminergic neurodegeneration - Lack clear motor deficits	- Study role of Parkin, PINK1, and DJ-1 - Used in combination with ASO or MPTP models to study interaction (64)	- Large number of identified mutations (65) -No NHP models
Nurr1 (66), **EN1** (67), **Pixt3** (68), **SHH** (69), **MitoPark** (70) Transcription factor deficiencies	Mice: - No α-synuclein inclusions - Dopaminergic neurodegeneration - Motor deficits	- Often heterozygous KO or targeted to dopamine neurons (DAT-Cre mice) - Study development and maintenance of the nigro-striatal system	- Not specific for PD - Nurr1 and Pixt3 models also used for schizophrenia (71, 72)

SNCA: α-synuclein, ASO: α-synuclein overexpression, LRRK2: leucine-rich repeat serine/threonine-protein kinase 2, WT: wild-type, UCH-L1: ubiquitin carboxyl-terminal hydrolase isozyme L1, PINK1: serine/threonine-protein kinase PINK1, DJ-1: protein/nucleic acid deglycase DJ-1, Nurr1: nuclear receptor-related 1, EN1: engrailed-1, Pixt3: paired like homeodomain 3, NHP: non-human primate, SNpc: substantia nigra pars compacta, KO: knockout.

Many have made efforts to replicate familial PD by generating transgenic mice carrying these mutations. However, these transgenic models do not display clear dopaminergic neurodegeneration or parkinsonian motor deficits (55). Nonetheless, they show altered neuronal function and α-synuclein aggregation. Mutated α-synuclein also disrupts cellular processes such as autophagy in A30P and A53T *in vitro* models (74) and is associated with the formation of tau fibrillary tangles in E46K transgenic mice (75). Truncated forms of α-synuclein are believed to be more toxic than full-length human α-synuclein, and transgenic mice expressing truncated α-synuclein show reduced number of nigro-striatal neurons due to cell loss during early development (57). Although these models are useful to elucidate the poorly understood α-synuclein function and the relation of PD to other synucleinopathies, the clinical relevance of this model for PD is questionable since the transgene affects early developmental stages instead of modeling late onset

neurodegeneration. As with other transgenic models, there is also a risk of compensatory mechanisms or additional deficits during development.

SNCA duplication or triplication (PARK4) is linked to PD with a dominant inheritance pattern (Table 2), showing that increased levels of WT α-synuclein can cause Lewy body pathology and PD. Interestingly, common polymorphisms in the SNCA promoter affecting gene expression levels are also associated with the risk of developing idiopathic PD (76). α-synuclein overexpression (ASO) can be modeled by knock-in transgene expression of human α-synuclein in rodents (77). ASO rat and mouse models affect both the development and maintenance of dopaminergic neurons and are useful models for early PD, and to study pathological cascades arising from α-synuclein. The spread of α-synuclein aggregates in these models depends on the integration site, the promoter used to drive SNCA transcription, and on the genetic background (78). The Thy1 promoter provides widespread SNCA expression and the formation of α-synuclein aggregates in the brain. Thy1-ASO mice also display some motor symptoms and olfactory deficits but lack dopaminergic neurodegeneration (79). The Thy1-ASO model unfavorably expresses α-synuclein in motor neurons in FVB mice but not in the C57Bl/6 background (77). In rats, the BAC-driven expression of E46K α-synuclein lacks dopaminergic neurodegeneration but displays α-synuclein aggregation, altered dopaminergic metabolism in the striatum, and evidence of oxidative stress (56).

Viral vector-mediated models

In addition to transgenic strains, α-synuclein overexpression can be induced by viral vectors. The advantages of this method include targeting of the nigro-striatal system, the induction of pathology in adulthood, and the possibility to adjust dosage (58). Recombinant adeno-associated virus (rAAV) and lentivirus (LV)-based vectors have been used to transfer SNCA in rodents.

Different AAV serotypes have been used for human α-synuclein transgene overexpression in the rat SNpc (59). Many have used AAV2 (59); however, the AAV6 serotype gave the largest dopaminergic cell loss (80% after 8 weeks) and profound motor deficits (80). Using a LV vector also provides α-synuclein aggregation but no clear dopaminergic cell loss or behavior changes with any of the α-synuclein genotypes (58). In addition to the viral vector, the genetic variant of the α-synuclein transgene influences the PD phenotype. rAAV-mediated expression of WT or A53T mutated α-synuclein seem to elicit a comparable pathology, while the expression of A30P mutated α-synuclein results in weaker and delayed pathology in rats (58, 81).

Efforts have been made to produce an equally strong rAAV-α-synuclein model in mice as in rats; however, most mouse models display weak neurodegeneration and no clear α-synuclein inclusions. Nevertheless, rAAV2/7-α-synuclein transduction in the SNpc produced strong dose-dependent dopaminergic neurodegeneration after 8 weeks and motor deficits after 12 weeks (82).

Viral vector-mediated α-synuclein models have also been applied to NHP. In the first model, rAAV2/2 vector was used to deliver WT or A53T α-synuclein (83). Similar to mice, after 16 weeks, marmosets showed some variability (30–60%)

in nigral dopaminergic cell loss, with matching dopaminergic striatal depletion and mild motor deficits (83). This model did not show functional recovery by 42 weeks post-injection. A second rAAV1/2-A53T α-synuclein NHP model has been produced and replicated in macaques (84). The macaque model displayed about 30% loss of dopaminergic nigro-striatal neurons, about 50% DA depletion, and 40% reduction of DAT, resulting in ON–OFF display of bradykinesia.

If viral vector-mediated α-synuclein models in NHP demonstrate robust motor deficits, they can become important preclinical models, complementing the current standard of MPTP-induced NHP models. The viral models have the advantage of producing α-synuclein pathology and can lead to the development of treatments targeting α-synuclein toxicity. A potential drawback of viral vector-based models is the unfavorable interaction with subsequent viral transductions used in gene therapy. Exposure to the first viral vector may change the response to future exposure to viral vectors, altering transfection and the reliability of experimental results.

Preformed fibril models

Evidence of α-synuclein toxicity, seeding, and anatomical spread (85) has led to the development of models directly introducing α-synuclein protein fibrils into the brain. α-synuclein is produced as a soluble protein that forms higher-order structures, including oligomers, fibrils, and filaments. Oligomeric structures seem to be the most toxic and aggregate into typical Lewy body inclusions. Mutations and truncations of α-synuclein increase its misfolding, aggregation, and toxicity (86). Administration of exogenous α-synuclein preformed fibrils (PFF), also called seeding, triggers formation of endogenous α-synuclein aggregates (59, 87). This prion-like propagation has been used to produce PD models with widespread bilateral α-synuclein inclusions to study their role in pathogenesis. In both mice and rats, introduction of α-synuclein inclusions in the striatum causes neuronal dysfunction, mitochondrial damage, and eventual retrograde degeneration of nigro-striatal dopaminergic neurons (88, 89). However, mice display more robust bilateral motor deficits than rats (59). The combination of viral vector-mediated overexpression of SNCA and seeding with PFF in rats induces progressive dopaminergic neurodegeneration and motor deficits alongside α-synuclein aggregation (61).

Two NHP models with α-synuclein fibrils have been published to date: macaques administered Lewy bodies extracted from PD patients into the striatum (60) and marmosets administered mouse A53T α-synuclein into the caudate and putamen (90). The macaques lost striatal dopaminergic fibers by 4 months and displayed neurodegeneration in the SNpc after 17 months. The marmosets also showed signs of nigro-striatal dopaminergic neurodegeneration. Overall, both of these experiments demonstrate a benefit in modeling PD using α-synuclein propagation, however, a limitation is that neither species showed clear motor deficits (91). The seeding of specific preformed structures of α-synuclein is a promising method to further explore the function, propagation, and role of α-synuclein in PD pathogenesis.

Autosomal dominant mutations: LRRK2 and UCH-L1

LRRK2 was identified as a monogenic cause of PD in 2004 (92). LRRK2 mutations linked to PD display autosomal dominant inheritance, but with incomplete and varying penetrance, depending on the population. This means that not all mutation-carriers will develop PD (93). The most common LRRK2 mutations are G2019S and R1441C/G.

Most LRKK2 transgenic mouse and rat models have been unsuccessful in replicating PD hallmarks (3, 62). Some success has been achieved with BAC-LRRK2-R1441G mice, which display some motor deficits and axonal pathology in the striatum, but lack clear dopaminergic neurodegeneration and formation of α-synuclein inclusions (94). A combination of transgenic models can enhance PD-like pathology, and overexpression of LRRK2 in A53T α-synuclein transgenic mice promoted dopaminergic degeneration and α-synuclein aggregation (95).

Due to the large size of the LRRK2 gene, a limited number of viral models have been generated using herpes simplex virus (HSV) and adenoviral vectors (58). In rodents, transfection of LRRK2-G2019S is more toxic than WT LRRK2, causing more neurodegeneration and formation of inclusions (96). Injection of HSV-LRRK2-G2019S in the mouse striatum has been shown to induce degeneration of about 50% of the dopaminergic neurons in the SNpc (97). LRRK2 models do not robustly replicate all PD hallmarks but are useful to understand the interplay between different genetic mutations and environmental factors and to untangle the mechanisms behind their functions in PD. Mutations in LRRK2 have not yet been used in PD models in NHP, but LRRK2 kinase inhibitors have been proposed as a potential therapeutic option in PD, and their effect has been explored in in macaques following MPTP exposure (98).

The pathogenic effects of UCH-L1 mutations have been debated due to their rarity in PD patients (23). However, transgenic mice with mutated UCH-L1 display a mild reduction in locomotion, reduced number of dopaminergic neurons in the SNpc, and dopamine depletion in the striatum, without α-synuclein inclusions (63). Mutant UCH-L1 also exacerbates pathology in MPTP and ASO mouse models (64).

Autosomal recessive mutations: PRKN, PINK1, and DJ-1

Autosomal recessive mutations in PRKN (Parkin), PINK1, and DJ-1 have been linked to familial PD. There are over 100 known mutations of PRKN and it is the most commonly mutated gene in early onset PD (50% of familial and 20% of idiopathic early onset cases) (65). PINK1 is the second most commonly mutated gene in early onset PD, present in 1–7% of the cases, while DJ-1 mutations are uncommon (81). Rare individuals who have two mutations in one of these three genes appear to have complete penetrance (62, 65, 99).

Mutations in all three genes give loss-of-function, and therefore knockout models have been generated. However, none of the knockout models display dopaminergic cell loss or motor deficits (62). Some DJ-1 and PRKN models display changes in dopamine neurotransmission and mitochondrial dysfunction (100–102). Interestingly, overexpression of mutated PRKN-Q311X leads to age-dependent dopaminergic neurodegeneration, α-synuclein aggregation, and some motor deficits, suggesting a gain of pathological function (103). Backcrossing

of DJ-1 nullizygous mice onto a C57Bl/6J background generates a new PD phenotype (104). These mice display strong early unilateral dopaminergic neurodegeneration that progresses into bilateral pathology in 15-month-old mice, demonstrating the age- and strain-dependent nature of some PD models.

Silencing gene expression of PINK1 or Parkin in adult mice using viral vectors has not yielded better PD models compared to knockout (58, 105), possibly because of less efficient silencing (86). However, both knockout and viral vector-mediated knockdown of PINK1 in mice rendered dopaminergic neurons more sensitive to MPTP (58, 105). PINK1 knockout mice, but not PINK1 silencing, enhanced neurodegeneration in the ASO model (106). Inactivation of PINK1 using RNA interference knocked down 71% of PINK1 in striatum and 68% in SNpc (86). These mice exhibited higher sensitivity to MPTP-induced neurodegeneration. Knockdown of DJ-1 also enhanced toxicity in the MPTP mouse model and could be rescued by reintroducing the gene with viral vectors (107). These examples of combinations of genetic and neurotoxic models highlight the complex interplay between multiple factors in PD pathology.

The overexpression of autosomal recessive genes, where loss-of-function is linked to PD, could become sources of treatments. For example, overexpression of PRKN via rAAV vectors had protective effects in the striatum of rAAV-α-synuclein-treated NHP (108).

Transcription factors

Nuclear receptor-related 1 protein (Nurr1), engrailed 1 (EN1), pituitary homeobox 3 (Pitx3), sonic hedgehog (SHH), and c-Rel are all transcription factors that play a role in the development and maintenance of the dopaminergic nigro-striatal system (3). Nurr1 knockout mice fail to differentiate dopaminergic neurons (109) and heterozygous Nurr1-deficient mice show a progressive dopaminergic cell loss (3, 66). DAT-Cre transgenic mice with Nurr1 knockout in dopaminergic neurons display rapid striatal dopamine depletion, loss of SNpc dopaminergic neurons, and motor deficits (110, 111). Meanwhile, silencing Nurr1 in dopaminergic neurons by using rAAV vectors gives progressive retrograde dopaminergic degeneration. However, none of the Nurr1-deficient models display α-synuclein aggregates.

Heterozygous deficiency of EN1 gives rise to a progressive retrograde degeneration with dystrophy of striatal dopaminergic axons as early as at 4 weeks, and clear dopaminergic cell loss by 16 weeks accompanied by motor deficits and depressive-like behavioral changes (67, 112, 113). The phenotype is seen in EN1-heterozygous SwissOF1 mice, but not in C57Bl/6 with the same mutation. The neuroprotection in C57Bl/6 is multigenic and could be useful for studying risk factors for idiopathic PD (114).

Knockout of c-Rel creates mice with disrupted production of cell survival factors (SOD2 and Bcl-xL). These mice have clear dopaminergic cell loss in the SNpc, dopamine depletion in the striatum, age-dependent motor deficits, and α-synuclein aggregation in the SNpc (115). Pitx3 knockout mice, also called aphakia mice, have abnormal dopaminergic systems. They display strong dopaminergic degeneration in the SNpc, 90% striatal dopamine depletion (68, 116), and clear motor deficits that are reversed with L-dopa administration (117).

SHH has a vital role in the embryonic development of dopaminergic projections in the midbrain (118) and ablation of SHH in dopamine neurons causes dopaminergic and cholinergic neurotransmitter dysfunction, progressive neuronal loss, and motor deficits (69). The MitoPark model disrupts mitochondrial function in dopaminergic neurons by knocking out TFAM in DAT-Cre mice (62). These mice display progressive dopaminergic degeneration, motor impairments, and formation of inclusions that lack α-synuclein (41, 70, 119).

Transgenic mice with deficiencies in dopaminergic transcription factors have a role in modeling PD but display other dopaminergic pathologies that undermine their specificity. Nurr1 and Pitx3 models have been suggested being applicable to cognitive disorders such as attention-deficit hyperactivity disorder (ADHD) and schizophrenia due to their involvement in other dopaminergic pathways (71, 72). Other transcription factors like SHH, TFAM, and NF-κB are relevant in the survival of many non-dopaminergic cells. These models can thus be useful to study defects in development and maintenance of dopaminergic neurons but are not specific to PD-related pathologies.

CONCLUSION

None of the animal models described above perfectly mimic the neuropathology of PD and the models cannot replicate the clinical syndrome. However, the wide variety of rodent, NHP, and non-mammalian models allows targeted studies of different pathological mechanisms of PD. The heterogeneity of these models can be seen as an advantage, since a clinical PD diagnosis reflects a heterogeneous group of patients with differences in onset, progression, symptoms, and neuropathology. The complex etiology of the disease, with interindividual variations in environmental and genetic risk factors, reflects the heterogeneity of PD and is seen both in idiopathic and monogenic cases. For example, some patients with DJ-1, Parkin, and LRRK2 mutations do not form Lewy bodies (62, 104). Diversifying the animal models can help understand various subtypes of PD and develop personalized treatments.

Current animal models of PD focus on degeneration of dopaminergic nigro-striatal neurons and formation of α-synuclein aggregates, but many lack one or the other. Classical neurotoxins (MPTP and 6-OHDA) (24) in rodents and NHP, and transcription factor deficiencies—Nurr1 (92), Pitx3 (68), SHH (69), MitoPark (102)—in mouse models cause an extensive loss of dopaminergic neurons in the SNpc and clear motor deficits but no α-synuclein aggregation. In contrast, mutated α-synuclein transgenic mouse models induce α-synuclein aggregation without neurodegeneration (55). Other models have struck a balance between the aggregation of α-synuclein and progressive degeneration of nigro-striatal neurons. These include chronic administration of MPTP in mice and NHP (10, 25), pesticide and herbicide rodent models (30, 31, 49), seeding of PFF in mice and NHP (59, 91), c-Rel knockout mice models (115), PRKN-Q311X overexpression (103), transfection of AAV-α-synuclein in rodents and NHP (80, 82, 84), and HSV- LRRK2 G2019S transfection in mice (97).

Furthermore, combined models can enhance PD hallmarks by hitting disease-associated pathways at several levels. For example, seeding of PFF in rats combined

with rAAV-α-synuclein transfection leads to enhanced α-synuclein aggregation and neurodegeneration (61). Many transgenic models (mutated LRRK2 [3] and UCHL1 [63]; DJ-1, PINK1, and Parkin knockout [62]) show some functional disruption in the nigro-striatal system but have been ineffective at inducing a robust display of neurodegeneration and α-synuclein pathology. However, UCHL1 mutations, DJ-1 knockout and PINK1 knockout, exacerbate ASO and MPTP mouse models (64, 86, 107).

