Parkinson disease: from pathology to molecular disease mechanisms

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A R T I C L E  I N F O

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A B S T R A C T

Parkinson disease (PD) is a complex neurodegenerative disorder with both motor and nonmotor symptoms owing to a spreading process of neuronal loss in the brain. At present, only symptomatic treatment exists and nothing can be done to halt the degenerative process, as its cause remains unclear. Risk factors such as aging, genetic susceptibility, and environmental factors all play a role in the onset of the pathogenic process but how these interlink to cause neuronal loss is not known. There have been major advances in the understanding of mechanisms that contribute to nigral dopaminergic cell death, including mitochondrial dysfunction, oxidative stress, altered protein handling, and inflammation. However, it is not known if the same processes are responsible for neuronal loss in nondopaminergic brain regions. Many of the known mechanisms of cell death are mirrored in toxin-based models of PD, but neuronal loss is rapid and not progressive and limited to dopaminergic cells, and drugs that protect against toxin-induced cell death have not translated into neuroprotective therapies in humans. Gene mutations identified in rare familial forms of PD encode proteins whose functions overlap widely with the known molecular pathways in sporadic disease and these have again expanded our knowledge of the neurodegenerative process but again have so far failed to yield effective models of sporadic disease when translated into animals. We seem to be missing some key parts of the jigsaw, the trigger event starting many years earlier in the disease process, and what we are looking at now is merely part of a downstream process that is the end stage of neuronal death.

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Defining Parkinson disease

Parkinson disease (PD)\(^1\) is the second most common neurodegenerative disorder after Alzheimer disease, with prevalence in industrialized countries of approximately 0.3% of the population. This rises with age from 1% in those over 60 years of age to 4% of the population over 80, illustrating the effect of aging. The mean age of onset is approximately 60 years; however, 10% of cases are classified as young onset, occurring between 20 and 50 years of age, which may represent a distinct disease group. PD is more prevalent in men than in women, with reports of ratios of 1.1:1 to almost 3:1 being quoted [135], which may be attributable to the protective effects of estrogen in women [121]. The socioeconomic cost of PD is high and in the United Kingdom is estimated to be approximately £3.3 billion. In the United States, the cost per patient per year is around $10,000, with a total economic burden of $23 billion. The greatest proportion of cost comes in the later stages of the illness for inpatient care and nursing homes and far less for medication [27].

This review aims to provide a current overview of the clinical, neuropathological, and biochemical features of PD, the genetic components of the disease, and risk factors for its development in relation to the pathogenic mechanisms thought to be involved in neuronal death. Currently drug treatment provides only symptomatic relief and we explore how knowledge of the pathogenic processes and the use of experimental models of PD interlink to assist in the search for neuroprotective/neurorestorative treatments.

Motor and nonmotor symptoms

Impaired motor function is classically used to make a clinical diagnosis of PD. The main features are bradykinesia, rigidity, tremor, and postural instability with an asymmetric onset spreading to become bilateral with time. Other motor features include gait and posture changes that manifest as festination (rapid shuffling steps with a forward-flexed posture when walking), speech and swallowing difficulties, and a masklike facial expression and micrographia [58]. A good response to dopaminergic medication is confirmatory of the diagnosis. Although this has been the classical textbook description of PD, more recently it has become recognized as a more complex illness encompassing both motor and nonmotor symptoms (NMS), such as depression, sleep disturbance, sensory abnormalities, autonomic dysfunction, and cognitive decline [74]. NMS affect all patients with PD, the frequency of which increases with disease severity, with late-stage patients exhibiting 6–10 NMS. NMS create the biggest demand on clinical resources, they are poorly diagnosed and treated and they are the major determinant of disease outcome, increasing disability, poor quality of life, and entry into long-term care [13]. Whereas the causes of motor dysfunction in PD are reasonably well understood (see later), the cause of NMS in PD remains poorly researched and they may largely relate to pathology outside of the basal ganglia.

From the perspective of this review, the most important feature of NMS is that some, for example, olfactory deficits, constipation, rapid-eye-movement sleep behavior disorder, and depression, may precede the onset of motor symptoms by many years (although they can occur at the same time as motor symptoms or follow the onset of motor abnormalities) [105]. NMS may in the future be used for the early diagnosis of PD, enabling neuroprotective strategies to be introduced at an early stage, and studies of large populations of apparently normal older individuals are ongoing at this time to enable such early detection to occur [6,143]. However, they provide another and perhaps vital clue to the search for the pathogenic processes that underlie PD, as they suggest that it is a disease of both the peripheral and the central nervous systems and that it is a multisystem disorder that spreads with time, affecting movement only at a relatively late stage in its course, and may thus be a target for early diagnosis and identification of at-risk populations. Current dopamine replacement strategies for treating PD are effective against the motor features of PD but are largely ineffective at addressing NMS. Hence a greater effort needs to be made not only in understanding the molecular mechanisms that cause NMS but also in how to treat them.

Spreading pathology

Neuronal loss in the substantia nigra pars compacta (SNc) and the subsequent loss of striatal dopamine content are accepted as being responsible for the classical motor features of PD. The neuropathological diagnosis of PD requires the detection of marked dopaminergic neuronal loss in the SNc and the presence of Lewy bodies, eosinophilic inclusions consisting of a dense core surrounded by a pale-staining halo of radiating filaments. The role of the Lewy body in pathogenesis remains unknown, but the discovery that misfolded α-synuclein is a major component of the radiating filaments and is also present in neuronal processes as Lewy neurites has altered views on their formation and role in neuronal loss and has led to a major shift in thinking about the onset and progression of the disease process from a pathological perspective and to the classification of PD as a synucleinopathy [21]. However, the neuropathological picture of PD has been known to be more widespread for many decades, with many nondopaminergic nuclei affected, including the locus coeruleus, reticular formation of the brain stem, raphe nucleus, dorsal motor nucleus of the vagus, basal nucleus of the Meynert, amygdala, and hippocampus [59]. Importantly, all of these nuclei degenerate with Lewy body pathology, suggesting a pathogenic process in common with that occurring in the SNc. It is the Lewy body pathology in these nondopaminergic areas that then results in some NMS. For example, hyposmia is associated with the presence of Lewy bodies and Lewy neurites in the olfactory bulb and brain centers such as the amygdala and perirhinal nucleus [78,162]. But it is the presence of Lewy bodies in peripheral tissues that takes the pathology associated with PD to another level. Orthostatic hypotension in PD is associated with
sympathetic autonomic denervation of the heart with the presence of Lewy bodies [28,112]. Lewy bodies are found in the myenteric plexus, reflecting the alterations in gastrointestinal motility occurring in PD. However, there may be a connection between changes originating in the periphery. For example, constipation as a common NMS is associated with neuronal loss and the presence of Lewy bodies in the dorsal motor nucleus of the vagus, which provides parasympathetic innervation to the stomach and intestine [10,20,159], and changes such as these can be initiated by injection of proteasomal inhibitors into the stomach wall and retrograde degeneration of the dorsal motor nucleus, at least in rats [102].

