



# Diagnosis and treatment of Parkinson disease: molecules to medicine

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**Parkinson disease (PD) is a relatively common disorder of the nervous system that afflicts patients later in life with tremor, slowness of movement, gait instability, and rigidity. Treatment of these cardinal features of the disease is a success story of modern science and medicine, as a great deal of disability can be alleviated through the pharmacological correction of brain dopamine deficiency. Unfortunately these therapies only provide temporary, though significant, relief from early symptoms and do not halt disease progression. In addition, pathological changes outside of the motor system leading to cognitive, autonomic, and psychiatric symptoms are not sufficiently treated by current therapies. Much as the discovery of dopamine deficiency led to powerful treatments for motor symptoms, recent discoveries concerning the role of specific genes in PD pathology will lead to the next revolution in disease therapy. Understanding why and how susceptible cells in motor and nonmotor regions of the brain die in PD is the first step toward preventing this cell death and curing or slowing the disease. In this review we discuss recent discoveries in the fields of diagnosis and treatment of PD and focus on how a better understanding of disease mechanisms gained through the study of monogenetic forms of PD has provided novel therapeutic targets.**

## History

Parkinson disease (PD) is a chronic, progressive neurodegenerative disorder that affects at least 1% of people by age 70 (1–3). James Parkinson provided the first detailed description of the disease in his 1817 monograph “An Essay on the Shaking Palsy.” In the latter part of the nineteenth century, Charcot further refined the description of this disorder and identified the cardinal clinical features of PD including rest tremor, rigidity, balance impairment, and slowness of movement (reviewed in ref. 4). An early clue to the pathology of the disease came from Brisaud, who speculated that damage in the substantia nigra (SN) might lead to PD (5, 6). Eosinophilic inclusions (Lewy bodies) later were identified in the brains of PD patients (7) and, along with abnormalities in the SN, became a recognized pathologic marker of the disease (8).

A major advance in the understanding of PD came when dopamine deficiency was discovered in the corpus striatum and SN of brains taken from patients (9). Later studies demonstrated the connection between the SN and the striatum, thus suggesting that dopaminergic cell loss in the SN directly leads to dopaminergic deficiency in the striatum (10). The determination that PD is a disease of dopamine loss led to the development of ratio-

nal therapies aimed at correcting this deficiency (11). After some initial uncertainty, the dopamine precursor levodopa proved to be a powerful PD treatment (12). Subsequent advances in therapy included combining levodopa with a peripheral decarboxylase inhibitor, such as carbidopa or benserazide (13, 14). This combination significantly reduced the nausea and vomiting associated with levodopa therapy and allowed a greater proportion of levodopa to enter the brain. Similarly, catechol-O-methyltransferase (COMT) inhibitors, which prolong the half-life of levodopa and dopamine were found to enhance the effect of a given levodopa dose (15–17). In addition to increasing the level of dopamine precursors, the focus of therapeutic design was also on limiting the breakdown of endogenous dopamine. The monoamine oxidase type B (MAO-B) inhibitor selegiline works in this fashion and provides symptomatic benefit (18). Finally, the development of dopamine agonists that directly stimulate postsynaptic dopamine receptors, thus bypassing dopamine synthesis completely, further illustrates how novel therapies can be borne from knowledge of pathology (19, 20).

Surgical therapies that reduce tremor and rigidity in PD patients were used prior to the advent of levodopa treatment. Meyers pioneered surgical lesioning procedures that targeted symptoms and spared patients from the hemiparesis that resulted from earlier surgical approaches. These procedures largely were abandoned once levodopa therapy became more common (21). Recent advances in the understanding of basal ganglia physiology and the development of new technologies has led to a reemergence of surgical PD therapies in the form of deep brain stimulation (DBS) (22, 23). DBS has become increasingly common in patients whose disease is difficult to manage with medical therapy alone.

Despite these landmark advances in symptomatic PD therapy, the ability of these treatments to facilitate an acceptable quality of life for the patient wanes with time. This is due to the development of motor complications including wearing-off (the

**Nonstandard abbreviations used:** COMT, catechol-O-methyl transferase; CoQ10, coenzyme Q10; DBS, deep brain stimulation; GDNF, glial cell line–derived neurotrophic factor; LRRK2, leucine-rich repeat kinase 2; MAO-B, monoamine oxidase type B; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson disease; PINK-1, PTEN-induced putative kinase 1; rTMS, repetitive TMS; SN, substantia nigra; SPECT, single photon emission computed tomography; TMS, transcranial magnetic stimulation; UCH-L1, ubiquitin carboxyterminal hydrolase L1.