When choosing an animal model for PD, genetics of the selected species also needs to be taken into consideration, since the genetic background of mouse strains has been shown to alter the phenotype of different PD models. One example of strain effects on a monogenic PD model is the strain-dependent α-synuclein toxicity in Thy1-ASO mice (77). Functional recovery in MPTP-exposed mice is also determined by the strain used, highlighting the importance of interaction of environmental factors with the genetic background (34). Attempts to map the genetic factors underlying strain-dependent susceptibility to PD-like phenotypes include linkage analysis in mice treated with MPTP (120) and paraquat (121) as well as in EN1-hemizygous mice (114). In addition, backcrossing DJ1-null mice to the C57Bl/6J background generated a progressive unilateral to bilateral neurodegeneration in a subset of mice that segregated with exonic variants in five genes (104). Genes identified as conferring strain-dependent susceptibility to PD models are strong candidates as risk-modifying genes in PD.

Functional recovery in neurotoxic models and the lack of comparable phenotypes in mice carrying mutations that cause PD in humans highlight the difficulty of replicating PD in animal models. There is also a lack of models that include non-motor symptoms, which significantly affect the quality of life of patients with PD. This questions the relevance of animal PD models to human pathology. However, the development of PD models in NHP that have greater anatomic and genetic resemblance to humans, and new genetic technologies open new avenues towards creating models that display a complex pathology more similar to what is seen in PD patients (11). In addition, the panel of non-mammalian, rodent and NHP models offers the possibility to selectively study specific aspects of PD pathology. New technologies together with further refinement and combination of existing models could thus generate an array of animal models that can deepen our understanding of PD and help translate research into treatment.

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Section II

Diagnosis and Management

6 The Differential Diagnosis of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a common, progressive neurodegenerative disease. It is a movement disorder presenting primarily with a combination of bradykinesia, rigidity and tremor. However, it has a variable presentation, including the presence of non-motor symptoms such as cognitive impairment and sleep disturbance. The diagnosis is made clinically by the recognition of these key features and the exclusion of other causes of parkinsonism. This chapter describes how to recognize the core motor and non-motor features of PD, as well as atypical features that suggest an alternative cause of parkinsonism. The essentials of these alternative diagnoses are outlined, and the list of differentials is structured into sections on other neurodegenerative causes of parkinsonism, secondary causes of parkinsonism, genetic causes of parkinsonism, tremor disorders, and non-neurological differentials of PD.

Keywords: Diagnosis; Differential; Movement Disorder; Parkinsonism; Parkinson's Disease

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INTRODUCTION

Idiopathic Parkinson's disease (PD) is a common, progressive neurodegenerative disorder, affecting approximately 1% of people over 65 years of age (1). Patients present to health services in a number of settings, and the key to its diagnosis is recognition of its core features, as PD remains a clinical diagnosis with no diagnostic test. A definitive diagnosis of PD is only possible postmortem and relies on the degeneration of the substantia nigra with the presence of Lewy body pathology, the main constituent of which is alpha-synuclein (α-synuclein) (2). Both structural and dopaminergic imaging can be helpful in specific scenarios, but only once the clinical phenotype has been established. PD is traditionally classified as a movement disorder, which is reflected in the diagnostic criteria (see the section below and Table 1) (3). However, the non-motor features are now well established and often precede the onset of the motor syndrome and thus can help in making the true diagnosis (4), although equally the heterogeneity of its presentation and the disease course means that PD can mimic other conditions.

THE DIAGNOSIS OF IDIOPATHIC PARKINSON'S DISEASE

The first component to the diagnosis of PD is establishing that the patient has "parkinsonism." This is a clinical diagnosis and relies on three key elements: bradykinesia, tremor, and rigidity. Of these, bradykinesia must be present, with at least one of the other two. PD is an asymmetrical condition, so during the clinical assessment, the parkinsonism should be more apparent on one side and may be purely unilateral in early disease (3). Figure 1 is a classical illustration of parkinsonism, with a description by William Gowers.

Once it has been established that a patient has parkinsonism, in order to diagnose PD, it is vital to exclude other causes of this syndrome. This includes looking for atypical features in the history and on examination. Furthermore, in addition to the presence of parkinsonism, there are also additional features which contribute toward a positive clinical diagnosis of PD. The presence of nonmotor features is important, as these may be prominent even early in the disease course. The response to treatment is equally supportive, as is the evolution of the clinical syndrome over time. These features will be discussed more thoroughly in the sections below, and they are summarized in the clinical diagnostic criteria in Table 1 as exclusion criteria, supportive criteria, and "red flags" (3).

Bradykinesia

Bradykinesia is a slowness and lack of spontaneous movement. Patients may report difficulty with fine motor tasks, such as doing-up buttons; a change in speech with a quiet, monotonous voice (hypophonia); or an increase in saliva because of an infrequent swallow. Even before examining a patient, bradykinesia can be appreciated by noticing a lack of normal gestures and fidgeting, the loss of facial expression (hypomimia), and a decreased blink frequency. It is helpful to observe their gait as they walk into the clinic room; they may exhibit a reduced arm swing as well as short, shuffling steps and a stooped posture.

TABLE 1 **The clinical diagnostic criteria for Parkinson's disease, based on the Movement Disorder Society guidelines**

1. *Diagnosis of parkinsonism*
   a. Bradykinesia
      Plus one of
   b. Tremor
   c. Rigidity
2. *Exclusion criteria*
   a. Cerebellar abnormalities
   b. Supranuclear gaze palsy
   c. Diagnosis of behavioral variant of frontotemporal dementia or primary progressive aphasia within 5 years of disease onset
   d. Parkinsonian features restricted to the lower limbs for more than 3 years
   e. Treatment with a dopamine receptor blocker or dopamine depleting agent consistent with drug-induced parkinsonism
   f. Absence of a response to high-dose levodopa despite at least moderate disease severity
   g. Cortical sensory loss, clear limb ideomotor apraxia, or progressive aphasia
   h. Normal functional imaging of the dopaminergic system ("DAT scan")
   i. Diagnosis of alternative condition causing parkinsonism which could be causing the symptoms
3. *Supportive criteria*
   a. Clear beneficial response to dopaminergic therapy
   b. Presence of levodopa-induced dyskinesia
   c. Rest tremor of a limb
   d. The presence of either olfactory loss or cardiac sympathetic denervation on MIBG scintigraphy (although the latter is rarely done in current practice)
4. *Red flags*
   a. Rapid progression of gait impairment leading to wheelchair use within 5 years
   b. Absence of progression of motor symptoms over 5 years, unless related to treatment
   c. Early bulbar dysfunction
   d. Inspiratory respiratory dysfunction
   e. Severe autonomic failure within the first 5 years of disease
   f. Recurrent falls because of impaired balance within 3 years of onset
   g. Disproportionate anterocollis or contractures within 10 years of disease onset
   h. Absence of any of the common non-motor features despite 5 years of disease
   i. Unexplained pyramidal signs
   j. Bilateral symmetrical parkinsonism

For the diagnosis of clinically established PD	*For the diagnosis of clinically probable PD*
1. Parkinsonism	1. Parkinsonism
2. Absence of exclusion criteria	2. Absence of exclusion criteria
3. At least 2 supportive criteria	3. Balanced numbers of supportive criteria and red flags

Bradykinesia can be specifically elicited on examination using repeated hand movements, for example, finger tapping or pronation/supination, or in the lower limbs using repeated foot tapping. Patients with parkinsonian bradykinesia should show not only a slowness of movement but also a decrement in the speed or amplitude of movement (5). A change in handwriting is another manifestation of bradykinesia which can be demonstrated during the clinical examination; patients

Figure 1 A case of Parkinson's disease as described and illustrated by William Gowers: "… the aspect of the patient is very characteristic. The head is bent forward, and the expression of the face is anxious and fixed, unchanged by any play of emotion. The arms are slightly flexed at all joints from muscular rigidity, and (the hands especially) are in constant rhythmical movement, which continues when the limbs are at rest so far as the will is concerned. The tremor is usually more marked on one side than on the other. Voluntary movements are performed slowly and with little power. The patient often walks with short quick steps, leaning forward as if about to run (61)."

display small and cramped handwriting which becomes progressively smaller (micrographia) with a tendency of the sentence to fall off the line.

On occasion, the true bradykinesia of PD can be mistaken because the patient has another comorbidity leading to difficulty in repeated hand movements, for example, psychomotor slowness in depression, the pain of arthritis, or other neurological disease including cerebellar ataxia or dyspraxia. In ambiguous cases, it is therefore important to rely on other clues in the examination and assess the patient for global bradykinesia (6).

Tremor

A parkinsonian tremor is classically described as "pill-rolling" (pronation/supination) and low frequency (4–6 Hz). It should start unilaterally but can progress to involve both sides, although it should remain asymmetric. It is apparent in the most distal part of a limb, that is, the hand if the upper limb is involved. It should typically occur at rest and improve or disappear on action. In early stage disease, it is typically intermittent and patients often notice it while relaxing, for example, watching television, and consequently, it is useful to watch for the tremor during the entirety of the consultation. The tremor also becomes more prominent when the patient is

engaged in another task, such as counting backwards or walking. The parkinsonian tremor also often shows a re-emergence phenomenon whereby it disappears following an action, but then after a few moments becomes apparent again (7). It is seen most commonly in the upper limbs, including just a finger or thumb, but can also involve the legs, chin, or jaw. Although tremor is often identified as a key feature of PD, by health professionals and members of the public alike, it does not need to be present for a diagnosis of PD, and a proportion will never have a tremor (5, 8). One study reported that 69% had a rest tremor at disease onset, with 75% having a tremor at some point in their disease course (9).

Rigidity

Patients will often report unilateral pain or stiffness, commonly in the shoulder. This can represent the first presentation of PD, leading to referrals to rheumatologists or orthopedic surgeons, and initial diagnoses of frozen shoulder (10).

Rigidity needs to be appreciated on examination, with the patient in a relaxed position. It can be felt through a range of passive movements of a joint (flexion, extension, and rotation). It is often best felt distally at the wrist, and in early disease may only become apparent with a reinforcement manoeuver, whereby the patient is asked to perform an action on the contralateral side (e.g., to raise the arm up and down), although a slight increase in tone with this manoeuver is seen in many people without PD. The increase in tone should be independent of velocity and is described as "lead-pipe" resistance, often with the "cogwheel" phenomenon, where a superimposed tremor is also felt (5).

Non-motor features

The non-motor features of PD contribute toward the certainty of diagnosis. There is established evidence of a pre-motor phase, which is thought to represent the onset of the neurodegenerative pathology. Patients who go on to develop PD commonly have experienced depression, constipation, anosmia, and REM sleep behavior disorder in the years preceding their diagnosis (11). Of course, these symptoms are non-specific and common in the elderly population, with many potential causes, which complicate their use in predicting those at risk of subsequently developing PD. However, their presence gives support to the diagnosis of PD and should be specifically queried during the diagnostic consultation.

There are further non-motor aspects to PD which can develop at any point through the disease course. Subtle cognitive deficits can be present even at diagnosis and typically affects attentional, executive, visuospatial, and memory functions (12). Neuropsychiatric symptoms are also common and cover depression, anxiety, and apathy as well as psychosis. Psychosis has a spectrum of presentations from minor illusions, for example, where patients report that they glimpse things out of the corner of their eye that turn out not to be real, through to formed hallucinations and delusions with a lack of insight, leading in some cases to delusional paranoid behavior (13). Autonomic dysfunction can manifest as urinary frequency or urgency, constipation, orthostatic hypotension, drooling, erectile dysfunction, or abnormal sweating (14).

These non-motor features initially present insidiously and non-specifically, and the patient will often think they are unrelated to PD and not volunteer them

during a consultation. Moreover, they are typically not treated by the dopaminergic therapy which is prescribed primarily for the rigidity and bradykinesia (4). It is therefore important to actively screen for them, for example, checking a lying and standing blood pressure and asking about sleep, as they can have a substantial impact on the patient's quality of life (15), and similar to the motor features of PD, there are many good treatments available.

Disease progression

Progression is an important diagnostic feature of PD. By the time the patient presents to clinic, there should be a history describing a worsening of symptoms to the point of seeking medical attention. It is then important that at each subsequent review, this story of disease progression is reviewed, because if lacking or abnormally rapid, it may point toward an alternative diagnosis. A caveat to this is that although all cases do worsen, there is a large range in the rates at which this occurs. Ten years following diagnosis, about one-fifth of patients are doing very well and have very few complications associated with the disease, while 40% will have gone on to develop a dementia (16). Equally, patients who respond well to medication may appear to have many years of stable disease with little worsening of symptoms and this can sometimes be mistaken as a "cure" by the patient.

As the disease progresses, other motor features become apparent which are characteristic of PD. As mentioned above, gait impairment is common. However, this becomes more pronounced and patients adopt a characteristic stooped posture with reduced arm swing (see Figure 1 for a classical illustration of this posture). The arm itself can also adopt an abnormal flexed posture on walking. Classically, patients have a flexed neck and camptocormia, where there is truncal flexion at the thoracolumbar spine. The shuffling steps can progress so that patients display festination whereby successive steps are shorter. Postural instability develops, which has been shown to have a significantly detrimental effect on quality of life, and leads to an increased risk of falls and associated morbidity (15). Patients may progress to develop freezing of gait, which is also very disabling with an associated falls risk. This is where patients are unable to initiate or continue walking and describe a sensation like their feet are stuck to the floor. It commonly occurs on turning, walking through a narrow space (such as a doorway), or on starting to walk (17).

A positive clinical response to levodopa is supportive of the diagnosis of PD, and this is the case particularly for bradykinesia and stiffness. Levodopa has a more variable effect on tremor. Equally supportive are the presence of dyskinesias or additional choreoform involuntary movements, which can develop as the disease progresses in response to the dose and duration of dopaminergic therapy (18).

OTHER NEURODEGENERATIVE CAUSES OF PARKINSONISM

The following conditions can mimic idiopathic PD, and on occasion, the correct diagnosis only becomes apparent with time. However, there are key features on history and examination to recognize in order to discriminate between these conditions.

Dementia with Lewy bodies

Dementia with Lewy bodies (DLB) is the second most common cause of dementia, following Alzheimer's disease. It is characterized by progressive cognitive impairment together with fluctuating levels of attention, visual hallucinations, REM sleep behavior disorder, and parkinsonism. The predominant pathology in both PD and DLB is Lewy bodies, the main constituent of which is α-synuclein, and there is undoubtedly a clinical and pathological overlap. Current diagnostic criteria state that if the cognitive symptoms pre-date or develop within the first year of the emergence of parkinsonism, then the diagnosis is DLB. Should cognitive impairment develop in the context of established PD, then the diagnosis is Parkinson's disease dementia (PDD) (19).

The cognitive impairment of DLB affects attentional-executive and visuospatial functions. Patients typically perform poorly on the Stroop and the trail-making tasks as well as drawing tasks, classically clock-drawing or intersecting pentagons (see Figure 2). Fluctuating attention can manifest as inconsistent behavior and periods of "zoning-out", and in fact, the presence of major fluctuations in a patient's cognitive state is a very helpful diagnostic feature of this condition. The visual hallucinations that occur in approximately 80% of patients are complex and tend to involve humans or animals. Patients have a variable amount of insight into their presence (19).

Other supportive features include a sensitivity to neuroleptic medication, postural instability and falls, autonomic dysfunction, and other neuropsychiatric features that include depression, apathy, anxiety, delusions, and hallucinations of other modalities. Treatment is based on a multi-disciplinary approach (19). Of note, the parkinsonism is less responsive to dopaminergic therapies, which can worsen cognition and behavior. Cholinesterase inhibitors have a role in management of the neuropsychiatric features (20), as can low-dose quetiapine. In all cases, however, when this problem emerges, other reversible causes should be sought, such as an underlying infection or subdural hematoma.

Multiple system atrophy

Multiple system atrophy (MSA) is another α-synucleinopathy, although much rarer than PD (21). There are a range of associated clinical features, which include parkinsonism, cerebellar ataxia, autonomic failure, urogenital dysfunction, and corticospinal involvement. There is a relative preservation of cognition. It is subdivided

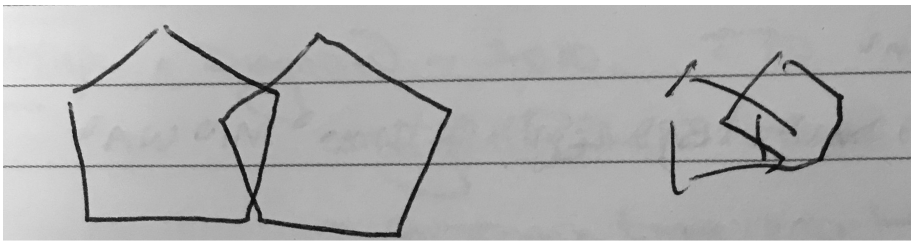

Figure 2 An illustration of the difficulties exhibited by patients with DLB when performing the interlocking pentagon copying task.

into MSA with predominant parkinsonism (MSA-P) and MSA with predominant cerebellar ataxia (MSA-C).