Indeed, what has become clear is that pathology in PD does not start in the SNc, but rather Lewy body pathology and the deposition of α-synuclein are proposed to originate in the olfactory bulb and lower brain stem, from where they spread in stages to involve the midbrain, eventually spreading to cortical regions. A staging of the caudorostral spread of Lewy body and α-synuclein pathology in PD was first proposed by Braak and colleagues in 2003 [11], when they proposed that α-synuclein deposition begins in the dorsal motor nucleus of the vagus (stage 1), from where it is thought to proceed in an upward direction via the pons (stage 2) to the midbrain (stage 3) and from there to the basal prosencephalon and mesocortex (stage 4), finally reaching the neocortex (stages 5 and 6). It is only at stage 3 after extensive loss of dopaminergic neurons in the SNc that motor features of PD become apparent. Although it is recognized that not all PD cases exactly follow this formal pattern of spread of PD pathology [68], it is now widely accepted that spreading pathology does occur in PD. This raises the important concept that PD pathology may be propagated from one neuron to another [34]. Exactly how this occurs is not known, but altered α-synuclein released by an affected neuron that is taken up by an adjacent unaffected neuron, or direct transfer between neurons, could act as a “seed” in a prion-like mechanism to perpetuate the cycle of α-synuclein misfolding and the spread of α-synuclein pathology [23]. Although this hypothesis is controversial some groups have demonstrated such a seeding mechanism in cell culture, animal models, and fetal mesencephalic cells transplanted into the PD striatum [23]. If such a hypothesis is correct, it will have a major impact on the molecular understanding of pathogenesis in PD.

Current approaches to treatment—no cure

The treatment of PD has not changed substantially in the past 30 years, with dopamine replacement therapy employing L-dopa and dopamine agonists as the mainstay, supported by the use of a series of enzyme inhibitors, namely peripheral decarboxylase inhibitors, catechol-O-methyl transferase inhibitors, and monoamine oxidase-B (MAO-B) inhibitors. These dopaminergic medications now come in a variety of enzyme inhibitors, namely peripheral decarboxylase inhibitors, providing differing degrees of drug delivery. Outside of the dopaminergic arena only anticholinergics and the weak N-methyl-D-aspartate receptor antagonist amantadine have had any use. But these are all symptomatic approaches to treating the motor deficits of PD with little effect on nonmotor symptoms and no proven effect on disease progression.

There have been several attempts to determine whether current therapy has any effect on the disease process. The dopamine agonists ropinirole and pramipexole show neuroprotective actions in preclinical models of PD of dopaminergic cell loss, but in clinical trial (REAL-PET; the Comparison of the Agonist Pramipexole versus Levodopa on Motor Complications of Parkinson’s Disease—CALM-PD) there was no slowing of progression of motor symptoms, although imaging studies suggested a lower rate of nigral cell degeneration compared to that seen in patients treated with L-dopa [117,161]. However, the drugs themselves turned out to affect the imaging markers employed [104]. Even in mild PD, the early use of pramipexole showed no advantage on disease progression over the later introduction of the drug (Pramipexole on Underlying Disease—PROUD study) [131].

L-dopa has had a checkered career as a potential reason for both acceleration of the cell death in PD and its slowing. For many years, the ability of L-dopa to generate free radicals and to kill dopaminergic cells in culture taints the drug with a neurotoxic label, but overall, based on a range of in vivo studies in animals, postmortem studies in humans, and clinical experience, this does not seem to be true, although the debate continues [1,108,120]. From the Earlier versus Late Levodopa (ELLDOPA) clinical trial, among others, early use of L-dopa seems to slow disease progression based on clinical rating scales, but not when using imaging endpoints [26].

It has been the MAO inhibitors selegiline and rasagiline, however, that have attracted most attention as potential disease modifiers. It was the ability of these drugs to prevent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity to nigral dopaminergic neurons through inhibition of 1-methyl-4-phenylpyridinium (MPP+) by blocking MAO-B activity [60]. However, both drugs also are associated with a range of actions that encompass antiapoptotic actions, antioxidant effects, antiglutamatergic effects, and neurotrophic actions, among others, that underlie a belief in their ability to alter the rate of loss of dopaminergic neurons [62,84]. Both selegiline and rasagiline have been examined in detail in clinical trials to assess whether disease modification occurs in humans. The Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) study initially suggested that selegeline treatment could delay the need for the introduction of L-dopa in early PD but this now seems largely due to a symptomatic effect of the drug, although open label extension studies in other clinical trials still suggest that disease progression is slower in those patients with PD that receive early selegline treatment [115,119]. Similarly, for rasagiline, the results of the TEMPO (TVP-1012 in Early Monotherapy for Parkinson’s Disease Outpatients) and ADAGIO (the Attenuation of Disease Progression with Azilect Given Once Daily) studies suggested that early use had a positive effect on clinical scores compared to later introduction of the drug, most notably in those patients with the highest degree of motor disability [109,118]. However, so far none of the studies undertaken have convinced the regulatory authorities to label either selegiline or rasagiline as disease modifying.

One other approach to current therapy deserves mention, namely, suppression of glutamatergic activity in PD. As will be explained later, excitotoxicity may contribute to the pathogenic process and as a consequence inhibition of glutamatergic function may influence disease progression. This has stimulated new interest in the actions of amantadine, which is currently used largely to suppress dyskinesia in PD. However, there is anecdotal evidence that treatment with amantadine from the beginning of therapy may lead to a better outcome than would otherwise be expected, and new clinical studies are being started with the drug to provide evidence-based medicine for its effects.

Risk factors for developing PD

Predisposing factors

Age represents the biggest predisposing factor for PD (young-onset cases may form a different patient population) for the majority of individuals developing the illness. However, it
remains unknown whether it is chronological age or the aging process that is responsible [69]. Loss of estrogen production in women with age may also remove a protective effect and there is some evidence that early menopause, hysterectomy, or removal of the ovaries increases risk in women that seen in men [121,123]. Genetic causes are becoming more common as gene hunting continues (see later) and may account for up to 40% of those at risk in the population from autosomal dominant, recessive, and susceptibility genes [41]. However, there are also environmental factors that contribute to the risk of developing PD [148].