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**Table 1**  
Diagnosing PD

**Cardinal features of PD**

- Bradykinesia (slow movements, decrement of frequency and amplitude of repetitive movements)
- Rest tremor (most commonly beginning in the hand)
- Cogwheel rigidity
- Postural instability (seen in later stage disease)

**Associated features in support of the diagnosis**

- Sustained and significant levodopa effect
- Reduced armswing
- Difficulty rising from a low chair
- Impaired olfaction
- Hypophonia
- Stooped posture
- Depression/anxiety
- Shuffling/festinating/freezing gait
- Unilateral symptom onset
- Difficulty turning in bed
- Drooling
- Constipation
- Micrographia
- Sleep disturbance/REM sleep behavior disorder
- Seborrhea

**Atypical features suggesting alternative diagnoses**

Limited, nonsustained, or no response to levodopa and:

**Symptom/sign**

- Early dementia with cognitive fluctuations, hallucinations, and delusions
- Early autonomic impairment and/or cerebellar ataxia, nocturnal stridor, rapid progression, dysarthria
- Stepwise progression, prominent lower extremity involvement, known vascular disease, possible levodopa responsiveness
- Cortical sensory impairment, automatic movements (alien hand phenomenon), apraxia, prominent akinetic limb dystonia
- Supranuclear gaze palsy, early falls, axial rigidity, pseudobulbar palsy, frontal lobe deficits
- Kinetic or postural tremor especially involving the head or voice, alcohol responsiveness, autosomal dominant pattern of inheritance
- Urinary incontinence, gait disorder, cognitive impairment, and brain imaging suggesting communicating hydrocephalus
- Symmetric onset with previous exposure to neuroleptics or other PD-mimicking drugs

**Consider**

- Dementia with Lewy bodies
- Multiple system atrophy
- Vascular Parkinsonism
- Cortical-basal ganglionic degeneration
- Progressive supranuclear palsy
- Essential tremor
- Normal pressure hydrocephalus
- Drug-induced Parkinsonism

return of PD symptoms too soon after a given levodopa dose), the presence of involuntary abnormal movements (dyskinesias and dystonia), and the emergence of treatment-resistant symptoms such as gait impairment, cognitive decline, autonomic dysfunction, and medication-induced psychosis. Clearly, the current symptomatic therapies cannot completely ameliorate later-stage symptoms, nor can they address the ongoing degeneration in the dopaminergic and nondopaminergic systems. For this reason, a good deal of current research has focused on finding the cause of dopaminergic cell loss and on exploring protective, restorative, and replacement therapies. Much in the same manner that understanding the cellular pathology of PD led to a revolution in symptomatic therapy, a better understanding of the molecular pathology of PD will lead to prevention and cure.

**Diagnosis**

Even with recent advances in our understanding of disease mechanisms, the diagnosis of PD is usually made based on patient history and physical examination alone. PD should

be considered if a person exhibits one or more of the cardinal features of the disease, including tremor at rest, bradykinesia, rigidity, and, in more advanced cases, postural instability. Perhaps more than any other single feature, the presence of a typical rest tremor increases the likelihood of pathologically supported PD, although approximately 20% of patients fail to develop a typical rest tremor (24). Supporting evidence also may come from a history that includes associated symptoms and the absence of findings that would suggest an alternative diagnosis (reviewed in ref. 25; see Table 1). Many of these associated symptoms include nonmotor complaints including disrupted sleep, depression, fatigue, constipation, and anxiety. Additionally, the early PD patient may complain of stiffness, slowness, tremor, and imbalance even when the neurological exam is normal (26). Finally, a significant and lasting clinical response to dopaminergic therapy is characteristic of PD, and the lack of such a response should prompt a search for alternative diagnoses. Despite careful examination, the rate of PD misdiagnosis is approximately 10–25% (27–29). Complicating diagnosis is the