The parkinsonism of MSA tends to be rapidly progressive and poorly responsive to levodopa. Bradykinesia and rigidity are usually predominant; a classic pill-rolling tremor is rarely seen. In the initial stages, MSA can be difficult to distinguish from PD, as symptoms are invariably asymmetric and up to 30% of patients do show an initial good response to levodopa treatment. MSA patients treated with levodopa can also exhibit dyskinesias, although these are often atypical and can manifest as prolonged facial dystonia or torticollis (22).

Autonomic failure is virtually always a feature at presentation, which tends to occur later in the disease course in PD. This is seen as an orthostatic drop in blood pressure, urinary incontinence, or erectile dysfunction. Additional features which point toward MSA and are not seen in PD are stridor, a positive Babinski sign, and cerebellar ataxia, which can manifest as a gait or limb ataxia, dysarthria, or oculomotor dysfunction. Myoclonus is also seen, particularly as "poly-mini-myoclonus": stretch-sensitive jerks affecting the fingers. This is unusual in PD (22, 23).

Imaging can be useful in discriminating PD and MSA. Structural imaging using MRI can reveal changes in the brainstem and basal ganglia which are supportive of MSA, including the "hot cross bun" sign, although this feature is only normally recognized by radiologists familiar with these types of syndrome (23).

Overall, the clue that the patient has MSA rather than PD is the poor response to levodopa and the involvement of systems outside the striatum and thus the prominent gait and autonomic problems early on in the disease course.

Progressive supranuclear palsy

This neurodegenerative disorder is a tauopathy which usually has a distinct presentation from PD. Patients usually present in their 60s with walking difficulties, unsteadiness, falls, and visual symptoms—often dry eyes and blurred vision (24). The parkinsonism of PSP tends to be symmetrical, involves the axial musculature, and is poorly responsive to levodopa therapy. This axial rigidity means that PSP patients tend to have a very different posture to classically stooped PD patients; they are upright, occasionally with retrocollis. Although postural instability occurs in PD and is a key marker of disease progression, in PSP it can be early and pronounced. Patients fall frequently, often backwards, especially when turning. It is tested clinically by the pull test. Equally, freezing of gait can be prominent and poorly responsive to levodopa (25).

Eye movements in PSP are abnormal. Early in the disease, this can be subtle and manifest only as square wave jerks or slowing of vertical saccades. However, as it progresses, patients develop a restriction of vertical eye movements, which can be overcome with activation of the vestibulo-occular reflex. However, it should be noted that a slight reduction in upgaze is common in the elderly with no underlying pathology in the central nervous system (25).

PSP is associated with a behavioral and cognitive syndrome, which can be apparent at the time of initial presentation. Apathy is characteristic, occurring in 80% of patients. Paradoxically, so is impulsivity. The most common cognitive deficits are in executive function and verbal fluency (26).

Similar to MSA, neuroimaging can be helpful, with mid-brain atrophy and the "hummingbird sign," (where the atrophied midbrain viewed in the sagittal

plane resembles the head and beak of a hummingbird) being supportive of the diagnosis (27).

Overall, the clue that the patient has PSP rather than PD is a poor response to levodopa, early falls, eye movement abnormalities, and rapid progression.

Corticobasal syndrome

Corticobasal syndrome (CBS) is another tauopathy which is both clinically and pathologically heterogenous. As well as a classical CBS presentation, there are three further clinical phenotypes all associated with corticobasal degeneration pathology: a frontal behavioral-spatial syndrome, a non-fluent primary progressive aphasia, and a PSP syndrome (28).

The diagnosis of CBS is clinical and based primarily on its motor features. It causes asymmetric limb rigidity or akinesia, which may resemble the parkinsonism of PD. However, this is frequently associated with dystonia and myoclonus, and the parkinsonism is typically levodopa-resistant and may also involve the axial musculature. Tremor may also be a feature, although it is not the typical rest tremor of PD (28).

Furthermore, there are multiple higher cortical features, which are key in distinguishing this condition from PD. These include apraxia, cortical sensory loss, progressive non-fluent aphasia, and alien limb phenomenon, where the patients have the feeling that the limb does not belong to them; has a will of its own; or independently performs complex, unintentional tasks. Cognitive impairment is heterogenous and has been shown to affect a range of modalities including executive function, episodic memory, visuospatial function, and cognitive flexibility. Behavioral changes may also be prominent and include apathy, irritability, antisocial behavior, personality changes, and hypersexuality (28).

Imaging may be helpful, typically showing an asymmetric pattern of atrophy primarily involving the frontal and parietal cortices. However, it is not diagnostic (29).

SECONDARY CAUSES OF PARKINSONISM

A number of secondary causes of parkinsonism need to be considered before making the diagnosis of idiopathic PD.

Drug-induced parkinsonism

Drugs that can act on dopamine receptors in the central nervous system can cause parkinsonism, as well as other movement disorders including tardive dyskinesia, akathisia, and dystonia. Antipsychotics are the most common cause of drug-induced parkinsonism, particularly the “typical antipsychotics” including haloperidol and chlorpromazine, although these drugs are now rarely used in clinical practice. However, atypical antipsychotics such as risperidone and olanzapine can also cause extra-pyramidal side effects, including parkinsonism.

Other causes of drug-induced parkinsonism include anti-emetics (e.g., metoclopramide and domperidone), calcium channel blockers (e.g., flunarizine and cinnarizine), anti-epileptics (e.g., sodium valproate and phenytoin), dopamine

depleting drugs (e.g., tetrabenazine), and selective serotonin reuptake inhibitor anti-depressants (30).

Drug-induced parkinsonism is said to typically cause a symmetrical parkinsonism syndrome with prominent bradykinesia and rigidity; however, this is of course not always the case, and it can closely mimic idiopathic PD. The symptoms typically resolve within weeks to months after withdrawal of the offending drug. However, there is also a proportion of patients in whom the drug has unmasked early underlying PD; in these cases, the parkinsonism will progress despite cessation of the drug. In cases where there is doubt over the diagnosis, a DaTSCAN is useful, as it is normal in drug-induced parkinsonism (30).

Toxins

A causative link between PD and exposure to toxins was first revealed in the 1980s in California when intravenous drug-users began presenting with acute parkinsonism, after injecting what was found out to be MPTP, a contaminant of their pethidine analog. This knowledge has since been used in research to create animal models of PD (31).

Epidemiological studies have gone on to reveal associations between environmental toxin exposure and the risk of developing PD. These include pesticides (rotenone, paraquat, organophosphates), solvents, and a limited amount of evidence for metals (lead, manganese, mercury). It seems that in these cases, environmental exposure is one of many potential etiological factors, but only explains the development of the disease in part (31). In these cases, MRI scans of the brain are often abnormal with signal change in the basal ganglia (32).

Acute carbon monoxide poisoning has been shown to cause parkinsonism, usually developing within 1 month of exposure. The clinical features can include bradykinesia, rigidity, hypomimia, and gait disturbance, but this is in the context of encephalopathy and frequent frontal lobe signs such as the grasp reflex. Prognosis is good, with most patients making a spontaneous full recovery (33).

Vascular parkinsonism

Parkinsonism caused by cerebrovascular disease is an important differential to consider in the elderly because it is common, accounting for an estimated 3–6% of cases of parkinsonism, and has a different prognosis and response to treatment. It is associated with increasing age and vascular risk factors, such as hypertension, previous TIAs, and diabetes mellitus. The syndrome is caused by a lesion affecting either the substantia nigra or its projections, which can include subcortical white matter disease or lacunar infarcts (34, 35).

Vascular parkinsonism has a distinct clinical phenotype. It tends to be bilateral, symmetrical and affects the lower limbs, so causing a predominant gait disorder. The gait is typically upright with a broad base, short steps, and normal arm-swing, compared to the stooped posture, narrow base, and reduced arm-swing of PD. Upper limbs are often spared, tremor is rare, and increased tone tends to be a combination of spasticity and rigidity, with gegenhalten paratonia. The clue is that the examination on the bed is relatively normal compared to the problems seen while mobilizing. Progression is typically stepwise and rapid. There are also often

supporting features, such as the presence of an extensor plantar response, brisk reflexes, a pseudobulbar palsy, and cognitive impairment on examination (34, 35).

Both PD and cerebrovascular disease are common in the elderly, so there can be an overlap, causing diagnostic difficulty. Vascular disease on brain imaging is supportive, but not conclusive, as these changes can also be present in patients with PD. DaTSCAN can be helpful, as it is usually normal in vascular parkinsonism. However, an abnormal scan could be caused by a focal basal ganglia infarct. Furthermore, although patients with vascular parkinsonism are said not to respond to levodopa therapy, there are a subset of patients who will get some therapeutic benefit. It is therefore recommended that all patients with suspected vascular parkinsonism receive a trial of levodopa. Otherwise, the management is aimed at rehabilitation and controlling vascular risk factors (34).

Normal-pressure hydrocephalus

Normal-pressure hydrocephalus (NPH) is diagnosed by the presence of a triad of clinical features (gait disorder, incontinence, and cognitive impairment or dementia) together with characteristic radiological appearances of enlarged ventricles. As well as this clinical triad, which can mimic PD, NPH is also associated with parkinsonism in approximately 70% of cases. Bradykinesia is the most common parkinsonian feature, but rigidity and postural instability are also frequently present. Furthermore, like PD, it is a slowly progressive disorder of the elderly (36, 37).

The gait disorder can appear very parkinsonian, with small, shuffling steps, difficulty turning, and falls. Urinary symptoms, which form part of the diagnostic criteria for NPH, are also common in PD. Furthermore, the cognitive impairment in NPH is similar to what is seen in PD, with associated psychomotor slowing, daytime somnolence, and apathy (36).

Structural brain is therefore important if there is an indication that the underlying cause of the parkinsonism is NPH, particularly as this is a treatable condition, with approximately 80% of patients experiencing an improvement in symptoms following surgical treatment with a CSF shunt (38). However, distinguishing between NPH and atrophy on a CT or MRI brain scan can be difficult, and if in doubt advice from a neurosurgical team familiar with NPH and CSF infusion studies should be sought.

GENETIC CAUSES OF PARKINSONISM

It is likely to be worthwhile considering genetic investigations in patients who present at a young age, less than 40, and who have at least one affected relative. There are also a number of atypical features that can give a clue regarding the underlying diagnosis.

Familial PD

Mutations in a number of genes have been found to be associated with familial PD, and although they can look phenotypically different from idiopathic PD,

some are identical to PD in terms of their presentation, for example, *LRRK2*. Some of the most well documented are outlined here.

The first gene identified was the *SNCA* gene, with mutations being linked to autosomal dominant early-onset parkinsonism. The clinical spectrum is broad, from classical PD to an aggressive syndrome with prominent cognitive impairment and autonomic instability which may mimic MSA. Atypical features of myoclonus and hypoventilation can also be present. A severe phenotype is associated with *SNCA* triplications, whereas duplications have a tendency to present as typical PD and are more common (39, 40).

Mutations in the *LRRK2* gene are another, more common, cause of autosomal dominant PD. These can lead to a typical clinical picture of PD, although with a broad range of onset age and a less severe clinical phenotype. The mutation is frequent in people of south European, north African, or Ashkenazi Jewish descent (39, 41).

Mutations in *parkin*, *PINK1*, and *DJ-1* are all associated with early-onset parkinsonism with autosomal recessive inheritance. They have a slowly progressive course, with a clinical picture which is typical of PD, and usually respond well to levodopa. Of these, mutations in *parkin* are most common and the clue to patients having this is their early onset, leg-dominant features, and a slow progression over decades (39, 40).

Wilson's disease

Wilson's disease is a rare autosomal recessive disease caused by a mutation in the *ATPB7* gene causing a toxic accumulation of copper. It typically presents in early adulthood, although can manifest up to the fifth or sixth decade of life. It has hepatic, psychiatric, and neurological manifestations. A primary neurological presentation has been reported to occur in up to 68% of patients and can involve a combination of dysarthria, tremor, dystonia, and parkinsonism. As the disease progresses, patients can subsequently develop choreoathetosis, ataxia, myoclonus, seizures, and eye movement abnormalities.

The parkinsonism of Wilson's disease has been shown to closely mimic idiopathic PD, with a typically asymmetric syndrome consisting of bradykinesia and rigidity. Rest tremor is less common, but can occur. However, the parkinsonian features rarely occur in isolation, without other neurological signs. Furthermore, patients may also have hepatic and psychiatric involvement, with subclinical liver disease being common in a primary neurological presentation. Diagnostic tests include initial copper studies; slit lamp examination for Kayser–Fleischer rings, which are invariably present in patients presenting with a neurological syndrome; and consideration of genetic testing. This is an important differential to consider, because there are effective treatments available (42).

Huntington's disease

Huntington's disease is a trinucelotide repeat disorder with autosomal dominant inheritance. Typically, the disease presents in middle age as a movement disorder, classically chorea, together with cognitive and behavioral features. However, parkinsonism is also a recognized feature, which can be present when the disease clinically manifests. Also, a longer triplet repeat is associated with a younger and

distinct phenotype, which is more akin to PD. This juvenile form of the disease, the Westphal variant, presents with a parkinsonian hypokinetic rigid syndrome usually before the age of 20, but unlike PD the patients typically also have a number of cognitive and behavioral problems (43, 44).

Spinocerebellar ataxia

Spinocerebellar ataxia (SCA) is a progressive disorder causing degeneration primarily in the cerebellum, leading to a predominantly ataxic syndrome. It has a large range of subtypes, with heterogenous clinical syndromes. Parkinsonism has been particularly reported in three of these: SCA2, SCA3, and SCA17, which can also appear to mimic MSA. These are all trinucleotide repeat disorders, inherited in an autosomal dominant fashion. Of these, SCA2 is the most common disorder associated with parkinsonism, which can be the presenting feature and mimic idiopathic PD, including responsiveness to levodopa. However, classical SCA2 manifests as cerebellar ataxia and peripheral neuropathy, and even in a parkinsonian presentation, ataxia tends to develop within a couple of years (45).

SCA3 is divided into a number of subtypes, of which type IV causes predominant parkinsonism, which is levodopa responsive. The other types are characterized by a combination of cerebellar ataxia and pyramidal signs with extrapyramidal rigidity and dystonia or a peripheral neuropathy. However, patients with type IV have also been shown to commonly have additional clinical features as well as parkinsonism, which would be atypical for idiopathic PD. These include eye movement abnormalities and peripheral neuropathy (45, 46).

As with SCA2 and SCA3, only a subgroup of patients with SCA17 present with predominant parkinsonism. But in those cases, it can be clinically typical, levodopa responsive PD. Other features of SCA17, which are more classical, include cerebellar ataxia, epilepsy, dementia, psychosis, chorea, and dystonia (45).

Fragile X-associated tremor/ataxia syndrome

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder characterized by cerebellar ataxia and intention tremor. It is caused by a premutation CGG trinucleotide repeat expansion of the fragile-X mental retardation 1 (*FMR1*) gene. The full mutation with >200 repeats is associated with intellectual disability with social and behavioral difficulties, usually from adolescence. The premutation, causing FXTAS, is 55–200 repeats, usually presents in middle age in people with normal intelligence, and demonstrates typical imaging findings, which include increased T2 signal in the middle cerebellar peduncle. As well as the cerebellar ataxia, it can also present with parkinsonism, with bradykinesia, postural instability, and rest tremor all being described. However, given the cerebellar features on examination, FXTAS is more commonly mistaken for MSA rather than PD (47).

Frontotemporal dementia with parkinsonism

Parkinsonism in frontotemporal dementia (FTD) is usually seen in the behavioral variant, rather than in association with primary progressive aphasia, and can develop either before or during the development of the classical FTD syndrome.

It can closely mimic idiopathic PD or have features suggestive of PSP or CBS. It is seen in association with underlying tau, TDP-43, or FUS pathology, as well as corresponding mutations in several genes, which include *MAPT, PGRN, C9ORF72, FUS,* and *TARDBP*. Rigidity and bradykinesia tend to be the more prominent parkinsonian features, with rest tremor occurring rarely. There is variable responsiveness to levodopa (48).

Neurodegeneration with brain iron accumulation

Neurodegeneration with brain iron accumulation (NBIA) patients present with a progressive extrapyramidal syndrome associated with iron deposition in the basal ganglia. The two main syndromes are outlined here, although there are additional syndromes including neuroferritinopathy and aceruloplasminemia. The most common of the NBIA disorders is pantothenate kinase-associated neurodegeneration (PKAN), resulting from mutations on the *PANK2* gene, accounting for 50%. The classic syndrome manifests in early childhood with a combination of pyramidal (spasticity, hyperreflexia) and extrapyramidal features (dystonia, parkinsonism). PKAN can also rarely present in early adulthood. There are typical MRI findings, with a central hyperintensity with surrounding low signal on T2 images in the globus pallidus, giving the so-called eye-of-the-tiger sign (49).

The second main type of NBIA is *PLA2G6*-associated neurodegeneration (PLAN). When onset occurs in infancy, PLAN causes progressive motor and mental retardation with cerebellar ataxia, seizures, and pyramidal signs. However, onset can occur later in life which leads to an atypical syndrome that may mimic PD, with rest tremor, rigidity, and bradykinesia and a good response to levodopa. However, patients also exhibit additional features including eye-movement abnormalities and pyramidal signs (49).