The discovery of the toxicity of MPTP to nigral dopaminergic cells generated interest in environmental causes of the disease, although no other MPTP-like toxins have been discovered [53]. The closest has been the ability of the structurally related parquat, a commercial weed killer, to destroy dopaminergic cells in rodents and this links nicely to the increased risk of developing PD that repeatedly emerges from epidemiological studies, although there is no association with any specific compound [90,149,150]. The same might apply to the complex I inhibitor rotenone as a constituent of derris, which is commonly used by gardeners as a pesticide, but no cases have specifically been reported [7]. In contrast, exposure to annonacin derived from fruits in Guadeloupe was shown to produce parkinsonism [75]. Other environmental causes include solvent exposure (n-hexane, methanol), carbon monoxide poisoning, hydrogen sulfide intoxication, and perhaps manganese, although the resulting syndrome involves striatal cell degeneration and dystonic features and there now seems to be no increased risk in welders, who can be exposed to manganese vapor [70].

One last factor worthy of note is head trauma, as it has repeatedly emerged as borderline significant for increased risk of PD in population studies. A recent study in ex-National Football League players in the United States suggests that head trauma does increase the risk of developing PD [81] and so this may be a more relevant factor than previously thought, although controversial [128].

Protective factors

Factors that protect against the development of PD may reveal a lot about underlying pathogenic mechanisms. Perhaps the protective effect of cigarette smoking is the best documented and most reproducible of observations [72]. Nicotine is the immediate candidate for a neuroprotective action and there is currently interest in developing nicotinic agonists, for example, α-7 nicotinic agonists, for both symptomatic treatment and neuroprotection [122]. However, cigarette smoke contains more than 4000 components, so other agents may contribute to the protective effects, for example, smoking decreases MAO-B activity in brain [165]. Additionally, it may be that a genetic disposition against reward-seeking habits, such as cigarette smoking, may prevail in those who go on to have a clinical diagnosis of PD. Caffeine intake also appears protective against the development of PD—more so in men than in women and more so in those not using hormone replacement therapy [114]. Caffeine has been demonstrated to exert neuroprotective effects in a variety of experimental models of PD, possibly via the involvement of the adenosine A2a receptor [14]. This has highlighted the potential neuroprotective effect of A2a antagonists, such as istradephylline and preladenant, which are currently under development as symptomatic treatments for PD.

Other potentially protective factors are not so well defined but there have been suggestions of an effect of antihypertensives (notably calcium antagonists), nonsteroidal anti-inflammatory drugs, and antilipidemics, among others, although all are balanced by negative reports on these compounds [5,146,148,169]. Perhaps surprisingly, but of enormous potential interest, is a role for exercise and metabolic factors associated with the risk of developing PD [169].

Genetics of PD—gene mutations and genome-wide association studies (GWAS)

Investigation of familial PD has so far revealed at least 17 autosomal dominant and autosomal recessive gene mutations responsible for variants of the disease [47]. These include α-synuclein mutations and triplication, parkin, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), DJ-1, phosphatase and tensin homolog-inducible kinase 1 (PINK1), leucine-rich repeat kinase 2 (LRRK2), and glucocerebrosidase (GBA) (see Table 1). Of these parkin and LRRK2 are probably the most common genetic link to young-onset and late-onset PD, respectively, whereas GBA mutations may be the most common risk factor. Autosomal dominant mutations are typified by mutations in α-synuclein and LRRK2. Although α-synuclein mutations are only rarely encountered, their discovery led directly to α-synuclein being identified as a major component of Lewy bodies and Lewy neurites and the labeling of PD as a synucleinopathy, underpinning much of the consensus on the final stages of neuronal loss in PD being related to altered protein aggregation [33,35]. Mutations in LRRK2 seem to alter kinase/GTPase of this mixed lineage-like kinase found in cytoplasm and outer membrane of mitochondria [17,39]. LRRK2 interacts with another PD-related protein, parkin, and mutant LRRK2 induces apoptotic cell death in cultured neurons. Autosomal loss of function mutations include those in the ubiquitin E3 ligase parkin, which in combination with the ubiquitin-conjugating enzyme causes the attachment of ubiquitin as a marker on proteins destined for destruction by the proteasome. Additionally mutations in the mitochondrial PINK1 protect cells from mitochondrial stress/dysfunction, and the rarest mutation, in the redox-sensitive chaperone Dj-1, protects cells against oxidative stress [17,39]. Familial forms of PD and the associated gene mutations currently account for approximately 10% of cases and they have distinct clinical and pathological phenotypes. However, there is sufficient overlap with sporadic PD that investigations into such gene mutations have revealed important clues as to the molecular mechanisms that underlie the disease process in PD (see Fig. 1). Indeed, many of these familial PD neurodegeneration mechanisms overlap with the pathogenic mechanisms discovered in sporadic PD, such as mitochondrial dysfunction, oxidative stress, and altered protein handling (see below and Fig. 1) [47]. Furthermore a combination of advances in genetic analysis techniques, the ability to genotype cost

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus/disease</th>
<th>Mode of inheritance</th>
<th>Age of onset (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNCA</td>
<td>PARK1/4</td>
<td>Autosomal dominant</td>
<td>20–85</td>
</tr>
<tr>
<td>LRRK2</td>
<td>PARK8</td>
<td>Autosomal dominant</td>
<td>32–79</td>
</tr>
<tr>
<td>GRN</td>
<td>FTDP-17</td>
<td>Autosomal dominant</td>
<td>45–83</td>
</tr>
<tr>
<td>MAPT</td>
<td>FTDP-17</td>
<td>Autosomal dominant</td>
<td>25–76</td>
</tr>
<tr>
<td>DCTN1</td>
<td>Perry syndrome</td>
<td>Autosomal dominant</td>
<td>35–61</td>
</tr>
<tr>
<td>PARK</td>
<td>PARK2</td>
<td>Autosomal recessive</td>
<td>16–72</td>
</tr>
<tr>
<td>PINK1</td>
<td>PARK6</td>
<td>Autosomal recessive</td>
<td>20–40</td>
</tr>
<tr>
<td>DJ1</td>
<td>PARK7</td>
<td>Autosomal recessive</td>
<td>20–40</td>
</tr>
<tr>
<td>FBXO2</td>
<td>PARK15/PPS</td>
<td>Autosomal recessive</td>
<td>10–19</td>
</tr>
<tr>
<td>NRR4A2/NRR1</td>
<td>Unknown</td>
<td>Unknown</td>
<td>45–67</td>
</tr>
<tr>
<td>POLG</td>
<td>Unknown</td>
<td>Unknown</td>
<td>20–26</td>
</tr>
</tbody>
</table>

FTDP-17, frontotemporal dementia with parkinsonism linked to chromosome 17; PPS, pallidoppyramidal syndrome.
effectively, and the formation of large patient sample consortia, for example, the International PD Genomics Consortium, has facilitated highly powered GWAS in idiopathic PD, which have revealed some 14 “risk gene” loci for PD, including α-synuclein, LRRK2, human leukocyte antigen (HLA), and tau [55,106] (see Table 2). Although the presence of such risk gene loci is not a predictor for the development of PD, they highlight the fact that PD is a complex disease involving both genetic risk and environmental factors in its etiology.