**Table 2**  
Potential biomarkers of PD

**Imaging**

Reduced striatal metabolism on <sup>18</sup>F-deoxyglucose imaging  
 Reduced dopa uptake and decarboxylation on <sup>18</sup>F-dopa PET  
 Altered dopamine receptor binding on <sup>11</sup>C-raclopride PET  
 Reduced level of dopamine transporter ligand uptake  
 on <sup>123</sup>I β-CIT SPECT  
 Mineral deposition in the SN on transcranial ultrasound  
 Cardiac denervation on cardiac scintigraphy

**Clinical**

Personality questionnaires  
 Neuropsychiatric testing  
 Sleep evaluation  
 TMS/evoked potentials  
 Olfactory screening

**Biochemical**

Genetic screening  
 Mitochondrial complex I measurement  
 α-Synuclein levels and isoforms in blood

<sup>123</sup>I β-CIT, iodine 123-2β-carbomethoxy-3β-(4-iodophenyl)tropane.

clinical heterogeneity of PD. Patients may present with a tremor-predominant clinical picture or lack tremor completely. Indeed patients presenting with early postural instability/gait difficulty (PIGD) or rigidity/bradykinesia follow a more rapid course of disease than do those presenting with early tremor (30).

Inaccuracy in PD diagnosis and the desire to identify presymptomatic patients have prompted the search for disease biomarkers that include imaging techniques and laboratory-based or clinical assays. Brain imaging studies using both PET and single photon emission computed tomography (SPECT) are able to distinguish those subjects with PD from normal controls with greater than 95% sensitivity (31). The results are not as good, however, when imaging is used to distinguish PD from similar disorders such as progressive supranuclear palsy or multiple system atrophy. In these cases multiple techniques and careful data analysis may be required to clearly identify whether idiopathic PD or another disorder is the cause of a Parkinsonian syndrome (32). Studies are being conducted to search for other possible diagnostic aids in PD, of which some of the most promising use transcranial ultrasound (33), examine deficits in olfaction (34), and determine oligomeric α-synuclein in blood from PD patients (35). A list of potential biomarkers under study is shown in Table 2 (for a more detailed review see ref. 36).

The screening of affected and presymptomatic individuals for known genetic mutations may aid in PD diagnosis. With the discovery that mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene are more common than expected in certain populations (37, 38), there is little doubt that screening patients at risk will become increasingly common. The research implications are great, as investigators will be able to follow presymptomatic patients over time to assess biomarkers, risk factors, and potential protective therapies. Until disease- and risk-modifying therapies are available, and until more is known about penetrance rates, mutation-phenotype correlations, and gene frequency, genetic screening for known mutations likely will have little clinical impact on the average PD patient.

**Pathogenesis**

The pathologic examination of brains from PD patients demonstrates neuronal cell loss, especially of the dopamine-rich, pigmented neurons in the SN, and the presence of Lewy bodies and Lewy neurites in multiple brain regions (39, 40). Lewy bodies and neurites stain with antibodies to α-synuclein, ubiquitin, and a variety of other biochemical markers and are found in many areas of the PD brain: not only the SN, but also the dorsal motor nucleus of the vagus, locus ceruleus, raphe and reticular formation nuclei, thalamus, amygdala, olfactory nuclei, pediculpontine nucleus, and cerebral cortex, among others (39, 41). Indeed pathology is present outside of the brain as well, involving autonomic and submucosal ganglia (41, 42). Despite this widespread pathology, much of the research into the PD pathogenesis has focused on the cell loss and Lewy bodies seen in the dopaminergic SN. The past focus on dopaminergic deficits is related to the prominent motor manifestations of PD for which patients seek treatment. However, it is clear that PD is more than just a syndrome of dopaminergic deficiency and that future research and therapy will need to address the multiple neuronal systems affected in PD.

Some studies suggest that environmental factors lead to PD. The occurrence of postencephalitic Parkinsonism supports this view, as a particular viral infection in the early twentieth century placed patients at a higher risk for developing nigral cell loss and some Parkinsonian clinical features (43). Some pathologic features were atypical of PD, however, including a lack of Lewy bodies and a prominence of neurofibrillary tangles (44). In addition, the discovery of toxins that induce a Parkinsonian condition both in animal models and in humans further supports the possibility of an environmental trigger. The most widely studied toxin is a meperidine analog, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which, when mistakenly injected, leads to the clinical features of PD (45, 46). In addition, exposure to other toxins such as rotenone, paraquat, maneb, and epoxomicin can induce a Parkinsonian syndrome in experimental animals (47–49).