Mitochondrial disorders

Mutations in the *POLG* gene have also been linked with early onset parkinsonism (50). This gene is implicated in the synthesis and repair of mitochondrial DNA, and a large number of clinical phenotypes are associated with its mutations. These include progressive external ophthalmoplegia, ataxia, and peripheral neuropathy. However, parkinsonism can also occur with bradykinesia and rigidity. This has been described as atypical for idiopathic PD, involving a symmetrical distribution with a postural (not rest) tremor and a poor response to levodopa (51).

Idiopathic basal ganglia calcification

This is a heterogenous disease associated with mineral deposition in the basal ganglia, as well as in other brain structures. There is a strong familial component, with causative mutations identified in *SCL20A2* and *PDGFRB*. Patients commonly have a movement disorder, with parkinsonian features of akinesia and rigidity which show a variable response to levodopa. Other features include cognitive impairment, gait disorder, pyramidal signs, and a psychiatric presentation. Imaging is crucial in diagnosis to identify the areas of calcification, with CT imaging being more useful than MRI (52).

TREMOR DISORDERS

Tremor remains the most well-known feature of PD, among both health professionals and the general public. This is despite the fact that a proportion of patients with PD will never develop a tremor. In some, however, it is the first reported symptom; therefore, it is important to distinguish the resting tremor of PD from other causes of tremor.

Essential tremor

Essential tremor (ET) is common, with an increasing incidence with age, although it may develop at any age from childhood. It typically progresses slowly until later in life. The neuropathological basis of this tremor is unknown, although there is a strong familial component. People with ET often don't present to a clinician unless there is also a concern about PD or the tremor is very disabling (53).

This tremor is typically present in both upper limbs and occurs on action, either while holding a sustained posture or while performing a voluntary movement. Patients commonly notice the tremor while performing actions such as writing or holding a cup. The tremor may improve with small amounts of alcohol and, as with all tremors, it gets worse with anxiety, illness, or tiredness. Tremor can also occur in other locations, such as the lower limbs, head, and it can affect the voice. For diagnosis, there should be at least a 3-year history and no other neurological signs. There is evidence that ET is a more heterogenous syndrome, including the presence of soft neurological signs in some patients; yet, currently there is no consensus regarding the inclusion of these signs in the diagnosis (8).

ET is easy to differentiate from the classical rest tremor of PD; however, some patients with PD also have a postural-action tremor. It is also important to note that cogwheeling rigidity is common with any tremor syndrome, including ET. Furthermore, a re-emergent tremor is also a feature of PD, which is visible several seconds following maintaining a posture (7). In cases where there is difficulty in distinguishing ET from PD, particularly in tremor-dominant, slowly progressive forms, a DaTSCAN can be useful, as it is normal in ET.

Dystonic tremor

Another common cause of tremor presenting to neurology services is dystonic tremor. This is a tremor occurring in a part of the body affected by dystonia. Therefore, when assessing someone with tremor, it is key to look for the presence of an abnormal posture, which may be subtle. The tremor is caused by rhythmic muscle contractions, often inconstant and described as "jerky." It is exacerbated by attempts to maintain a normal posture. For example, dystonic tremor commonly occurs with cervical dystonia, causing a dystonic head tremor, which may include upper limb involvement. Another clue to the presence of dystonia is looking for a "geste antagoniste," a voluntary movement that corrects the dystonic posture (8, 54).

Where there is diagnostic doubt, as with ET, DaTSCAN is normal. The treatment of choice is typically with local Botox injection.

NON-NEUROLOGICAL DIFFERENTIALS OF PARKINSON'S DISEASE

As described at the beginning of the chapter, PD is a heterogenous condition, with a range of non-motor features as well as the motor syndrome of parkinsonism. It has been shown that in approximately 20% of patients, the non-motor symptom is the presenting feature (55). With this range of potential symptoms, PD also commonly presents to specialties other than neurology, notably rheumatology and psychiatry.

Arthritis

Pain has been shown to be one of the most common non-motor symptom at presentation in PD, and so, a patient's initial referral is often to a rheumatologist or orthopedic surgeon. Pain is usually related to the affected motor side and, together with the associated rigidity, can resemble a stiff joint. Frozen shoulder, degenerative spinal disease, and osteoarthritis are some of the most common initial diagnoses in cases that later are revised to be PD. These misdiagnoses have also been shown to be related to unnecessary procedures, including steroid injections and spinal surgery (55, 56).

Furthermore, rheumatological disease can also resemble PD, and it is possible for someone with a primary underlying polyarthropathy to be mistaken as having PD. Joint stiffness can mimic the rigidity and can make it difficult during clinical examination to distinguish it from the bradykinesia of PD. To help to separate these conditions, it is key to look for decrement on the examination of bradykinesia, which is specific for PD, and to explore other supportive features and disease progression in the history. Finally, it is also important to remember that PD patients can develop musculoskeletal disorders secondary to parkinsonian bradykinesia and rigidity (57).

Depression

Depression is common in PD, affecting up to an estimated 40% patients, and can pre-date the motor syndrome (4). The two conditions therefore commonly coexist, but equally they can be mistaken for each other. The psychomotor slowing in depression can mimic the bradykinesia of PD and vice versa. For example, the lack of facial expression and flat affect in depression can be confused with the hypomimia of PD. They also share multiple comorbid features; both depression and PD are associated with sleep disturbance, anxiety, fatigue, apathy, and poor concentration (58).

Obsessional slowness

This is a rare motor presentation of obsessive–compulsive disorder, which can mimic PD. The motor slowness of obsessional slowness (OS) is not related to the obsessions and compulsive rituals of OCD, but is instead a separate phenomenon, although

occurring in patients who commonly have the classic features of OCD. Patients exhibit slowness of movement, poor speech production and have difficulty in initiating voluntary actions. However, the motor slowness does not display the decrement during repetitive actions which is seen in PD, and DATScan is normal. Motor speed can equally appear normal for reflexive actions or in times of stress (59).

Psychogenic parkinsonism

Like other psychogenic movement disorders, the etiology of psychogenic parkinsonism (PP) is presumed to be a conversion or somatoform disorder, without conscious awareness or control. It can occur, but not exclusively, in the presence of other psychological or psychiatric disorders, and stress plays a role in most by precipitating, maintaining, and exacerbating symptoms.

A positive diagnosis is made by the identification of clinical features. The slowness of PP is effortful, without decrement, and improves with distraction, as does the rigidity, which is felt as an active resistance against passive movement. The tremor has a number of features which are not typical of PD; it tends to start abruptly; it has a variable frequency, direction, and distribution; and it also improves with distraction. Testing for postural stability may be helpful, as patients commonly have bizarre or exaggerated responses, without falling. Finally, there may be other clinical features of functional neurological disorders, such as "give-way" weakness, a positive Hoover's sign, or non-anatomical sensory loss (60).

CONCLUSION

Parkinson's disease is a clinical diagnosis, the accuracy of which, as in much of neurology, relies on a detailed history and thorough examination. The certainty of this diagnosis improves following a positive response to treatment and a clinical picture of steady symptomatic decline when the patient is seen in subsequent appointments. The differential diagnosis of PD outlined here is broad, with many rare causes of parkinsonism. It is certainly not appropriate to investigate all cases of PD, but it is critical to keep an open mind regarding unusual features which may lead you to consider one of these differentials.

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Pharmacological Treatment of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is one of the common chronic degenerative conditions of the nervous system. There is currently no cure for PD, but a number of drugs offer benefits in terms of controlling the motor symptoms. While these drugs can offer significant improvements to motor function, they may lead to problematic adverse effects, particularly as disease progresses. In this chapter, we focus on the drugs that are currently employed for the treatment of PD, including discussion on their mode of action, clinical utility, and adverse effects. We also cover some interesting emerging approaches that are currently under investigation.

Keywords: Clinical trials; Dopamine; Emerging treatments; Management; Parkinson's disease; Pharmacological treatment

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INTRODUCTION

Parkinson's disease (PD) is a gradually progressive neurodegenerative condition. The etiology and pathogenesis remain incompletely understood. There are currently no disease-modifying treatments for PD, and medical management is predominantly focused on controlling the motor symptoms using drugs. The long-term duration of disease means that patients may take sophisticated medication regimes aimed at controlling the motor symptoms, with a likelihood of problematic side effects. The movement disorder of PD occurs largely due to the selective loss of neurons in the substantia nigra pars compacta, with consequent depletion of dopamine in the striatum (1–3). Dopaminergic drugs designed to replace the action of dopamine in the deplete striatum form the mainstay of PD treatment at present.

This may be achieved through drugs that are metabolized to dopamine, that activate the dopamine receptor, or that prevent the breakdown of endogenous dopamine (4–6). There is no gold standard of treatment strategy, with medication regimes being tailored to the individual patient, based on the severity and temporal nature of their symptoms, as well as the side effects that they experience (1–4). In this context, this chapter will discuss treatment strategies involving pharmacological agents, with major emphasis on dopamine replacement therapies.

DOPAMINE BIOSYNTHESIS AND METABOLISM

Dopamine is incapable of crossing the blood–brain barrier (BBB), and it must be produced within the central nervous system (CNS) in order to act in the striatum. It is primarily synthesized in dopamine-producing neurons (dopaminergic neurons) within the brain, with small amounts of dopamine also being produced in the medulla of the adrenal glands (7). Here, we discuss the pathway pertaining to dopamine synthesis within the CNS.

In the classical biosynthetic pathway of dopamine, the direct metabolic precursor is L-dihydroxyphenylalanine (levodopa or L-DOPA) which is synthesized either directly from tyrosine (a non-essential amino acid) or indirectly from phenylalanine (an essential amino acid) (8). L-phenylalanine is converted into L-tyrosine in the liver, by the enzyme phenylalanine hydroxylase (PH) in the presence of oxygen, iron, and tetrahydrobiopterin as cofactors (8, 9). Tyrosine produced in the liver is then transported by an active transport mechanism into the dopaminergic neurons within the brain. This is followed by the conversion of L-tyrosine into L-DOPA through hydroxylation at the phenol ring by the enzyme tyrosine hydroxylase (TH). Subsequently, L-DOPA is converted into 3,4-dihydroxyphenethylamine (dopamine) through decarboxylation by the enzyme L-3,4-dihydroxyphenylalanine decarboxylase (DOPA decarboxylase) in the pre-synaptic terminal (8). DOPA decarboxylase is also known as aromatic-L-amino acid decarboxylase (AADC) due to its action on all naturally occurring aromatic L-amino acids, in addition to L-DOPA. Furthermore, under specific conditions, dopamine can also be synthesized by a minor pathway, in which

L-tyrosine is converted into p-tyramine (mediated by AADC), with subsequent hydroxylation to dopamine by the enzyme CYP2D6 (Cytochrome P450 2D6) which is found in the substantia nigra of human brain (10–12).

Dopamine is metabolized after reuptake into dopaminergic neurons or glial cells (13). It undergoes oxidative deamination, catalyzed by the enzyme monoamine oxidase (MAO) in the presence of flavin adenine dinucleotide (FAD), to produce reactive aldehyde 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is inactivated by conversion to 3,4-dihydroxyphenylethanol (DOPET) by alcohol dehydrogenase (ADH) or to 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase (ALDH) (13). DOPAC is then degraded to the biologically inactive metabolite homovanillic acid (HVA) by the enzyme catechol-O-methyl transferase (COMT). Alternatively, dopamine is metabolized to 3-methoxytyramine by COMT, which is in turn converted to 3-methoxy-4-hydroxyacetaldehyde by MAO. The aforementioned ALDH then converts this to HVA, which is excreted in the urine (8). These pathways are illustrated in Figure 1.

Multiple components in this pathway have been targeted for the treatment of PD. For example, genes encoding the rate-limiting enzymes for dopamine synthesis, TH and AADC, formed part of the experimental lentiviral gene therapy ProSavin (Oxford Biomedica) which has been trialled in PD patients (14). Levodopa forms the mainstay of PD treatment regimes, with inhibitors of the metabolic enzymes MAO-B and COMT also being used. These drugs are discussed in detail in the remainder of this chapter.

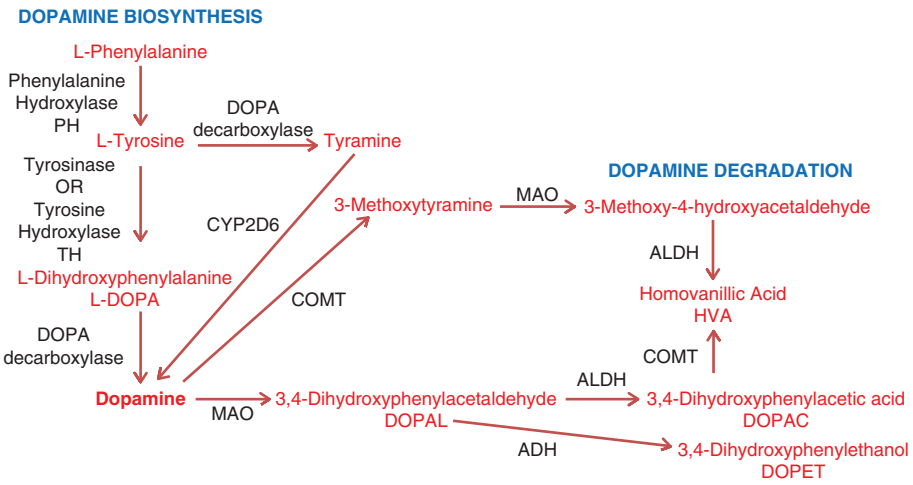

Figure 1 Metabolic pathway of dopamine synthesis and clearance. Dopamine is synthesized from phenylalanine or tyrosine via sequential reactions catalyzed mainly by PH, TH, and DOPA decarboxylase. It can also be synthesized from tyramine in a minor pathway by CYP2D6. Dopamine is effectively degraded into the main inactive metabolites DOPAC and HVA via a series of reactions mediated predominantly by the enzymes MAO, COMT, ALDH, and ADH.

CURRENT TREATMENTS

There are currently no disease-modifying drugs for PD, but the treatments that are used can offer significant symptomatic relief of the motor symptoms. They offer little clinical benefit in terms of the non-motor manifestations of PD. It is usual practice to delay the initiation of treatment until the patient's symptoms become troubling, to reduce the impact of adverse effects.

Levodopa

The mainstay of current PD treatment are levodopa-based preparations, designed to replace the dopamine in the depleted striatum. As is described above, dopamine itself is unable to cross the BBB and cannot be used to treat PD (2). In contrast, the dopamine precursor levodopa is able to cross the BBB and can be administered as a therapy. After absorption and transit across the BBB, it is converted into the neurotransmitter dopamine by DOPA decarboxylase (6) (Figure 1). It is usual practice for patients to be commenced on a low dose of levodopa, with the dose being titrated up based on the patient's response to treatment, balanced against the adverse effects experienced. Most patients require a dose in the range of 150–1000 mg daily, divided into multiple doses (15). Increasing doses result in elevated risk of developing problematic adverse effects, as discussed below (15). Generally, the clinical effect of levodopa is noticed quickly, and may last for several hours, particularly in the early stages of disease (15). However, as disease becomes more advanced, the effect of the drug usually wears off after shorter durations, and an increased frequency of dosing is often required.

Levodopa, though effective, comes with significant side effects that constitute an important part of the illness experienced by the patient, particularly in advanced disease. Some of its associated side effects result from the conversion of levodopa to dopamine outside the CNS (peripheral conversion) by DOPA decarboxylase (6, 16). These effects are minimized by administering levodopa in combination with peripheral inhibitors of DOPA decarboxylase, as is discussed below. Prolonged use can result in significant motor complications, including dyskinesias, and severe on–off motor fluctuations (6).

Dyskinesias are involuntary twisting hyperkinetic movements, which usually occur when the drug is at peak dose (but may also occur as the drug is wearing-off or even during off-periods) (17, 18). The emergence of problematic dyskinesias may be treated by a reduction in levodopa dose, meaning that a difficult balance must be struck between optimizing the control of the motor symptoms, while minimizing the adverse effects. In patients that have previously responded well to levodopa, but that have developed problematic dyskinesias, deep brain stimulation may be considered, which may allow for the control of motor symptoms on a reduced dose of levodopa. This is discussed in detail in Chapter 8. The on–off phenomenon refers to the fact that patients with advanced PD may experience rapid fluctuations in their motor function. During the "on" state, motor symptoms are controlled relatively well, but rapid wearing-off of the effect of levodopa leaves the patient in the "off" state, in which they have severe Parkinsonian motor features. These fluctuations can be particularly problematic and severely

limit function. The probable causes of these motor symptoms are variable drug absorption and transit across the BBB, and resultant fluctuations in pre-synaptic and post-synaptic dopamine levels in the nigrostriatal pathway (19–25). Other important side effects include gastrointestinal disturbances such as nausea and vomiting, and orthostatic hypotension. Neuropsychiatric features including anxiety and hallucinations may occur due to "off-target" effects of dopamine acting in extranigral brain regions (6, 16).