**Mechanisms of neurodegeneration**

**Oxidative stress in Parkinson disease**

Oxidative stress remains a cornerstone of the concepts underlying the loss of dopaminergic neurons in PD. Since the 1980s there has been an exponential growth in publications that implicate the formation of reactive oxygen species as a final step in neuronal death of whatever origin. Starting from the idea of free radical production resulting from increased chemical and enzymatic oxidation of dopamine, through to the mechanism of action of toxins such 6-hydroxydopamine (6-OHDA) and paraquat, to evidence from postmortem and clinical investigations, oxidative stress and the resulting oxidative damage have emerged largely unscathed [36,61,63,64,141]. Whereas direct evidence for oxidative stress remains hotly contested, with both neuronal and glial sources being implicated, but there seems little doubt that the most likely contributor is increased radical formation originating from mitochondria (see later) and, perhaps, endoplasmic reticulum. Altered accumulation of iron in SNc, changes in calcium channel activity, altered proteolysis (proteasomal and lysosomal), changes in α-synuclein aggregation, and the presence of mutant proteins (for example DJ-1) are all examples of how oxidative stress might be induced in PD [126,133,146,152]. This emphasizes the diverse nature of the causes of cell death in this illness but also the common final pathways through which neuronal loss occurs. There is nothing specific about a role for oxidative stress in PD, as it also contributes to cell degeneration in a variety of other disease states. However, it may offer the opportunity to influence disease progression although attempts to date have been unsuccessful in clinical trials despite a multitude of encouraging reports from both in vitro and in vivo models of PD. However, ongoing studies with centrally active iron-chelating drugs and the elevation of urate levels through administration of uric acid or inosine administration offer new hope.

**Altered mitochondrial function in PD**

Mitochondrial involvement in cell death in PD has returned to center stage and provides part of a unifying concept of how neuronal loss occurs in both sporadic and inherited disease. The discovery of the neurotoxicity of MPTP through its metabolite MPP+ identified a role for the inhibition of complex I in pathogenesis that is shared by other substances toxic to dopaminergic neurons, including rotenone and annocanin [32,76,124]. Very quickly, an inhibition of complex I was shown to be present in the SNC that
was tissue and disease specific, although also detectable in platelets and muscle in PD [85,129,130,132]. A key Krebs cycle enzyme, α-ketoglutarate dehydrogenase, was also shown to be impaired [103]. The construction of cybrids using mitochondrial DNA (mtDNA) from patients with PD has clearly demonstrated the encoding of the complex I defect [38], but no consistent alterations in mtDNA have been demonstrated, although deletions associated with complex IV and oxidative damage occur in PD. Perhaps importantly, only about 30% of patients with PD have a clear complex I defect, suggesting, perhaps, that they form an important subgroup within the disease that offers a specific target for interference with the disease process.

More recently, interest in the role of mitochondria has stemmed from genetic investigations in familial PD. Notably mutations in α-synuclein, parkin, PINK1, and DJ-1 and perhaps LRRK2 have been associated with altered mitochondrial function [129,130,133] (see Fig. 1). These mutations can lead to altered protein localization in mitochondria in PD, abnormalities in mitochondrial structure and function, and a decrease in complex I assembly and activity. Loss of function of, notably, DJ-1, but also parkin and PINK1, decreases mitochondrial protection against oxidative stress, which in turn increases mitochondrial dysfunction. Another important role for mitochondria in PD, abnormalities in mitochondrial structure and function, and a decrease in complex I assembly and activity. Loss of function of, notably, DJ-1, but also parkin and PINK1, decreases mitochondrial protection against oxidative stress, which in turn increases mitochondrial dysfunction. Another important role for mitochondria in PD, abnormalities in mitochondrial structure and function, and a decrease in complex I assembly and activity. Loss of function of, notably, DJ-1, but also parkin and PINK1, decreases mitochondrial protection against oxidative stress, which in turn increases mitochondrial dysfunction.

Altered proteolysis in PD—proteasomal and lysosomal

The presence of multiple proteins in Lewy bodies, most notably α-synuclein, led to the idea that the catabolism of unwanted, damaged, or mutated proteins might be disrupted in PD, leading to cellular aggregation and neuronal death [31,136,140]. This led to investigation of the roles of the ubiquitin–proteasome system (UPS) and lysosomes in pathogenesis in PD. UPS involvement was supported by the discovery of mutations in parkin and UCH-L1, which function as a ubiquitin–protein ligase and in ubiquitin recycling. Examination of the 26S proteasome in PD revealed selective changes in its catalytic activity and composition in the SNc that were associated, perhaps wrongly, with impaired degradation of α-synuclein [97,100,151]. In cell culture and after direct intracerebral injection, proteasomal inhibitors, such as lactacystin and proteasome inhibitor 1 (PSI), were found to selectively destroy dopaminergic neurons [88,94,98,163]. In cell culture, the initiation of cell death using proteasomal inhibitors led to a cascade of events involving increases in oxidative and nitrative stress and damage and alterations to mitochondrial function [50]. Indeed, there is a close association between mitochondrial electron transport activity and the regulatory caps of the 26S proteasome, which are ATPases. The potential role of the UPS has been confirmed many times over and supported by microarray and transcriptional studies on laser-captured microdissected nigral dopaminergic neurons from PD [37]. However, the concept lost credibility when a slow, progressive degeneration of neurons in the brain that resembled that occurring in PD was claimed after systemic administration of proteasomal inhibitors [99], but this was never substantively reproduced (compare [73,86] with [12,167]).

As a consequence attention turned to lysosomes and to autophagy (macro-autophagy, micro-autophagy, and chaperone-mediated autophagy) (see [133]). Because autophagy-related proteins and autophagosomes are present in Lewy bodies, this seemed a viable target for disruption related to dopaminergic cell death. Indeed, the expression of the chaperone-mediated autophagy proteins lysosome-associated membrane protein type 2A (LAMP2A) and heat shock protein 70 is reduced in SNc in PD [2]. In addition, silencing of LAMP2A activity in a dopaminergic cell line reduced chaperone-mediated autophagy and increased the half-life of α-synuclein. Altered handling of α-synuclein can also be induced by disruption/mutation of the lysosomal membrane protein ATPase (ATP13A2), which is affected in one form of familial PD [154]. The potential involvement of altered lysosomal function in PD was supported by studies in MPTP-treated mice [18,19]. Toxin treatment caused a depletion of lysosomes in dopaminergic cells secondary to the onset of oxidative stress that led to the accumulation of autophagosomes and cellular degeneration. The toxicity of MPTP was attenuated by pharmacological and genetic treatments that enhanced lysosomal formation. Alterations in autophagy and mitophagy also occur in response to nigral neuronal degeneration induced by 6-OHDA [87,139].

Another strand supporting lysosomal involvement in neuronal loss in PD comes from Gaucher disease, a lysosomal storage disorder due to mutations in the GBA gene. This leads to a deficiency of the lysosomal enzyme GCase, which catalyzes the

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**Table 2**

GWAS risk genes for Parkinson disease.

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPT</td>
<td>rs2942168</td>
<td>Microtubule-associated protein tau</td>
</tr>
<tr>
<td>SNCA</td>
<td>rs356221</td>
<td>α-Synuclein</td>
</tr>
<tr>
<td>BST1</td>
<td>rs4698412</td>
<td>Bone marrow stromal cell antigen 1</td>
</tr>
<tr>
<td>LRKK2</td>
<td>rs1491942</td>
<td>Leucine-rich repeat kinase 2</td>
</tr>
<tr>
<td>GAK</td>
<td>rs11248051</td>
<td>Cyclin G-associated kinase</td>
</tr>
<tr>
<td>HLA-DRB5</td>
<td>rs1279882</td>
<td>Major histocompatibility complex, class II, DR B5</td>
</tr>
<tr>
<td>ACMSD</td>
<td>rs10928513</td>
<td>Aminocarboxymuconate semialdehyde decarboxylase</td>
</tr>
<tr>
<td>STK39</td>
<td>rs2102808</td>
<td>Serine threonine kinase 39</td>
</tr>
<tr>
<td>HIP1R</td>
<td>rs10847864</td>
<td>Huntingtin-interacting protein 1-related</td>
</tr>
<tr>
<td>MCCC1/LAMP3</td>
<td>rs11711441</td>
<td>Lysosomal-associated membrane protein 3</td>
</tr>
<tr>
<td>SYT11</td>
<td>rs34372095</td>
<td>Synaptotagmin-11</td>
</tr>
<tr>
<td>GPNMB</td>
<td>rs156429</td>
<td>Glycoprotein (transmembrane) NMB</td>
</tr>
<tr>
<td>STX1B</td>
<td>rs4889603</td>
<td>Syntaxin 1B</td>
</tr>
<tr>
<td>FGFP20</td>
<td>rs591323</td>
<td>Fibroblast growth factor 20</td>
</tr>
</tbody>
</table>

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metabolism of the sphingolipid glucosylceramide to ceramide. Gaucher mutations lead to a 20- to 30-fold increase in the risk of developing PD and 5–10% of patients with PD have a GBA mutation (see for example [156]). Recently, a GCase deficiency was reported in the substantia nigra of patients with PD and GBA mutations but, importantly, also in those with sporadic PD [30]. Because not all individuals with GBA mutations develop PD, the probability is that these lead to increased susceptibility to other factors involved in the pathogenic process, such as α-synuclein accumulation and oxidative stress occurring as a result of altered mitochondrial function. Indeed, GCase deficiency leads to α-synuclein accumulation both in vitro and in vivo [89,164].

Inflammatory change

The concept of inflammatory change in the brain in PD started with the description of activated HLA-positive microglia in SNc [92]. Subsequently, alterations in the cytokines interleukin-1α (IL-1α), IL-1β, and tumor necrosis factor-α (TNF-α) were found in the brain and cerebrospinal fluid, and postmortem studies showed inducible nitric oxide (NO) synthase to be present in activated microglia [45]. Inducible NO synthase is a source of NO, which in turn can react with superoxide from glial or neuronal sources to form the highly reactive peroxynitrite. This can nitrate proteins and other biomolecules, and 3-nitrotyrosine adducts are found in the SNc in PD. In addition, there is evidence for the presence of nitrated α-synuclein in Lewy bodies [31]. Microglia activation and inflammatory change were thought to be a consequence of neuronal destruction but there is evidence for a more general systemic inflammatory reaction in PD, suggesting it to be a primary cause of neuronal loss in some cases. In addition, peripheral inflammation may enhance the adverse effects of inflammatory change occurring in the substantia nigra [43]. This point was brought into focus by recent GWAS that showed HLA to be a risk factor for the occurrence of PD [40].

Additionally, there seems to be some cross-talk between the central nervous system innate inflammatory response and the peripheral immune system. Microarray gene expression studies have identified modified neuroimmune signaling in peripheral blood mononucleated cells from PD patients, and changes in neuroinflammatory markers in PD have more recently been detected by studies demonstrating increased concentrations of IL-2 [145], TNF-α, IL-6 [22], osteopontin, and RANTES/chemokine (C–C motif) ligand 5 [125] in serum in PD. In particular, serum levels of RANTES, a chemokine produced by activated microglia, were not only higher in PD compared to normal subjects but significantly correlated with Unified Parkinson's Disease Rating Scale scores [125]. Such findings have supported the concept that a selective blood biomarker could be developed and used to refine the clinical diagnosis of PD.

Multiple experimental studies have been utilized to investigate the role of inflammatory change in PD in greater detail. Initially toxins that directly destroy dopaminergic neurons, such as 6-OHDA, MPTP, and rotenone, were shown to activate microglia and induce inflammatory change. But it has been the use of lipopolysaccharide (LPS) in both in vitro cellular models and in vivo studies that focused on glial activation [44,56,57,95,96]. These studies have shown that dopaminergic neuronal loss can occur as a direct consequence of microglial activation that is accompanied by increased cytokine formation, increased production of reactive oxygen and nitrogen species, and decreased secretion of trophic factors responsible for the normal maintenance of neuronal viability. A potentially important finding is that damage after toxin application to SNc is accompanied by the presence of monocytes, suggesting blood–brain barrier damage and permeability. Indeed, altered gene and protein expression for the adhesion molecule intercellular adhesion molecule-1 occurs in PD and in experimental models of the illness [101]. The current consensus seems to be that a mild to moderate peripheral inflammation can exacerbate the loss of dopaminergic neurons initiated by another toxic insult.