Some strategies used to counteract the adverse effects of levodopa include using the minimum effective dose, fractionation of the dose, and the use of alternative dopaminergic treatments. Historically, temporary withdrawal of levodopa was used ("levodopa holidays"), but this is no longer recommended (6, 26). To reduce its peripheral side effects, levodopa is administered in combination with DOPA decarboxylase inhibitors such as benserazide and carbidopa. These compounds do not cross the BBB, but selectively prevent the peripheral conversion of levodopa to dopamine, thereby reducing the peripheral side effects (27, 28). The most frequently prescribed combination drugs are carbidopa/levodopa (co-careldopa [trade names Sinemet, Pharmacopa, Atamet]) and benserazide/levodopa (co-beneldopa [trade name Madopar]) (29). These compounds are available in several formulations, including modified release preparations, which can be useful for controlling symptoms overnight, and limiting early morning symptoms, as well as suspensions, which can be useful for patients that have swallowing difficulties (6, 29–31).

More recently, continuous intestinal infusion of levodopa gel (Duodopa [AbbVie Limited]—a combination of levodopa with carbidopa) has shown to be effective in terms of decreasing severe motor fluctuations when compared to oral levodopa—probably a result of more consistent levodopa absorption. However, this treatment is currently prohibitively expensive for widespread use (29). Researchers continue to focus on the development of other long-acting oral preparations as well as other modes of drug delivery, which may allow for improved clinical efficacy and side-effect profiles in the future (29).

Dopamine agonists

Dopamine receptor agonists came into the market for the treatment of PD in 1978. The commonly used agonists contain an ethanolamine moiety, and they may be categorized into ergot and non-ergot derived, based on receptor specificities (see Table 1) (32). These drugs stimulate the activity of the dopamine system by binding to the dopaminergic receptors and, unlike levodopa, do not need to be converted into dopamine (2, 6). Dopamine agonists are often prescribed as an initial therapy for PD, particularly in younger patients (6, 33). This approach allows for a delay in the use of levodopa, which may reduce the impact of the problematic motor complications, discussed above (6, 33). Some of the drugs listed in Table 1 are no longer used in clinical practice, as significant idiosyncratic adverse effects were observed. For example, pergolide was withdrawn as a treatment in 2007, after studies found that it was associated with a risk of pericardial, retroperitoneal, and pleural fibrosis (34).

Some of these drugs are available in controlled or prolonged release formulations in the form of tablets, patches, and injections. Rotigotine patches,

TABLE 1 Classification of dopamine agonists

Ergot derived	Non-ergot derived
Bromocriptine (Parlodel, Oral)	Apomorphine (Apokyn, Subcutaneous)
Pergolide (Permax, Oral)	Pramipexole (Mirapex, Oral)
Cabergoline (Oral)	Ropinirole (Requip, Oral)
Lisuride (Oral)	Rotigotine (NeuPro, Transdermal Patch)

for example, are useful in patients that are unable to take oral medications, for example, when they are kept nil-by-mouth in preparation for surgery. Switching a patient onto rotigotine patches in this setting requires calculation of the levodopa-equivalent dose of their existing treatment regime, to ensure that they are adequately medicated. While they may be less effective than levodopa in controlling the motor symptoms of PD, with the majority of patients ultimately requiring levodopa therapy, dopamine agonists can be useful in patients with minor symptoms, in those that are unable to tolerate levodopa, or as an adjunct to levodopa therapy.

The drug half-life, and therefore duration of action, varies with patients and the type of agonist prescribed (6). On initiation of dopamine agonists, the dose is usually gradually increased, based on the patient's response and the side effects experienced (6). Of the commonly prescribed dopamine agonists, the usual dosing is as follows: 9–16 mg (maximum 24 mg) total daily dose for ropinirole, divided into three to four doses; up to 3.3 mg total daily dose of pramipexole, divided into three doses; and 4–6 mg once daily for rotigotine (35). Apomorphine is used less frequently but can be useful in relieving severe "off" episodes when given as a subcutaneous injection, or in patients with severe motor fluctuations (despite optimization of other medications), as a subcutaneous infusion. Some preclinical and imaging studies have suggested that dopamine agonists may possess antioxidant properties and lead to reduced loss of dopaminergic neurons, though there is no convincing evidence that these drugs offer a disease-modifying effect (36–40).

Treatment with dopamine agonists has been shown to result in a reduced incidence and severity of dystonia, motor fluctuations, and dyskinesia in comparison to levodopa (6, 33). However, they may cause other severe adverse effects (41). Common side effects include nausea and vomiting (which occurs due to stimulation of the area postrema, situated in the medulla at a site in which the BBB is disrupted), dry mouth, insomnia, peripheral edema, constipation, fainting, hallucinations, and sleepiness (2, 6, 33).

Perhaps, the most important adverse effect of dopamine agonists is the development of compulsive and impulsive behavioral problems (impulse control disorder [ICD]). Symptoms may include hypersexuality, gambling, binge eating, compulsive buying/shopping, punding, and hobbyism (compulsive Internet use, artistic endeavors, and writing) (42–48). It is important that clinicians are vigilant for such problems after initiation of dopamine agonists because they can result in

significant issues for the patient, from a financial and social perspective. ICD may result in problems with interpersonal relations, caregiver well-being, and quality of life. Certain behaviors may be more common in males (e.g. hypersexuality) or females (e.g. compulsive shopping), and ICD is more common in those with a history of addiction (e.g. to alcohol or gambling) (41). ICD occurs in 15–20% of PD patients taking dopamine agonists (there is also an increased risk of ICD with levodopa, though this is much less than that associated with dopamine agonists) (49, 50). Proposed mechanisms have included the action of dopamine agonists on the mesolimbic dopaminergic pathway, orbitofrontal cortex, and opiate and serotonin systems (42, 47).

Another important consideration is the risk of dopamine agonist withdrawal syndrome (DAWS), which may occur when a person with compulsive or impulsive behavior either stops taking or reduces the dosage of dopamine agonists (48). Symptoms of withdrawal syndrome may include anxiety, panic attacks, insomnia, irritability, dysphoria, agitation, fatigue, orthostatic hypotension, diaphoresis, and drug cravings (51). Thus, withdrawal of dopamine agonists must be performed cautiously, with clinical vigilance for these problems. As with ICD, DAWS can result in significant psychosocial consequences.

Monoamine Oxidase B (MAO-B) inhibitors

Other PD medications work by inhibiting the enzymes involved in dopamine metabolism, which preserves the levels of endogenous dopamine. One such class is the MAO-B inhibitors. As is discussed above, MAO-B is one of the main enzymes involved in the breakdown of dopamine, and reducing the activity of this enzyme therefore results in increased dopaminergic activity within the striatum, mediated by endogenous dopamine (see Figure 1) (6). Their use relieves motor symptoms in PD patients, and as with dopamine agonists they may be used as an initial treatment option, to delay the need for levodopa therapy, to reduce the risk of levodopa-induced motor complications (33). While they are sometimes sufficient for control of symptoms in early disease, most patients ultimately require levodopa-based treatment. MAO-B inhibitors may also be used in combination with levodopa-based preparations, to allow for a reduction in the levodopa dose.

Commonly used MAO-B inhibitors include selegiline (Deprenyl, Eldepryl, Zelapar) and rasagiline (Azilect). More recently, the drug safinamide (Xadago) was also approved for use in PD, which appears to have multiple modes of action, one of which is thought to be inhibition of MAO-B (52). The recommended dosage for selegiline is 5–10 mg daily, and for rasagiline it is 0.5–1 mg once daily (35). MAO-B inhibitors are generally well tolerated, with gastrointestinal side effects being the most common problem. Other adverse effects include aching joints, depression, fatigue, dry mouth, insomnia, dizziness, confusion, nightmares, hallucinations, flu-like symptoms, indigestion, and headache (6, 33).

Catechol-O-methyl transferase inhibitors

As is discussed above, another enzyme that is involved in dopamine degradation is COMT (Figure 1). Inhibitors of COMT therefore also offer a therapeutic means of preserving endogenous dopamine levels, by reducing its breakdown (6, 33).

These are predominantly used as adjunctive therapy to levodopa, prolonging its duration of action by increasing its half-life and its delivery to the brain. In some patients, this allows for control of motor symptoms, with a reduction in off time in comparison to standard levodopa/DOPA decarboxylase inhibitor combinations (36). They are often prescribed to the patients when end-of-dose "wearing-off" is a particular problem, with levodopa therapy alone.

COMT inhibitors come in the form of tablets and are not generally prescribed as monotherapy, as on their own they offer only limited effect on PD symptoms. Examples of COMT inhibitors include entacapone (Comtan), tolcapone (Tasmar), and opicapone (Ongentys). Entacapone is often used in a combination preparation along with carbidopa and levodopa (Stalevo, Sastravi) (6, 35). The typical dosage for entacapone is 200 mg four to eight times a day with each levodopa dose and 100 mg three times a day in the case of tolcapone—the two most commonly used COMT inhibitors. It should be noted that COMT inhibitors can lead to the amplification of levodopa-induced side effects, including dyskinesias, and it may be that they necessitate a reduction in the levodopa dose (35). Tolcapone is associated with an uncommon, but potentially serious, risk of hepatotoxicity, and as such entacapone is generally preferred (35). Treatment with tolcapone therefore warrants monitoring of liver function tests. Other uncommon side effects include sleepiness, nausea, loss of appetite, diarrhea, dizziness, orange urine discoloration, hallucinations, abdominal pain, headaches, confusion, dry mouth, and chest pain (6, 35).

Anticholinergics

The medications that have so far been discussed are all designed to increase dopaminergic activity in the striatum. There are a small number of drugs used in the treatment of PD that act through non-dopaminergic mechanisms. One such class of drugs are the anticholinergics. These reduce the activity of the neurotransmitter acetylcholine, by acting as antagonists at cholinergic receptors (35). While their role is limited and they are now prescribed infrequently, they may offer some benefit in improving rigidity and tremor in PD (53). Loss of dopaminergic neurons results in disturbance of the normal balance between dopamine and acetylcholine in the brain, and anticholinergic drugs may lead to restoration and maintenance of the normal balance between these two neurotransmitters (33).

The main role of these drugs is in young patients at early stages of the disease for the relief of mild movement symptoms—particularly tremors and muscle stiffness (35). Anticholinergic drugs play more of a role in tremor-predominant PD, where they may be used as monotherapy in the early stages. However, when anticholinergics are used, they are usually done so in combination with levodopa and the other aforementioned medications. They are generally avoided in elderly patients or those with cognitive problems, due to an increased risk of confusion with this class of drugs (35). Tablet and oral suspension preparations exist. Examples of anticholinergics include benztropine, orphenadrine, procyclidine, and trihexyphenidyl (Benzhexol) (35). The common adverse effects include blurred vision, dry mouth, constipation, drowsiness, trouble urinating, urinary retention, confusion, cognitive impairment, hallucinations, dizziness, trouble swallowing, dyskinetic movements, and memory problems. Although dry mouth is listed as an adverse effect of anticholinergics, in patients in whom drooling is a

particular problem, the reduced salivation brought on by anticholinergic drugs is a desirable effect, and they may actually be used in the treatment of this symptom (33, 35, 36).

Amantadine

Initially, amantadine (Symmetrel) was developed as an antiviral drug for treating flu, but it has subsequently been used for the treatment of PD. It may be used for the treatment of rigidity, rest tremor, and sometimes fatigue, and may offer a short-lived improvement in symptoms. It may also allow for a lower dose of levodopa to be used, reducing the risk of dyskinesia. However, its most useful property is probably the fact that it can be used to limit the severity of levodopa-induced dyskinesias (54). It should be noted that the evidence for the use of amantadine in controlling PD symptoms is limited, with a 2003 Cochrane review concluding that there was insufficient evidence to recommend its use in PD (55).

Chemically, it is the derivative of adamantine as 1-adamantylamine or 1-aminoadamantane. It is not known how amantadine may have an anti-Parkinsonian effect, but it acts as a weak glutamate antagonist at the N-methyl-D-aspartate receptor (NMDAR) (54). Like most of the other anti-Parkinson drugs that have been discussed, it is started with a low dose and is titrated up. It comes in the form of tablets and liquid syrup. While generally well tolerated, possible side effects associated with the use of amantadine include hallucinations, confusion and impaired concentration, livedo reticularis, leg swelling, blurred vision, nausea and vomiting, appetite loss, insomnia and nightmares, sweating, agitation, and headache (35).

EMERGING TREATMENTS

The drugs that have been discussed are used to control the symptoms of PD, but none of them alter the course of disease. While there are currently no disease-modifying treatments for PD, a number of promising novel approaches are currently under investigation (56, 57). As well as new experimental compounds, there is also much interest in drug repurposing—the use of drugs that have an established clinical indication—in a new setting. Because such drugs have been used previously, safety data already exist, so progress through clinical trials may potentially be expedited. In addition to new drugs, there are a number of regenerative approaches currently in, or about to enter, clinical trials. These include gene therapies, such as ProSavin—a lentivirus vector carrying the genes encoding DOPA decarboxylase, TH, and guanosine triphosphate cyclohydrolase-1 (GTPCH1) and stem cell approaches (with the latter being discussed in detail in Chapter 9) (14, 57). These regenerative treatments are not designed to offer a disease-modifying effect but to restore dopaminergic activity in the striatum in a more physiological fashion than what is currently achieved with dopaminergic medications, theoretically with a reduced risk of the adverse effects of levodopa (58).

There is a plethora of evidence suggesting that alpha-synuclein (α-synuclein) aggregation plays a central role in the pathogenesis of PD (59, 60). Thus, there is

much interest in how this process may be targeted by potential therapies. Therapeutic approaches have been developed aiming to:

(i) reduce α-synuclein production
(ii) inhibit α-synuclein aggregation
(iii) increase intracellular and extracellular degradation of α-synuclein aggregates
(iv) reduce uptake of extracellular α-synuclein by neighboring cells (56)

Immunotherapies targeting α-synuclein are now beginning to enter clinical testing. Recently, a Phase 1 clinical trial with the synthetic vaccine AFFITOPE PD03A, containing an α-synuclein mimicking peptide, has been completed by Affiris (61). This formulation was tested in 36 patients with early stage PD, who received the vaccine subcutaneously, and it was found to be very well tolerated, with only mild side effects. An α-synuclein-targeting passive immunotherapeutic agent PRX002 (Prothena) has also been tested in Phase 1a and Phase 1b clinical trials. This is a humanized monoclonal antibody with which a 96.5% reduction in free serum levels of α-synuclein was observed (62, 63). No major side effects or toxicity occurred, and the drug has progressed to Phase 2 clinical trials (64). Another α-synuclein-based passive immunotherapy, BIIB-054 (Biogen), was found to be well tolerated with a satisfactory pharmacokinetic profile (65, 66). A number of other experimental immunotherapeutic agents are also under investigation (67–69). In addition, drugs aiming to result in increased extracellular degradation of α-synuclein are being considered as potential therapeutic options for PD, for example, the serine protease Kallikrein 6 (KLK6 or neurosin) (70, 71).

In addition to increasing α-synuclein clearance, another potential avenue would be to reduce α-synuclein production, which may be achieved through RNA interference (RNAi) technology. Although this has not reached in-human trials, *in vitro* and animal studies have generated some interesting results. For example, short hairpin RNA (shRNA) targeting α-synuclein has been delivered via a lentiviral vector to rats, which resulted in silencing of the expression of ectopic human α-synuclein in the striatum. Small interfering RNA (siRNA) delivered into the mouse hippocampus also decreased the expression of endogenous α-synuclein after a 2-week infusion, with no signs of toxicity (72, 73). Subsequent use of siRNA in non-human primates demonstrated a reduction in α-synuclein levels by 40–50%. However, this approach did not progress toward clinical trials due to lack of funding (74). Rats treated with shRNA also demonstrated a reduction in α-synuclein levels of 35% (75). Of course, the concern with suppressing α-synuclein levels to such a degree is that the normal function of the protein is lost. In some studies, significant reduction in α-synuclein levels was accompanied by escalated neurotoxicity, with some even showing degeneration of nigrostriatal system (76–78). Extensive preclinical safety data will therefore be necessary if these techniques are to enter clinical trials.

Another approach for reducing α-synuclein production involves reducing its expression at a transcriptional level. Beta-2-adrenoreceptor (beta-2AR) agonists, such as clenbuterol, have been suggested to do this, and have achieved a greater than 35% reduction in α-synuclein expression in a neuroblastoma cell-line and in rat cortical neurons (79). It has been postulated that they act by histone 3 lysine 27 acetylation of α-synuclein promoters and enhancers. Supportive evidence for a potential benefit of these drugs comes from two epidemiological studies carried out in Norway involving very large numbers of patients, suggesting that beta-2AR

agonists warrant further investigation, and that they may play a role in PD treatment in the future (79).

Of the other existing drugs being considered for repurposing, two have entered clinical trials—the chemotherapy agent, nilotinib, and the glucagon-like peptide-1 receptor agonist, exenatide. Nilotinib is a c-Abl tyrosine kinase inhibitor used in the treatment of chronic myelogenous leukemia (CML). Activity of c-abl has been found to be enhanced in brain tissue of PD patients, which may lead to increased phosphorylation and aggregation of α-synuclein, and/or reduced function of the Parkin protein involved in mitochondrial biogenesis (80). Nilotinib attenuated α-synuclein levels in A53T transgenic mice and also provided a degree of neuroprotection (81). It has been observed to be well tolerated by PD patients albeit at a much lower dosage than what is usually prescribed for the treatment of CML (82). Following these observations, nilotinib has entered a Phase 2a trial in 2017, and there is much hope about its treatment potential (83). However, a potential limitation to the use of c-abl inhibitors in PD is the poor transit across the BBB, and novel agents may need to be developed to circumvent this.