All of these data suggest that one way of attempting to reduce cell loss in PD may be by reducing inflammatory change, and certainly this seems to work in experimental systems (but see later). This may be relevant to both the initiation and the progression of neuronal destruction irrespective of whether this starts at the level of neurons or glia. The importance of the glial activation in PD might lie in its long duration. In both humans and nonhuman primates, activated microglia can be detected years after exposure to MPTP, suggesting a propagating role in ongoing pathogenic change [93]. Conversely, this long-term activation may prevent microglia from performing their normal support role for neuronal viability, for example, through the release of trophic factors. Although the emphasis on the role of glia has centered on activated microglia, it should be borne in mind that astrocytosis also occurs in PD and it too may play a significant role in the sequence of events that lead to cell death.

Excitotoxic mechanisms

Excitotoxicity is always included in the list of pathogenic mechanisms that are thought to contribute to cell death in PD. However, the degree of direct evidence for this is small. The major contributor is the overactivity of the subthalamic nucleus (STN), which releases glutamate and which innervates the substantia nigra pars compacta and the internal segment of the globus pallidus [8,142]. In addition, there is evidence of altered glutamatergic input from the corticostriatal pathway. Excitotoxicity might also arise from the dysfunction of mitochondria that occurs in PD. However, experimental studies show that excitotoxins can cause destruction of nigral dopaminergic neurons and that this process can be blocked by various classes of glutamate antagonists [48,67,142,153,157,158]. Similarly, cell loss induced by toxins acting through other mechanisms, such as 6-OHDA, is diminished by drugs that either modulate glutamate release or diminish its action on target cells through actions on either inotropic or metabotropic glutamate receptors. The scenario would be that as PD develops with the onset of neuronal loss in the SNc, the increased activity of the glutamatergic input from the STN acts to amplify cell death. Indeed, lesions of the STN have been shown to reduce the loss of dopaminergic neurons normally induced by the injection of 6-OHDA into the SNc [12,99,135]. Overactivity of the glutamatergic output pathways from the STN to the globus pallidus also contributes to the evolution of some of the clinical symptoms of PD. Indeed, deep brain stimulation (DBS) can correct the overactivity of the STN, producing a clinical benefit in PD. Perhaps more importantly, recent investigations utilizing DBS in PD have associated it with a lack of progression of motor symptoms [147]. Assuming that DBS would also decrease the overactivity of the STN pathways innervating the SNc, this could add some support to the concept that excitotoxicity plays a role in cell death in PD.

Bringing it all together?

In the past 20 years, there have been significant advances in the understanding from a mechanistic perspective of the events that lead to the death of nigral dopaminergic neurons in PD. It is worth remembering, however, that the pathology of PD is widespread and that the same rigor has not been applied to looking at the underlying causes of cell death in nondopaminergic nuclei, such as the locus coeruleus and raphe nuclei. Indeed, there has been a presumption that because these neuronal groups also degenerate with the appearance of Lewy bodies, the same cell death sequences are operative, but in reality, this is not known. Cartoons of the sequence of events
that underlie nigral cell loss have been published regularly (and to which we have added in this review) but these have not changed much over the years in terms of the overall concepts espoused, as there has been a trend to make new information on pathogenic processes in PD fit into the existing framework of conceptual belief. This may or may not be correct.

Certainly the events that are commonly related to pathogenesis in PD fit nicely together—for example, mitochondrial dysfunction with oxidative stress and altered proteolysis. The evidence from gene mutations identified in inherited PD has added to this accepted interaction and has again highlighted mitochondrial abnormalities and altered protein handling as key events. The general acceptance of PD as a synucleinopathy has added to the weight of the arguments for a common thread underlying nigral cell loss originating in both sporadic and inherited disease [33,35]. There is also a degree of comfort associated with containing novel information within an accepted arena. But is this the right thing to do?

A skeptical view would be that it is not surprising that all the major organelles of a cell are somehow involved in the demise of the neuron. This is emphasized by a range of studies in which cell death is initiated at a point in the accepted cycle of pathogenic events in PD. These illustrate very nicely that it is difficult to distinguish what is horse and what is cart. For example, if cell death is initiated by inhibiting complex I, then this entrains changes in oxidative and nitrative stress and proteasomal activity. Inhibiting proteasomal activity leads to altered mitochondrial function and increased oxidative and nitrative stress and so on, with the same applying to the introduction of mutant parkin or α-synuclein into cell lines [50–52,79,80].

A more difficult conclusion—and one that would decrease an author’s chances of publication—would be that many of the key events that are identified as being involved in cell death in PD and that result from gene mutations represent separate pathogenic pathways that define different forms of what is currently deemed sporadic PD. The general acceptance of PD as a synucleinopathy has added to the weight of the arguments for a common thread underlying nigral cell loss originating in both sporadic and inherited disease [33,35]. There is also a degree of comfort associated with containing novel information within an accepted arena. But is this the right thing to do?

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A more difficult conclusion—and one that would decrease an author’s chances of publication—would be that many of the key events that are identified as being involved in cell death in PD and that result from gene mutations represent separate pathogenic pathways that define different forms of what is currently deemed to be PD. Indeed, the current view is that PD is not a single disease but rather a syndrome of multiple causes and manifestations.

A simple example is the general absence of Lewy bodies in the SNc in cases with parkin mutations [42]. Another example from sporadic PD is that only 30% of individuals have a complex I defect that is outside of the normal range. Our own limited investigations failed to show any correlation in postmortem nigral tissue between iron accumulation, the extent of oxidative stress, and mitochondrial dysfunction [65].

Perhaps in what is termed sporadic PD, we are missing key pieces of the jigsaw and what we are observing are secondary changes that are the result of one or more primary events that remain unknown. It becomes increasingly possible that pathogenic events in SNc do not originate in the brain but are the result of some peripheral event that is then "transmitted" into brain, initiating cell death that propagates itself in a stoichiometric fashion through synucleinopathy to eventually reach the SNc and that explains the long prodromal period and the occurrence of nonmotor symptoms that are now accepted part of what used to be considered a movement disorder (see earlier).