Similarly, exenatide, an established treatment for type 2 diabetes mellitus, is emerging as a promising therapeutic option for PD. Neuroprotective potential has been seen in preclinical models of the disease, with persistent clinical improvements observed in an initial clinical trial (84). Thereafter, it was taken forward to a single-center, randomized, double-blind, placebo-controlled Phase 2 clinical trial in which a weekly dosage of 2 mg was administered to patients subcutaneously (85). Similarly, improvements in motor scores were observed, which persisted even beyond discontinuation of treatment.

CONCLUSION

The current treatments available for PD are designed to restore dopaminergic activity in the dopamine-deplete striatum of PD patients, with consequent improvement in motor symptoms. Disappointingly, there is a paucity of pharmacological options for treatment of the non-motor features, which are unfortunately often the most disabling aspects of disease. At the present time, there are no established treatments able to slow, stop, or modify the disease course. Commonly used drugs for PD include those based on exogenous administration of compounds with dopaminergic activity (e.g. levodopa, dopamine agonists), and those that inhibit the metabolism of endogenous dopamine (e.g. COMT, MAO-B inhibitors) (6). While levodopa can cause significant adverse effects, the vast majority of patients ultimately require treatment with this drug. It is important to note that there is no standard treatment regime for PD, with each patient being treated with a tailored approach taking into account the severity of their symptoms and temporal nature of these, the side effects that they experience, and their personal priorities.

While there have been few major developments in the field of PD treatment since the introduction of levodopa, numerous experimental therapeutic approaches are currently under investigation. These include drugs that specifically target α-synuclein pathology—widely considered to be the driver of neurodegeneration in PD. These drugs offer hope that a disease-modifying agent will be identified in

the short- to medium-term future. In combination with a number of regenerative approaches, including stem cells and gene therapies, therapeutics of PD is likely to see significant advances over the coming years, with a number of novel, effective options likely to become available to clinicians in the foreseeable future.

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8

Considerations for Patient and Target Selection in Deep Brain Stimulation Surgery for Parkinson's Disease

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Abstract: Deep brain stimulation (DBS) is an effective treatment for improving motor symptoms of Parkinson's disease among well-selected patients. Over the past 30 years, several anatomical regions have been targeted with DBS based on prior experience in lesional neurosurgery and characteristic changes in subcortical motor regulation in Parkinson's disease. In this chapter, we provide an overview of the patient selection process and surgical procedure for DBS. We also discuss the various surgical targets for DBS in patients with Parkinson's disease. The subthalamic nucleus and the globus pallidus interna are the most

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common surgical targets among patients with Parkinson's disease and have equivalent beneficial effects on motor symptoms. Most studies report 30–60% improvement in motor score evaluations after DBS among well-selected patients. After subthalamic nucleus DBS, patients are able to reduce medications by 50% on average. In patients with globus pallidus interna DBS, stimulation has an anti-dyskinetic effect, although medication doses remain similar. DBS of the subthalamic nucleus is generally avoided in patients with a history of depression or neurocognitive impairment. Thalamic DBS ameliorates tremor, but has little effect on bradykinesia or rigidity. Finally, the pedunculopontine nucleus DBS is an emerging experimental treatment for postural and gait instability in Parkinson's disease.

Keywords: Deep brain stimulation; Globus pallidus interna; Parkinson's disease; Pedunculopontine nucleus; Subthalamic nucleus; Ventralis intermediate nucleus

INTRODUCTION

Idiopathic Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder after Alzheimer's disease (AD). PD affects an estimated 600,000 people in the United States alone, with an estimated global total of 6.8 million people affected in 2015 (1, 2). The typical combination of motor features of PD is called "parkinsonism," which requires the presence of bradykinesia and the variable coexistence of other clinically defined signs: resting tremor, muscle rigidity, and postural instability. In addition, several other motor and non-motor features occur during the course of the disease, such as cognitive changes, sleep disturbances, and autonomic nervous system disorders. The hallmark pathological feature in PD is profound degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). Loss of these neurons, which project widely within the striatum and pallidum, produce a state of low dopamine within the brain (3).

Levodopa and other dopamine replacement medications have been used since the 1960s and have revolutionized the treatment of PD (4, 5). Patients initially respond well to dopamine replacement treatment; however, over time, the beneficial effects are associated with complications such as motor fluctuations and levodopa-induced dyskinesias (LID) (6). Motor fluctuations include early wearing off, delayed on and sudden on/off phenomena, while dyskinesias occur usually during peak dose of levodopa in the form of involuntary hyperkinetic movements. These complications occur in a variable spectrum of severity and affect most, if not all, patients during the progression of the disease.

Surgical treatments such as pallidotomy and thalamotomy were used to alleviate the motor symptoms of PD and were historically introduced even prior to the development of dopamine replacement therapy (7–13). These procedures were temporarily abandoned after the introduction of dopamine replacement therapy; however, as the complications of pharmacological therapies were recognized, there was a resurgence in surgical treatments for PD (14–16). During lesioning procedures, such as thalamotomy, electrical stimulation was commonly used to test for effects and side effects prior to making permanent lesions.

Also, high-frequency stimulation of the thalamus induced reduction in tremor severity in patients with PD (17). These findings, along with advances in implantable pulse generator devices, led to the development of deep brain stimulation (DBS) systems as we know today. DBS has been used to treat more than an estimated 120,000 neurological patients worldwide (18).

This chapter first discusses the multidisciplinary approach to patient selection for DBS surgery and the general surgical procedure for device implantation. Next, the two most common surgical targets for DBS in PD, the subthalamic nucleus (STN) and the internal segment of the globus pallidus (GPi), are discussed. Finally, studies that compared these two sites and other potential DBS targets such as the thalamus and pedunculopontine nucleus are covered.

PATIENT SELECTION

The Core Assessment Program for Neurosurgical Interventions and Transplantation in Parkinson's Disease (CAPSIT-PD) recommends that patients considered for surgical intervention should have a disease duration for at least 5 years. This time frame allows for atypical forms of parkinsonism to fully manifest, and during this time, most patients receive levodopa therapy. Patients who are candidates for surgery should show a positive response to dopamine replacement medications. Response to levodopa is generally considered to be a greater than 30% improvement in the Unified Parkinson's disease rating scale motor score (UPDRS part III). Clinical motor improvement following DBS surgery for PD closely parallels the improvement seen after levodopa challenge. Finally, CAPSIT-PD recommendations suggest that patients with preexisting dementia and severe depression should be excluded.

There is currently no evidence to indicate DBS for any of the other disorders that may mimic the classical symptoms of PD, including multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal degeneration, and Lewy body dementia (Table 1). As such, it is paramount that the diagnosis of PD is confirmed by a specialist with experience in the field of movement disorders (19). Patients with a confirmed diagnosis of PD should have their symptom responsiveness to levodopa determined by a standardized levodopa challenge test. The results of this test reflect the expected potential benefit achievable with surgery (20). Another important criterion for patient selection is the presence of disabling motor fluctuations and dyskinesias despite trials of all relevant medications tested at therapeutic dosages by a specialist with experience in the field. All patients who are deemed good candidates for DBS surgery should undergo magnetic resonance imaging of the brain prior to surgery in order to rule out any secondary diagnosis or structural concerns within the brain.

A preoperative assessment by a neuropsychiatrist and neuropsychologist is also necessary for further risk stratification and identification of patients who may require closer follow-up in the postoperative period. Many patients with PD may have mild cognitive impairment or mood/behavioral impairments that should be identified and managed. In general, stable and milder forms of these abnormalities do not necessarily mean contraindication for surgery; on the other hand, more

TABLE 1 Differential diagnosis of Parkinson's disease

Neurodegenerative disorders
Idiopathic Parkinson's disease
Familial Parkinson's disease
Multiple system atrophy (MSA)
Progressive supranuclear palsy (PSA)
Lewy body dementia
Corticobasal degeneration
Alzheimer's disease
Drug or toxin related
Haloperidol
Metoclopramide
Reserpine
MPTP
Manganese
Vascular
Infarctions of the basal ganglia or midbrain
Infectious or inflammatory
Post-encephalitis
Multiple sclerosis

DBS has been shown to be effective in reducing motor symptoms among well-selected patients with idiopathic Parkinson's disease. It is important that Parkinson's disease is clearly distinguished from other disease entities that can mimic the symptoms of Parkinson's disease, which do not respond to DBS.

severe or progressive changes may represent risk factors for further deterioration triggered by the surgical process and/or stimulation.

Although there are no specific age limits for DBS surgery, initial clinical trials included patients who were 50–65 years old (21), while fewer studies included patients older than 75 years (22). This may reflect a general concern among referring neurologists and neurosurgeons regarding serious surgical complications, physiological reserve to recover, and rapid deterioration of motor symptoms among older patients. However, trials that have included older patients demonstrate that these patients still benefit equally from surgery without apparent further risks (22). DeLong et al. analyzed the Thomson Reuters MarketScan national database including more than 1700 patients who underwent DBS for PD between 2000 and 2009 (23). This study analyzed reported 90-day complication rates and did not find any association with increasing age (23). Further studies are needed to project the long-term outcomes of DBS surgery among elderly patients with PD.

More recently, there is evidence to suggest that DBS can improve quality of life and motor function when motor complications become evident, but before they become disabling. In a multicenter, randomized study, Schuepback et al. demonstrated that patients with early, mild PD symptoms had significant improvements in quality-of-life assessments and UPDRS part III scores compared to best medical therapy (24). Patients included in this study were younger and had shorter disease duration than those enrolled in previous, large clinical trials (discussed below) (24). This study challenges the concept that disease severity should be

disabling before surgical intervention is delivered; however, it underlines the need for proper diagnosis before enrolling patients in surgery.

As with all elective brain surgeries, patient-specific medical conditions must be considered before proceeding to surgery. Patients who are at increased risk of perioperative complications should be medically optimized prior to any planned surgical intervention. Patients with a history of angina or coronary vascular disease should first be evaluated by a cardiologist. Patients who are taking antiplatelet agents for cardiovascular disease or anticoagulants for atrial fibrillation, pulmonary emboli, or deep venous thrombosis must temporarily stop taking these medications prior to surgery, or should be appropriately bridged with a reversible agent. Co-morbid conditions such as hypertension and diabetes should be adequately controlled to avoid intra-procedural and postoperative complications. Patient's age and overall physical condition should also be considered when risk stratifying a patient for consideration of surgery. Although there is no age limit for consideration for DBS surgery, younger patients may have better results and may tolerate the procedure better. Elderly patients or patients with significant dementia may not be good candidates for DBS surgery.

One of the most important factors in obtaining satisfactory outcomes for patients with PD after DBS is managing expectations (25). It is important to identify the patient's most disabling symptoms and to assess the patient's postoperative expectations. It is crucial to explain what the most reasonable outcomes are after surgery. This includes a candid explanation of what symptoms are likely to improve, the magnitude of improvement, and which symptoms may not improve or even worsen after surgery. If there is discord between expectations and surgical results, patients will likely be disappointed with their condition after surgery. Care should be taken when extrapolating published results during conversations with patients because of selection bias. In general, it should be emphasized that DBS serves as an "add-on" therapy, supplementing but not replacing the current therapy of the patient, aiming at improving the motor but not the non-motor symptoms of PD. It comes with risks of complications, during the implantation and during long-term care. Of note, DBS should not be presented to the patient as a cure, and despite optimal programming, the underlying PD will progress.

A critical element in determining successful surgical outcomes for patients with PD is having the support of a multidisciplinary team that specializes in the care of patients with DBS devices. This team typically includes a neurologist who is a specialist in movement disorders, a neuropsychologist who has excellent knowledge of PD and its non-motor features, and a neurosurgeon who has a specialty training in stereotactic and functional neurosurgery. Since DBS for PD was approved by the FDA, there has been a growing trend for DBS surgery to be performed at smaller hospitals with lower volumes of movement disorder surgery. Large, database studies examining the National Inpatient Sample (NIS) have demonstrated that patient outcomes after DBS surgery for PD are better when these operations are performed at hospitals with moderate or high volumes for these procedures (26). While these studies provide evidence for improved early postoperative outcomes at high-volume centers, such as favorable patient disposition and low early complication rates, it does not account for long-term patient outcomes. Device programming, managing patient medications, and general postoperative care are arguably better when provided by expert teams.

SURGICAL PROCEDURE

The surgical procedure for implanting DBS devices can be performed with several approaches in terms of stereotactic systems, intraoperative confirmation of lead position, use of anesthesia, and staging procedures. The key components of the fully implanted system are (i) a precisely implanted intracranial electrode in the target area, (ii) implantation of lead extension wires that connect the intracranial leads to a power generating and programming source, and (iii) implantation of an internal pulse generator (Figure 1).

Figure 1 Current deep brain stimulation devices from Medtronic, Boston Scientific, and St. Jude's Medical. Images demonstrate internal pulse generator (indicated with *) and intracranial electrode with multi-contact configurations (indicated by black arrow). Images provided by Boston Scientific, Medtronic, and St. Jude's Medical and are used with permission.

Surgery is most commonly performed while the patient is awake; however, several centers offer surgery under general anesthesia (27). There is considerable variation in the procedural details across different centers. At the Toronto Western Hospital, the surgical procedure begins by rigidly fixing a Leksell stereotactic to the patient's head under local anesthesia. The patient then undergoes stereotactic imaging with the frame in place. Several software packages are available to plan electrode targets and trajectories based on coordinate frame-based, frameless, or robotic stereotaxy.

Once the surgical plan has been made, the patient is brought to the operating room for surgery. The patient is placed in a semi-recumbent position, the hair is clipped and the scalp is prepared with betadine solution. We plan a coronally oriented incision spanning Kocher's point bilaterally, although several other incision strategies have been described. The scalp is generously blocked with local anesthetic, opened to expose the frontal bone of the skull, and the skull is trephined approximately 1 cm anterior to the coronal suture and at least 2 cm lateral from the midline. The dura is coagulated and opened. Care is taken to minimize cerebrospinal fluid loss during dural opening and throughout the procedure as this can cause brain shift.

Once dural opening is complete, a guide cannula is inserted into the brain 1–1.5 cm above the desired target. Microelectrode recording can then be used to identify the electrophysiological signature of the target structure and map the dorsal–ventral borders. Once a suitable tract is identified, the microelectrodes are removed and a permanent macroelectrode is inserted to the target structure. Test clinical stimulations are then performed at each of the contacts to assess for negative side effects and for clinical efficacy. Once the DBS electrode is properly situated in its final position, as verified by intra-operative fluoroscopy, it is secured to the skull and the incision is closed.

During a second procedure while the patient is under general anesthesia, the distal ends of the intracranial electrodes are connected to extension wires that are tunneled subcutaneously behind the ear down to the chest. A second incision is made 2–3 cm below the clavicle, and a subfascial pocket is made to house the internal pulse generator (IPG). The extension wires are connected to the IPG and the impedances for the system are assessed. Once completed, patients will typically spend one to two nights in the hospital for observation. They are brought back to the clinic 6–8 weeks later to turn on the device and begin programming.

SUBTHALAMIC NUCLEUS

The STN is a small, glutamatergic nucleus involved in the subcortical motor circuitry, measuring approximately 7 mm × 5 mm × 3 mm in dimension. As its name implies, it resides ventrally below the thalamus and zona incerta at the level of the red nucleus in the midbrain. It is bordered anteriorly by the cerebral peduncle and posteriorly by the medial lemniscus. The STN receives excitatory fibers from the cerebral cortex via the hyperdirect pathway and inhibitory inputs from the external segment of the globus pallidus (GPe) as a part of the indirect pathway. The STN sends its excitatory projections to the basal ganglia output nuclei (GPi and substantia nigra pars reticulate [SNr]).

Experimental evidence in rodent and primate models of PD suggests that the STN is critically involved in organizing basal ganglia output and has pathologically increased activity in PD (28–32). These findings were among the rationale for chronic electrical stimulation of this target which was first reported by Benabid et al. in 1994 (33). Since then, several centers have published large case series, and two large, multicenter, randomized controlled trials comparing STN DBS with best medical management have been published (Table 2) (21, 22).

In 2006, Deuschl et al. reported a randomized trial of neurostimulation versus best medical management among 156 patients with PD. Patients who received DBS treatment had significant improvements in UPDRS part III scores and significantly higher quality of life on PDQ-39 assessments compared to patients who received best medical therapy alone (21). Weaver et al. similarly conducted a large, multicenter, randomized trial comparing STN DBS with best medical therapy among patients with advanced PD. This study enrolled 255 patients and included patients with advanced age. Overall, among patients who received STN or GPi DBS, 71% had a >5 point improvement on the UPDRS part III scores (compared to 32% in best medical treatment group). Furthermore, the DBS group also experienced significant improvements in quality of life (22). The results from these two studies provide solid evidence favoring the use of STN DBS among patients with medication-refractory PD with disabling motor symptoms, dyskinesias, and motor fluctuations.

Apart from the large, randomized clinical trials, several additional studies have concluded that motor function was significantly improved after STN DBS, by 25–60% in the "off" medication state compared to baseline motor scores (34–44). After STN DBS, patients were able to reduce dosages of dopamine replacement medications by approximately 50%. Non-motor symptoms of PD did not improve among patients treated with STN DBS, and there were reports of worsening verbal fluency, mood disorders, suicide, hypophonia, and worsening postural and

TABLE 2 Studies with outcome data for STN DBS

Reference	Study design	Patients, *N*	Study duration	UPDRS part III improvement
20	Randomized control trial	156	6 months	41%
21	Randomized control trial	255	6 months	29%
33	Case series, blinded evaluation	18	10 years	25%
34	Case series, blinded evaluation	30	1 year	30%
35	Case series	23	5 years	55%
36	Case series	20	2 years	57%
38	Case series	7	1 year	41%
39	Case series	24	1 year	60%
42	Case series	19	2.3 years	28%
43	Case series	15	1 year	74%

STN, subthalamic nucleus; DBS, deep brain stimulation; UPDRS, unified Parkinson's disease rating scale.

gait disturbances. Since these initial reports demonstrating significant benefits after STN DBS surgery, several studies have assessed the long-term efficacy at 5-year and 10-year time points (34, 36, 38, 45). These studies indicate that STN DBS has continued positive benefits for patients by improved motor scores and in quality-of-life assessments.

GLOBUS PALLIDUS INTERNUS

The internal segment of the GPi is a wedge-shaped nucleus that is one of the main inhibitory output centers of the motor circuit in the basal ganglia. It is bordered laterally by the external segment of GPe and medially by the posterior limb of the internal capsule, and resides superiorly above the optic tract. The GPi receives input from the striatum, GPe, and STN and projects inhibitory fibers to the motor thalamus (Vop, Voa, and PCfc) via the ansa lenticularis and lenticular fasciculus.

Pallidotomy has long been known to improve the motor symptoms of PD (11, 12), and early studies comparing unilateral pallidotomy with unilateral pallidal DBS demonstrated equipoise in terms of improvement of motor symptoms (46). In 1994, Siegfried et al. first reported GPi DBS for the treatment of PD (47). Several case series have demonstrated significant improvements in motor function compared to preoperative motor evaluations (48–50). UPDRS part III scores are reported to improve by 30–50%, with approximately 50% improvement in rigidity and 80% improvement in tremor. Significant improvements are also noted in dyskinesia with GPi DBS. However, unlike STN DBS, there were no decreases in dopamine replacement medications, but there were fewer overall concerns for cognitive and mood changes with GPi DBS.

COMPARISON OF STN AND GPi DBS

DBS targeting both STN and GPi has been reported to be effective in treating motor symptoms in the "off" medication state in PD patients. However, based on the initial clinical studies that described improvement in motor scores and complication rates, it was unclear whether one site provided superior outcomes compared to the other. Several studies have now been published that compared treatment outcomes with STN versus GPi DBS, including several controlled trials and a large, multi-centered, randomized trial (Table 3) (51–56).

In 2010, Follett et al. reported on 299 patients who were randomized to receive STN DBS (147 patients) or GPi DBS (152 patients) and were followed clinically for 24 months (53). Blinded assessments were performed at 6 and 24 months. In this study, there was no difference in the degree of motor improvement in the "off" medication state comparing STN with GPi stimulation at the 6- or 24-month study time points. Overall, there were no differences in complication rates between the two sites, with 56% of patients who received STN DBS reporting a serious adverse event compared to 51% of patients who received GPi DBS. Among patients who received STN DBS, there was a significant reduction in dopamine replacement medication required at 24 months compared to

TABLE 3 Studies comparing STN and GPi DBS

Reference	Study design	Patients, *N*	Study duration	Conclusions
50	Randomized, blinded	10	12 months	Similar response
51	Randomized, blinded	134	3 months	Both sites had significant improvements
52	Randomized, blinded	316	24 months	Both sites had similar levels of improvement
53	Randomized	128	12 months	No difference in ADL, and STN DBS had more significant improvement in UPDRS III
54	Retrospective, case control	133	12 months	Overall outcome similar for dyskinesia control

STN, subthalamic nucleus; GPi, globus pallidus; DBS, deep brain stimulation; ADL, activities of daily living; UPDRS, unified Parkinson's disease rating scale.

GPi DBS, which is consistent across several studies. In addition, patients who received STN DBS demonstrated a significant decrease in visual motor processing speed compared to changes seen among patients who received GPi DBS. Patients with STN DBS also had worsened depression scores compared to patients with GPi DBS (53).

Odekerken et al. (53) also performed a multicenter, randomized trial with video-blinded assessments and compared STN with GPi DBS. The primary outcomes in this study were (i) measure of functional health and (ii) composite evaluation of cognitive, mood, and behavioral effects. Secondary outcomes were symptom scales and patient questionnaires. One hundred twenty-eight patients were enrolled of whom 65 received GPi DBS and 63 received STN DBS. There were no differences in primary outcomes comparing GPi and STN DBS; however, there were larger improvements in the 'off' condition UPDRS score among patients with STN DBS. Based on these findings, the authors suggested that STN could be the preferred target for patients with advanced PD.

Most studies directly comparing STN and GPi DBS suggest that motor evaluations and improvements in quality-of-life assessments are equitable (51–56). Among patients with medication-induced dyskinesias, where one of the goals of treatment is to reduce levadopa equivalent dosages, STN DBS may be the preferred treatment. On the other hand, if reducing medication dosages is not the primary goal of treatment, GPi DBS may be used as an anti-dyskinetic therapy, without reducing medication doses.

Patients who are candidates for DBS surgery should be evaluated by a neuropsychologist for depression, cognitive impairments, and dementia. For patients with a history of depression, suicidal ideation, or suicide attempts, it is imperative to determine whether these patients are clinically stable with regard to their psychiatric health and have proper social support in place prior to enrolling in surgery.

Among patients with a significant history of depression or mild dementia and among patients with advanced age who are otherwise good surgical candidates for DBS, some surgeons advocate for GPi DBS surgery compared to STN DBS to minimize the risks of worsening depression or cognitive changes postoperatively (57). Several early studies cited worsening depression (with suicide attempts) and impaired cognitive function after STN DBS, which prompted investigators to question whether GPi DBS may be a more suitable treatment among this patient population. However, several recent meta-analyses comparing STN and GPi DBS failed to demonstrate a significant difference between these two targets. Despite these studies, many teams continue to implant GPi DBS when there is a question of cognitive impairment, advanced patient age, or history of depression.

VENTRALIS INTERMEDIATE NUCLEUS AND PEDUNCULOPONTINE NUCLEUS

Tremor is a prominent motor feature of PD, and for patients with tremor dominant forms of PD, it may be the most disabling symptom. Thalamic lesioning procedures have been reported for more than 50 years among PD patients for relief of disabling tremor and are still used today (9, 13, 58). Thalamic surgery for movement disorders is most commonly used among patients who have essential tremor (ET) (59, 60). The target is the ventral intermediate (Vim) thalamic nucleus. The Vim nucleus is roughly 4 mm in the anterior–posterior dimension and approximately 10–12 mm in the rostral–caudal dimension. It resides anterior to the ventrolateral caudalis nucleus, which receives somatosensory fibers from the medial lemniscus, and posterior to the ventralis oralis posterior (Vop) nucleus, which receives input from the basal ganglia. The Vim nucleus receives unconscious proprioceptive fibers from the dentatorubrothalamic tract.

Among the early studies using chronic electrical stimulation for movement disorders, the Vim nucleus was targeted as a treatment for ET and PD. However, among patients with PD, Vim stimulation was only able to alleviate tremor symptoms and was not effective for the management of the other cardinal motor symptoms such as akinesia and rigidity, which are often the more disabling symptoms of PD, especially the more common akinetic-rigid forms of PD. Accordingly, Vim DBS is most commonly used for patients with ET (59, 60), while STN DBS, which is also highly effective in treating tremor symptoms related to PD, is the treatment of choice among patients with PD. Vim DBS in the context of PD is reserved for those patients with tremor-dominant PD, in whom the tremor has the largest impact on their quality of life and the other motor symptoms of PD are either mild or do not significantly impact the quality of life. It is important that patients understand the goal of the surgery is tremor control and that as PD progresses they may need additional electrodes placed at STN or GPi in the future to improve quality of life with respect to the other motor manifestations of PD.

The pedunculopontine nucleus (PPN) is a cholinergic/glutamatergic cell mass that resides caudal to the substantia nigra. It receives inhibitory and excitatory fibers from the main basal ganglia output centers (GPi and STN) and projects to

the striatum, thalamus, spinal cord, and brainstem (61). The PPN is thought to be an important relay center involved in coordinated locomotion.

Shuffling gait and freezing are prominent, disabling features of PD; and current DBS targets do not have a significant positive impact on these symptoms when resistant to dopaminergic medications. In an effort to address this clinical need, several groups have tested PPN DBS as a treatment for gait instability related to PD (62–66). Among the centers that have reported outcomes, there has been considerable variability in patient selection, procedures performed (unilateral vs bilateral), and PPN targeting methods (67). Thus far, in studies that have conducted blinded assessments, there have not been any objective improvements; however, when PPN is stimulated with lower frequencies and in combination with STN DBS, there may be improvements in gait freezing and the number of falls (64). Further work is needed to define the role of PPN DBS in gait disturbances in PD, and at this time, it remains an investigational but promising target.

CONCLUSION

DBS is an effective treatment for controlling many of the motor symptoms associated with PD among well-selected patients. Optimal patient outcomes can be best achieved by multidisciplinary teams (which include neurologists, neuropsychologists, and neurosurgeons), that are highly experienced in postoperative management of patients with PD. The most common targets for DBS in PD are the STN and the GPi. Both of these targets are equally effective in controlling motor symptoms in the "off" medicated state in PD. However, STN DBS is effective in reducing levodopa requirements postoperatively, while GPi DBS may have fewer negative effects on mood and cognition, especially among older patients. Vim DBS is reserved for patients whose primary disabling symptoms are tremor, and PPN DBS is an experimental treatment for patients with gait and postural instability.

Current DBS therapy is delivered as a continuous train of electrical pulses; however, many people with Parkinson's disease have fluctuations in their symptoms throughout the day. As more information about pathological neuronal firing is elucidated in Parkinson's disease, there is an effort to create DBS systems that can detect pathological brain activity and deliver electricity when these features are present. This strategy, termed closed-looped or responsive DBS, has the potential to improve symptoms of Parkinson's disease while minimizing potential complications of continuous stimulation.

Advances in DBS device engineering have improved the configuration of the intracranial electrodes and are progressing towards smaller devices with longer battery life. Currently, several device manufacturers employ "directional leads" whereby current settings can be preferentially selected to create a diverse and selective electrical field and zone of effectiveness. This may allow further programming options to minimize side effects while maximizing benefits of stimulation. Smaller internal pulse generators with longer battery lives are on the horizon, which may obviate the need for extension wires and chest implants that need to be replaced every 3–5 years.

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Stem Cell Treatments for Parkinson's Disease

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Abstract: Parkinson's disease (PD) manifests with a typical movement disorder, due to the loss of dopaminergic neurons of the substantia nigra. There are no disease-modifying treatments, and current management is centered on symptom control using predominantly dopaminergic drugs. While effective at improving the motor symptoms of PD, these treatments result in significant adverse effects, due to non-targeted and non-physiological delivery of dopamine to the brain. For many years, there has been interest in cell grafting as a potential means of restoring dopamine to the striatum in a physiological manner, which would theoretically treat the symptoms of PD that are due to dopamine deficiency, without the motor and neuropsychiatric adverse effects that are seen with dopaminergic medications. A number of cell sources have been trialed in PD patients, but lack of efficacy, ethical and logistical barriers have meant that most of these do not offer useful treatment options. Stem cell-based treatments are emerging as the most

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promising approach for the development of a useful regenerative treatment that could be used in a large number of patients. Although progress in this field has been slow, a number of exciting clinical trials are now on the horizon, and there is genuine hope that stem cells will enter the clinic in the short- to medium-term future.

Keywords: Embryonic stem cell; Induced pluripotent stem cell; Neural grafting; Parkinson's disease; Regenerative therapies

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease, which manifests with a characteristic motor syndrome consisting of bradykinesia, rigidity, rest tremor, and as the disease becomes more advanced, postural instability and falls (1). These motor symptoms constitute the syndrome of parkinsonism, which occurs in PD due to the relatively selective loss of dopaminergic neurons of the substantia nigra pars compacta, which results in a reduction in dopamine levels in the striatum (2, 3). There are a number of additional non-motor manifestations that occur due to neurodegeneration in other regions, including the cerebral cortex (3). These include sleep disturbance, anosmia, neuropsychiatric features, and cognitive decline (4). Because the motor symptoms of PD occur due to the loss of a specific population of neurons, there has been long-standing interest in whether the function of these neurons could be replaced by regenerative treatments that would restore the dopamine levels in the brain of PD patients (5). This chapter discusses some of the stem cell approaches that are being investigated as a potential means of doing this and where they may fit in the future landscape of PD management.

RATIONALE FOR CELL-BASED THERAPIES FOR PARKINSON'S DISEASE

There are currently no disease-modifying treatments for PD. The mainstay of current treatment involves the use of dopaminergic drugs to restore the striatal dopamine concentration, with consequent improvement in motor function. These drugs include levodopa (the precursor of dopamine), administered with a peripheral dopa-decarboxylase inhibitor to reduce peripheral side effects, dopamine agonists, and inhibitors of the enzymes that metabolize dopamine (e.g., monoamine oxidase B and catechol-O-methyltransferase). Although these drugs do not alter the course of disease and do not treat the non-motor aspects of PD, they convey a significant benefit in improving the motor symptoms of the majority of patients, particularly in the early stages of the disease.

However, while these drugs offer significant symptomatic benefits, they also result in problematic side effects (6–8). The majority of patients are treated with

levodopa-based regimes. Administration of levodopa, however, results in dopamine delivery to areas of the brain other than the dopamine-deplete striatum. Consequently, patients experience side effects including hallucinations and cognitive impairment, due to off-target effects of the drug (6). Furthermore, plasma levels of levodopa can be erratic due to variable absorption of the drug, and transit across the blood–brain barrier can be inconsistent (9). This means that patients may develop significant motor fluctuations, with dramatic wearing-off of the drug, which can be particularly problematic (9). Finally, additional involuntary movements may develop in the form of dyskinesias, which are thought to occur due to the continuous nature of dopamine delivery with administration of dopaminergic drugs, which contrasts with the normal pulsatile release of dopamine from nigrostriatal neurons (7, 8). Some of these effects can be circumvented by the use of levodopa-intestinal gel, although this is currently very expensive, meaning that it is only used in a minority of patients (10). Deep brain stimulation (DBS), which is discussed in Chapter 8, may also be used in patients with problematic levodopa-induced side effects, although this may also result in significant neuropsychiatric adverse effects and speech problems (11, 12).

There is therefore a need for a means of the targeted delivery of dopamine to the striatum, with the dopamine being released in a physiological manner. This would theoretically offer improvements in the motor symptoms of PD patients, while minimizing the off-target side effects, motor fluctuations, and dyskinesias that are seen with levodopa therapy. This could be achieved by the introduction of a graft containing dopamine-producing cells into the patient's striatum. A number of cell sources have been investigated as potential means of doing this (see Table 1). While most of these trials have been unsuccessful for a number of reasons (discussed below), they have offered crucial insights into what is necessary for a cell-based therapy to be effective, and have allowed for significant progress toward a useful cell-based treatment for PD.

TABLE 1 Sources of experimental cell-based treatments for Parkinson's disease

Cell types trialed in humans	Cell types with forthcoming trials in humans
Autografts	Embryonic stem cell-derived neural progenitors (53)
Adrenal medullary cells (31–38)	
Carotid body cells (42, 43)	Induced pluripotent stem cell-derived neural progenitors (53)
Mesenchymal stem cells (61)	
Allografts	
Human fetal ventral mesencephalon (19–25, 27, 28)	
Retinal pigment epithelial cells (40, 41)	
Xenografts	
Porcine ventral mesencephalon (44)	

HISTORY OF CELL-BASED THERAPIES FOR PARKINSON'S DISEASE

In the 1980s and 1990s, there was much interest in the investigation of human fetal ventral mesencephalon (FVM) as a potential source of dopaminergic cells that could be grafted into PD patients. In preclinical studies, it had been seen that the dopaminergic cells in FVM grafts could survive and make synaptic connections in the brains of rodents and that they could result in motor and behavioral improvement (13–18).

Following the promising results of the preclinical trials, human FVM grafting began in patients, with a number of small open-label trials being performed in Sweden. No improvement was seen in the first patients to receive such grafts (19), but by using a greater volume of tissue, and tissue from an earlier gestational age, the majority of patients derived clinical benefits, with some able to discontinue their medications (20–25). At postmortem, it has been seen that these grafts were able to survive for decades, albeit with the development of Lewy body pathology in 11% of the grafted neurons (26).