## Translation into animal models of PD

### Toxin relevance

Numerous toxins are in use in experimental models of PD largely based on their ability to destroy dopaminergic neurons through a variety of relevant pathogenic mechanisms. In cell culture and in primary neuronal cultures, the concentration of toxin to which cells are exposed and the duration of that exposure can be controlled, although the time course of such studies is seldom more than a few days. In this manner, it is possible to study the role of specific pathogenic pathways in the induction and progression of cell death, although the environment is entirely artificial and the cells may not be representative of the susceptibility of dopaminergic neurons in the aging brain in humans that are affected in PD. For example, cell lines may not have a full dopaminergic phenotype, they may not be derived from a neuronal source, and they will not be accompanied by glial cells. Primary cultures are derived from fetal sources, they are immature and may be from species in which susceptibility to toxin action is different from that in humans. Even the cell culture medium may not be reflective of the normal environment for cells in that it may be high in iron and low in antioxidants and so provide a highly pro-oxidant environment without the natural antioxidant defense mechanisms being operative [16]. Nevertheless, as a primary means of assessing cell death processes, these are highly useful techniques and capable of being manipulated, for example, by gene transfection, to study both sporadic and familial PD.

The use of toxins in in vivo models of PD is also a highly useful way of investigating dopaminergic cell death and drug action, but has limitations that have proved difficult to overcome (see [9,24,91] for recent reviews; see Table 3). Most toxins require direct intracerebral injection into the SNc, medial forebrain bundle, or striatum. Most need to be injected in concentrations that rapidly kill the majority of dopaminergic neurons to allow motor deficits to be observed. It is much more difficult to control the toxin concentration, the degree of neuronal exposure, and the time course of toxin effect than when using in vitro culture systems. Certainly, specific pathogenic mechanisms, for example, oxidative stress and complex I inhibition and inflammatory change, can be modeled using 6-OHDA, MPP+ and LPS, respectively, but toxin exposure is usually acute, in young animals, and in a species that differs in susceptibility to humans. The biggest drawback is that most approaches are one off and do not in any way reflect the progressive nature of cell loss in PD. One or two exceptions are the injection of 6-OHDA in to the striatum, which causes retrograde degeneration of dopaminergic neurons, and the use of infusion pumps to provide longer periods of toxin exposure. But even here, we are talking about days rather than weeks or months or years. In addition, most pathogenesis-based toxin studies are focused entirely on the SNc and there have been only limited attempts at destroying other neuronal systems affected in PD, which do not mimic one of the key features of PD, that being altered protein deposition.

There are, however, exceptions to most of the above criticisms. The peripheral administration of 6-OHDA to neonatal rats destroys dopaminergic and noradrenergic neurons as it passes the incomplete blood–brain barrier. MPTP can be used systemically in both mice and monkeys to destroy dopaminergic neurons in the SNc although, again, the time course of these studies is short and the effects specific to dopaminergic systems. Peripheral administration of LPS has been reported to induce nigral

### Table 3

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<th>Pathology modeled</th>
<th>Toxin</th>
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<td>Oxidative stress</td>
<td>6-OHDA and paraquat</td>
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<tr>
<td>Nitrative stress</td>
<td>LPS</td>
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<tr>
<td>Mitochondrial dysfunction</td>
<td>Rotenone, MPTP, and MPP+</td>
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<td>Excitotoxicity</td>
<td>Quinolinic acid, ibotenic acid</td>
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<tr>
<td>Proteasomal dysfunction</td>
<td>PSL, epoxymycin, lactacystin</td>
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<td>Glial cell activation</td>
<td>LPS</td>
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dopaminergic cell loss but not consistently, although it may increase susceptibility to the central actions of other toxins. Systemic administration of rotenone provides an interesting possibility despite its inherent toxicity [7]. There seems to be an agreement that with careful administration, rotenone kills nigral dopaminergic cells as well as affecting neurons in other nuclei and perhaps with sequential pathology [54,116]. The biggest question is whether these are relevant to PD or more reflective of the widespread pathology occurring in multiple-system atrophy, another Parkinson-like disorder [46]. Most intriguing is the use of proteasomal inhibitors such as epoximycin and PSI. There is no doubt that these are toxic to dopaminergic neurons in vitro and on stereotoxic injection into the SNc (see above). But reports of the slow progressive onset of neuronal loss in a pattern that resembles that occurring in PD have proved difficult to reproduce in some laboratories but not others. This would be a marked advance in toxin-based models of PD if viable, as cell death progresses through the brain, as suggested by Braak and is accompanied by α-synuclein aggregate formation. In fact, recent data suggest that PSI does not enter the brain but rather such proteasomal inhibitors can induce Lewy body-like pathology in the gut and other organs (unpublished observations) and this then invades the brain through the dorsal motor nucleus of the vagus [102]. If this is a reality, it would explain much that has consumed the debate over the progression of PD in recent years.

**Gene relevance**

Understanding the function of wild-type and mutated proteins associated with familial PD has been possible through their transfection into cell lines and this has led to advances in discerning their role in the pathogenic process through alterations in oxidative stress, mitochondrial function, and altered proteasomal and lysosomal activity. But it has been the translation into in vivo experimental models that has proved more daunting. Despite promising findings in *Drosophila caenorhabditis elegans*, there are no transgenic, overexpression, knock-out, or knock-in models of wild-type or mutant α-synuclein that have fully recapitulated the pathological picture of PD (see [9,83,166] for recent reviews). Viral vector-induced expression of wild-type and mutant α-synuclein in rats and primates has been associated with nigral dopaminergic cell degeneration but this has not been a consistent finding. The picture has been similar when the same approaches have been attempted using parkin, DJ-1, PARK1, and LRRK2. Changes in dopaminergic function in striatum, altered morphology of dopaminergic neurons, changes in other monoaminergic systems, degeneration of motor neurons, gliosis, and inflammatory changes occur, among others, along with mild motor abnormalities, but not the expression of PD that was hoped for. However, there is still value in this approach, as the generation of these animals allows an in vivo approach to explaining the roles of mutated proteins in the pathogenic process defined using other techniques. It also provides a test bed in which to examine potential neuroprotective therapies, for example, kinase/GTPase inhibitors in LRRK2 transgenic animals [77,82] and the role of passive vaccination in α-synuclein transgenic mice [155]. There also seems to be a significant role for studying nonmotor symptoms of PD in genetic models of PD, with reports of everything from cognitive impairment to altered gastrointestinal motility and constipation [91,160]. It could be questioned whether mice provide the right background to test the pathogenic potential of mutated proteins in PD, as total knockout of single wild-type proteins does not seem to result in alterations in dopaminergic function in the brain of mice that are null for parkin, DJ-1, and PINK1; nothing untoward seems to occur as they age [71]. Increased susceptibility to subsequent toxic insult may be enhanced and it may be that combinations of genetic models and toxin-based approaches will yield important information on pathogenesis related to nigral cell death. However, one recent study suggests that models more closely related to PD can be generated under appropriate conditions. A subset of DJ-1 null mice backcrossed into a normal background was reported to show early onset of unilateral nigral dopaminergic cell loss that became bilateral with time and later involved the locus coerules and the onset of motor deficits [127]. It should also be remembered that genetic changes not directly linked to PD have yielded important animal models in which nigral dopaminergic cell death does occur and which again add to the knowledge of pathogenic mechanisms. These include the aphaikia mouse lacking the homeobox transcription factor Pitx3, the mitopark mouse lacking mitochondrial transcription factor A, and Nurr-1 deficient mice [29,49,66].

**From pathogenesis to neuroprotection?**

Based on the extent of knowledge of pathogenic mechanisms leading to dopaminergic cell death in PD, it would seem likely that treatment strategies aimed at stopping or slowing the progression of the disease process would be available but this is not the case. Neuroprotection has proved a more difficult nut to crack than most had envisaged and the current situation is starting to look like that in stroke. Based on experimental studies, a range of compounds have been advanced into clinical development, but so far, none have proved to have a convincing effect on the worsening of motor symptoms [107,110,111,134,144]. This has involved antioxidants, dopamine agonists, monoamine oxidase inhibitors, agents affecting mitochondrial function, calcium antagonists, antiapoptotic agents, and neurotrophic factors, among others. So far, only the monoamine oxidase B inhibitor rasagiline has been shown to have some effect on motor scores, but this was not sufficient for it to be labeled as disease-modifying [109]. Although the pace has lessened, there are a number of ongoing studies looking to see whether centrally active iron chelators, precursors of urate, or stimulators of glial cell-derived neurotrophic factor production in brain are able to do what has eluded previous attempts. But perhaps the worrying issue is whether the current concepts of the pathogenic processes in PD and the nature of PD itself are correct.

We base the animal models of PD on the processes that we believe to be operative in cell death in humans. Classically, there is great reliance on using MPTP-treated mice and the 6-OHDA-lesioned rats to model oxidative stress and mitochondrial dysfunction, respectively. But these may have deceived us as so far there has been no translation into clinical effect. The surprising thing is that we continue to use these models to find further potential neuroprotective strategies despite their history, perhaps because there is nowhere else to turn in terms of toxin-related effect. Interestingly, there has been relatively little work in rotenone- or PSI-treated rodents, which may have more to offer. What is particularly worrying is the large numbers of compounds reported as positive in MPTP-treated mice—if it were that easy, we would have neuroprotection in PD by now. There is clearly a need to reassess the models employed and to ensure that we are working from a firm knowledge base from postmortem studies and the living PD patient with respect to the events that occur during neuronal loss and their time course. The last may be very important as it may be too late to expect a neuroprotective treatment to have much effect by the time a diagnosis of PD is made on classical grounds, as neuronal loss will be too advanced. Perhaps ongoing studies to identify premotor signs of PD will...
identify an earlier patient population in which to study neuro-protection and maybe the animal models will turn out to be correct in their prediction of effect.

The gene defects associated with familial PD may lead to new potential targets for neuroprotection and, at present, mitochondrial dysfunction and lysosomal defects seem attractive. The problem has been, and to a large extent remains, that most aberrant proteins identified do not offer an obvious target for therapeutic intervention and, in many cases, their physiological function remains unknown. This applies to α-synuclein, parkin, DJ-1, and PINK1, among others. LRRK2 mutations are linked to altered kinase/GTPase activity and this could be an attractive target, but to find a centrally active kinase inhibitor that focuses on the areas of the brain affected in PD, that does not impair the physiological function of the protein, and that does not affect other kinases, is challenging. Indeed, the pharmaceutical industry may find it difficult to identify viable targets at the current time on which to unleash the medicinal chemists and preclinical teams. The other danger is that the defects in familial disease have nothing to do with the processes that underlie neuronal loss in the majority of PD patients with sporadic disease. The means of assessing molecules that might potentially rectify the effects of gene defects also needs assessment. For example, it is perhaps not too surprising that kinase inhibitors prevent the neuronal loss in mice induced by the introduction of a LRRK2 mutation that increases kinase activity. The acceptance that PD is a synucleinopathy suggests that removal of the protein could slow disease progression whatever the initial cause. But what type of α-synuclein should be removed and how much of it? Whether this should be aggregates, fibrils, or protofibrils or inhibition of the formation of the protein, all of it, some of it, or none of it remains to be determined. Certainly approaches such as passive vaccination can remove α-synuclein and prevent the consequences of its aggregation in α-synuclein overexpressing mice, but will this work in PD? The worry is that several similar approaches using human monoclonal antibodies against amyloid have recently failed in Alzheimer disease despite encouraging preclinical data.

Finally, it may be the nature of PD that has so far prevented the translation of pathogenic mechanisms into a neuroprotective treatment. Clinical trials are still undertaken in the classical manner of comparing the effect of an agent between large groups of patients in a placebo arm and an active treatment arm, looking for a statistically relevant outcome. But this presumes that PD is a single illness resulting from the same pathogenic processes and this is increasingly being challenged. Rather there should be attempts to look at drug effect in individuals with common features, for example, a mitochondrial complex I defect. At present significant effects of a specific drug targeted at one component of the cell death pathway may be missed in a small proportion of patients in a clinical trial because of dilution of the outcome by the majority who have PD associated with other pathogenic mechanisms and who did not respond. Indeed, it may be that we have to think about neuroprotection for small portions of the PD population who have the disease for specific reasons and, notably, this may be those with inherited disease, for example, a LRRK2 mutation, as a druggable target. The downside is that such an approach may not be economically viable for the pharmaceutical industry. The alternative is to adopt the oncology approach and to use cocktails of drugs that exert multiple effects on the pathogenic cascade.

Concluding remarks

Clearly we have made great strides in improving our understanding of the nature and causes of PD. However, there are several fundamental gaps in our knowledge, which hamper our ability to develop effective neuroprotective strategies for PD. Importantly, we still do not understand the molecular mechanisms that account for the spreading pathology of the disease. Additionally, the failure to generate an accurate model of sporadic Parkinson disease and the clinical failure of neuroprotective strategies developed from such models highlight that we are missing a key section of the cell death mechanism jigsaw. Indeed, the mechanisms currently used to explain pathogenesis in PD may just be the downstream consequence of a so far unknown trigger. Certainly, it is clear that PD is not a single disease with a single cause but a syndrome in which the variants have in common nigral dopaminergic cell death and motor dysfunction, but around which multiple other neuronal systems degenerate and which have multiple primary causes involving a range of pathogenic processes.

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