The promising results in the Swedish trials led to the initiation of two sham surgery–controlled double-blinded trials in the USA (27, 28). In these studies, 56 patients ultimately received human FVM grafts. However, doubt was cast over this approach when these trials reported a high incidence of graft-induced dyskinesias, with little clinical benefit. The development of these dyskinesias has been attributed to the presence of serotoninergic neurons within the transplant, which highlighted the need for a pure population of cells for any graft product (29, 30). Additionally, in both of these trials, it was felt that a suboptimal amount of fetal tissue was used, along with inadequate immunosuppression (in one of the trials no immunosuppression was used). While these trials produced disappointing results due to these problems with trial design, they did offer important insight into what was necessary for a cell-based therapy to be effective.

After analysis of all of the available data from the FVM trials that had been carried out, a further open-label trial (TRANSEURO) was commenced in Europe (Clinical trials identifier NCT01898390), which is currently in its follow-up phase. In this trial, patients have received at least three fetal grafts per side, with 12 months of immunosuppression. Although the results of this trial are yet to be published, a positive result will provide convincing proof-of-principle that cell-based therapies can be effective in PD.

Around the same time as the initial preclinical studies of FVM grafts, there was parallel interest in the use of adrenal medullary cells (catecholamine-producing cells that release small amounts of dopamine) as an alternative source of a cell-based therapy for PD. Although these grafts actually survived poorly in the animal models, these adrenal medullary grafts progressed to open-label clinical trials prior to FVM grafts (31–38). Although initially reported to produce promising results, it became clear that any benefit conveyed by these grafts was short-lasting. Significant neuropsychiatric side effects occurred in a number of patients, and at postmortem, it was found that these grafts did not survive, and this approach was therefore abandoned (39).

Recognizing the failings of the adrenal grafts and the ethical and logistical challenges associated with FVM grafting, a number of other cell sources were considered as potential candidates to serve as the basis for a regenerative therapy for PD. Retinal pigment epithelial cells are able to produce levodopa, as well as potentially beneficial neurotrophic factors. These were initially delivered into the putamina of six PD patients using gelatin microcarrier constructs (40). Initial results were promising with a sustained motor improvement reported, which led to progression to a randomized phase II trial. However, in this trial, it was found that these grafts conveyed no benefit when compared to sham surgery controls and that graft survival was poor (41). Autologous grafts using carotid body cells (42, 43) and xenografts using porcine mesencephalon tissue (44) have also been performed in PD patients, with disappointing results.

Importantly, although it had become clear that FVM grafting was not scalable to be used as a mainline treatment for PD predominantly due to logistical barriers and inadequate supply of fetal tissue, the promising results seen in the Swedish trials provided proof-of-principal that neural grafting could serve as the basis for a useful symptomatic treatment in PD. It became clear that it was necessary to develop a renewable source of dopaminergic neurons (or their progenitors) that would be suitable for grafting into a large number of patients. The subsequent discovery of stem cells provided hope that these could fulfil this requirement, and stem cell-based approaches have now become the focus of the field of regenerative medicines for PD.

STEM CELL-BASED APPROACHES

Stem cells have the potential for self-renewal through unlimited replication, as well as the capacity to differentiate into any cell type within body. The ability to direct the fate of these cells to become a dopaminergic neuron potentially therefore offers an unlimited number of cells that could be used for neural grafting. Although a number of stem cell types have been considered as potential treatment options for PD, the most promising are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSC) (Figure 1).

Embryonic stem cells

Human ESCs were first isolated by Thomson in 1998 (45). These are harvested from the inner cell mass of the early blastocyst, and a number of human ESC cell lines have been generated from excess embryos from *in vitro* fertilization procedures. Over the following decade, differentiation protocols were developed attempting to direct these cells to become dopaminergic neurons (46). While it was possible to induce expression of the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH), the yield of TH-positive cells was highly variable (47–49). Nevertheless, these cells were shown to survive transplantation into rodents and to produce a degree of motor recovery (50).

As well as the variable efficacy of the differentiation techniques, it was also clear that although these cells could secrete dopamine, they were not authentic nigral neurons, failing to express *LMX1A* and *FOXA2*, which were considered

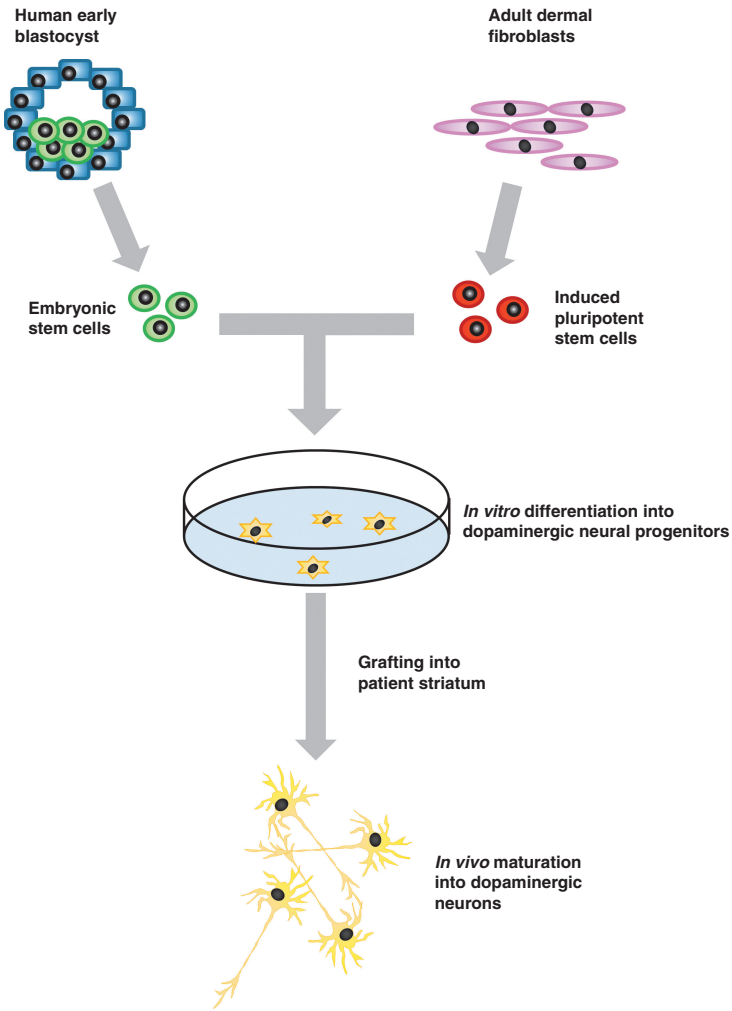

Figure 1 Approaches to stem cell-derived neural grafting in Parkinson's disease.

important markers for nigral dopaminergic neuron phenotype (5). However, the recognition that nigral dopaminergic neurons were of floor plate, rather than neuroepithelial origin, led to the development of new differentiation protocols and the generation of cells that closely resemble authentic nigral dopaminergic neurons (51).

However, as experience of ESC-derived neural cell grafting in animal models has grown, it has become clear that variable yield has remained a problem. An important retrospective study in which over 500 grafts were analyzed sought to identify the factors that determined a successful graft outcome (52). It was found that the grafts containing a high content of TH-positive cells were seen in those grafts that were enriched for neural progenitor cells expressing markers found in

the caudal midbrain (e.g., *CNPY1* and *EN1*) (52). The markers that had previously been used to indicate nigral phenotype including *LMX1A* and *FOXA2* were also present in rostral midbrain progenitor cells destined for subthalamic nucleus neuron fate. This discovery accounts for the heterogeneity that has been seen with graft outcomes historically and importantly means that it is now possible to generate dopaminergic neuron progenitors at high purity and with high efficiency.

Progress toward an ESC-derived therapy has clearly been iterative, with novel discoveries in neurodevelopmental biology changing the direction of the field several times, as discussed. However, having reached the stage of being able to develop dopaminergic neuron progenitors effectively, the attention is now turning to whether these cells can be clinically useful, and a number of clinical trials of this approach are due to begin (53).

Induced pluripotent stem cells

In 2007, the process for generating iPSCs was first reported, offering a new avenue for the development of a stem cell-based treatment for PD (and a number of other conditions) (54). iPSCs are generated by the reprogramming of an adult somatic cell (such as a dermal fibroblast) into a stem cell, through the expression of a number of transcription factors that could induce pluripotency (e.g., c-myc, klf-4, sox2, and oct4) (55). The iPSCs derived in this way can be differentiated into dopaminergic neurons using protocols similar to those used with ESCs, which could serve as the basis of a useful cell-based treatment for PD (56). The potential advantage of iPSC-derived over ESC-derived grafts is that it would be possible to generate autologous grafts, by using a patient's own fibroblasts to produce a neural grafting product, negating the requirement for immunosuppression that will be necessary with ESC-derived grafts. However, there are other biological and logistical challenges faced with the iPSC approach, which are discussed below.

iPSC-derived neural grafts have been trialed in primates with MPTP-induced nigral toxicity, with promising results (57). The neural progenitors grafted ultimately extended neurites into the striatum, did not form any tumors, and resulted in improved motor function at two years. As with the ESC-approach, clinical trials in humans are on the horizon and will begin in the next couple of years (53).

Other cell types

Mesenchymal stem cells can be derived from the bone marrow and have also been considered with regard to development of regenerative treatments in PD. However, although it is possible to generate TH-positive cells, it has not currently been possible to consistently generate authentic midbrain dopaminergic neurons from mesenchymal stem cells; hence, it is unlikely that these will be useful as a cell-replacement therapy for PD (58). These cells have been postulated to have other potential beneficial effects, including anti-inflammatory properties and neuroprotective potential through paracrine activity (59, 60). A small cohort of PD patients received mesenchymal stem cell grafts in an open-label study, which demonstrated short-term safety of this approach (61). However, no clinical benefit has been demonstrated, and the future role of these cells remains uncertain.

An alternative potential means of generating dopaminergic neurons for transplantation is through direct conversion of somatic cells to induced neurons (iNs), through forced expression of proneural transcription factors (62). As with iPSCs, these cells can be derived from patient fibroblasts; therefore, it would be possible to generate autologous neural grafts. Unlike iPSC-derived neurons, there is no stem cell stage to the generation of iNs; hence, they potentially offer an advantage over stem cell sources of dopaminergic neurons, as there is theoretically a reduced risk of tumorigenesis. However, iN reprogramming is less well established than the stem cell approaches, and the results are highly variable (62–66). Furthermore, the number of iNs generated is limited to the number of somatic cells available at the start of a conversion, which is in contrast to the unlimited number of neurons that can be derived from replicating stem cells. Given these limitations, iNs are unlikely to be useful as a cell-based treatment for PD, at least in the short- to medium-term future. There is some interest in the development of *in vivo* direct reprogramming techniques, in which astrocytes within the host brain are reprogrammed directly into neurons, through introduction of proneural transcription factors with viral vectors (67). While a fascinating prospect, this technique is many years away from being considered as a potential treatment approach, and extensive optimization and investigation of its safety will be necessary. Should the ESC- or iPSC-derived products that are due to enter clinical trials in the short-term future be shown to be safe and effective, it is unlikely that iNs or other cell sources will emerge as favorable options.

DISADVANTAGES OF STEM CELL APPROACHES

While the stem cell approaches described potentially offer promising treatment approaches, a number of problems must be overcome in order for them to be used as a mainline treatment for PD.

Of course, any grafting therapy will require a neurosurgical procedure, and it must be demonstrated that this can be achieved safely, with minimal risk. Additionally, for allogenic grafts a period of immunosuppression will be required, with the associated risk of infection and malignancy. Having said this, there is postmortem evidence of FVM graft survival for over two decades, with only a transient period of immunosuppression, and taking into account the fact that the central nervous system is an immune-privileged site, it is unlikely that this will be a major problem (26).

The need for immunosuppression can potentially be circumvented by using autologous grafts derived from iPSCs. This would require generation of a specific neural grafting product for each individual patient. This raises potential regulatory issues, and it may be necessary for each graft product to undergo extensive testing to demonstrate safety, which would make this approach prohibitively expensive (68). The more likely scenario is that it will be necessary to show that the iPSC-reprogramming and differentiation protocols are consistent between all individuals before these products could be approved, which would also carry significant economic expense. This would not be a problem with ESC-derived grafts, as this approach would lead to the development of a single grafting product that would be used for all patients.

It has been estimated that generating iPSCs from 150 human leukocyte antigen (HLA)-typed individuals could allow for the development of haplobanks which would be able to provide HLA-matched cell products for over 90% of a population (69). This would mean that rather than an autologous grafting product being produced for each patient, that an iPSC line could be selected with which they were HLA compatible to generate a matched cell product. However, in order to achieve this, a degree of HLA mismatch would be necessary, and a period of immunosuppression would therefore probably be required. Additionally, this would still have significant economic costs (70).

Another potential concern about the use of autologous grafts is that they will carry any genetic risk factors that contributed to the development of PD in the patient. Development of PD pathology has been identified in human FVM grafts at postmortem, and it may be that this process is accelerated in the presence of specific genetic risk factors (26). This may mean that the benefits conveyed by autologous grafts are lost earlier than they would be in allogenic grafts.

Of course, there are ethical considerations that must be taken into account with relation to cell grafting techniques. Historically, ethical objections to the use of human fetal tissue resulted in major challenges to the development of FVM grafting techniques. Generation of ESC-based cell products requires the destruction of a human embryo, which in some societies is also ethically unacceptable. However, in most countries the use of ESCs, derived from human embryos that would otherwise have been discarded, is ethically preferable to the use of fetal tissue, and it is unlikely that this will be a major barrier to the introduction of these treatments (71).

The major safety concern regarding stem cell-based treatments for PD is the potential for tumor formation, which may occur due to graft overgrowth, residual pluripotent cells in the graft, or acquisition of tumorigenic mutations during cell culture. Although tumor formation was seen in some of the early preclinical studies (47, 48, 72), improvements in differentiation protocols have led to refined cell products of increased purity, which have not caused tumors in animals. Having said this, while tumors have not been seen post-grafting in animals, it must be acknowledged that the grafts are only present in the animals for 1 or 2 years. In contrast, neural grafts would be expected to be present for decades in patients; therefore, rigorous safety data will be necessary to ensure that tumor risk is negligible, even over long periods of time.

It is possible for cultured cells to acquire mutations in oncogenes and tumor-suppressor genes, such as P53, and the significance and potential impact of this is unclear (73). There is a degree of uncertainty with regard to the extent of genetic testing that will be required on any stem cell product, with forthcoming trials planning to adopt different approaches (53). Some trials will perform full genetic sequencing, others will sequence specific genes, and others will perform karyotype analysis only. The significance of any genetic abnormalities identified will need to be established so that the approach to genetic testing can be optimized. For example, identification of a mutation in the *BRCA1* gene—a well-established oncogene associated in particular with breast cancer—may be of no clinical significance in dopaminergic neurons grafted into the brain. Uncertainty about the interpretation of genetic screening has led to the cessation of a planned clinical trial of iPSC-derived retinal pigment epithelial cells in patients with macular degeneration, due to the finding of a mutation in a cancer-associated gene.

Although there was no evidence of tumor formation in preclinical testing of this cell line, the risk that it posed to a potential recipient was not known, and the trial was halted (74).

Finally, if stem cell therapies are to be introduced for the treatment of PD, determination of the optimal patient group will be necessary. It is increasingly clear that PD is a heterogeneous condition, with clinical subtypes emerging (75). Some patients experience motor symptoms only, while others are at high risk of dementia and non-motor manifestations. The stem cell treatments that have been discussed may offer significant symptomatic benefit to those with predominantly motor symptoms but will be of little benefit to patients in which the main problems are non-motor. Public misconception may mean that patients view these treatments as a "cure" for PD; hence, it will be important to convey the fact that these approaches will be symptomatic treatments suitable in appropriately selected patients.

STEM CELL TREATMENTS IN THE FUTURE OF PARKINSON'S DISEASE MANAGEMENT

Although there are a number of challenges brought about with stem cell-based treatments for PD, it seems probable that these treatments will progress to the clinic in the short- to medium-term future. While development of optimized products has been necessarily slow and iterative, the field is now asking questions about how these treatments can be scaled and delivered—this demonstrates the progress that has been made with these approaches.

As has been discussed, the purpose of stem cell treatments is predominantly to treat the motor symptoms of PD. They will not have any disease-modifying effect and will not treat the major non-motor symptoms which can be particularly disabling in some patients. While these techniques can form one arm of the future of PD treatment, they will likely be combined with other novel treatments targeting alpha-synuclein pathology (e.g., immunotherapies and re-purposed drugs) (76). It may be possible for stem cell-based regenerative therapies to be employed to restore the function of dopaminergic neurons that have already been lost, while novel disease-modifying drugs could be used to prevent ongoing neuronal death.

CONCLUSION

For the past four decades, there has been much interest in the development of cell-based regenerative therapies, designed to treat the dopaminergic deficits of PD in a physiological manner. However, while a number of cell sources had initially seemed promising, ethical, logistical, and scientific barriers have meant that progress has been slow and iterative. Taking into account historic experiences with other cell sources, stem cell-based treatments have emerged as the most promising means of offering an effective regenerative therapy that could feasibly be scaled for treatment of large numbers of patients. While progress toward clinical trials has taken over a decade, these are now on the horizon. Rather than

asking whether it is possible to generate a useful cell product, questions asked of this field are now increasingly aimed at answering how these treatments can be delivered to patients, highlighting the progress that has been made toward a cell-based regenerative therapy for PD.

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