Genetic causes or predispositions also play an important role in PD. The disease is inherited in well-characterized kindreds (50, 51), and patients by chance have an affected family member more often than expected (52). Population-based association studies have identified genetic loci that may contribute to the development of “sporadic PD” (53). Thus far, genetic variability in tau, semaphorin 5A, α-synuclein, fibroblast growth factor 20, and nuclear receptor-related 1 genes are associated with increased disease risk (54–56). Perhaps more intriguing is the discovery of single gene mutations responsible for causing disease phenotypes that can be indistinguishable from sporadic PD. Understanding and comparing how mutations in specific genes can lead to Parkinsonism will provide new model systems and a better knowledge of the more common (i.e., sporadic) form of the disease (Table 3). These specific genes, their syndromes, and how their discovery impacts the search for the cause and treatment of PD are described below.

**α-Synuclein and the role of protein aggregation**

The first genetic mutation causing PD was found in the gene encoding α-synuclein and consisted of an alanine-to-threonine substitution (A53T) (57). This and 2 other point mutations (A30P and E46K) in the coding region of this gene, as well as gene duplications and triplications, cause a very rare, autosomal-dominant form of the disease (58–61). The clinical syndrome resulting from an A30P mutation can be indistinguishable from sporadic PD,



**Table 3**  
Monogenetic causes of PD

Gene/protein	Pattern	Prevalence	Pathology	Common features	Notes
$\alpha$ -Synuclein	AD	Very rare	Lewy bodies	Early-onset dementia; presentation variable with mutation type	Aggregation of protein in Lewy bodies from genetic and sporadic forms of PD
Parkin	AR (mostly)	18% EOPD (50% with family history)	Rare Lewy bodies, if any	Early onset, slow progression	Protein is involved in ubiquitination
DJ-1	AR	<1% EOPD	Unknown	Early onset, slow progression	Protein is involved in the cellular stress response
PINK-1	AR (carriers may be at increased risk)	2–3% EOPD	Unknown	Early onset, slow progression	Protein is a mitochondrial kinase
LRRK2	AD	Highly variable	Lewy bodies	Typical PD (mostly)	Protein is a kinase with multiple putative domains

AR, autosomal recessive; AD, autosomal dominant; EOPD, early-onset PD (usually before 50 years of age).

including typical motor findings and reported late-stage dementia and psychosis (62). On the other hand, the A53T mutation can lead to unusual features such as earlier onset (less than 45 years of age), myoclonus, and more severe autonomic dysfunction (63). The E46K mutation causes Parkinsonism with the added features of dementia and hallucinations. Patients harboring gene triplications have an early age of onset with prominent autonomic and cognitive dysfunction, a rapidly progressive course, and broader brain pathology compared with sporadic cases (64, 65). Those patients with gene duplications have a less severe phenotype and a later age of onset than those with triplications, suggesting that the level of expression of  $\alpha$ -synuclein correlates with disease severity (61, 66). In addition, polymorphisms within the  $\alpha$ -synuclein promoter are associated with an increased PD risk, further implicating altered  $\alpha$ -synuclein expression as a disease mechanism (67–69). This gene dosage effect, coupled with the important finding of  $\alpha$ -synuclein immunoreactivity in Lewy bodies (70), provides strong evidence that this protein plays an important role in the pathogenesis of both sporadic and certain inherited forms of the disease. Furthermore, mice engineered to lack  $\alpha$ -synuclein show resistance to the dopaminergic toxin MPTP (71), implicating  $\alpha$ -synuclein in the pathogenic mechanism that leads to MPTP-induced Parkinsonism.

$\alpha$ -Synuclein is a highly conserved, abundant presynaptic phosphoprotein that adopts an elongated, unstructured shape in solution (72). In Lewy bodies, however,  $\alpha$ -synuclein — the prominent structural component — is present in aggregated and insoluble filaments that are hyperphosphorylated and ubiquitinated (73, 74). It is likely that the abnormal aggregation of  $\alpha$ -synuclein into a toxic, misfolded form contributes to neuronal cell death in both overexpressed wild-type and missense mutated proteins (75, 76). Factors such as the presence of a pathologic  $\alpha$ -synuclein mutation, oxidative and nitrosative stress, phosphorylation, mitochondrial and proteasomal dysfunction, as well as dopamine can influence aggregation and folding of  $\alpha$ -synuclein into a variety of forms including protofibrils, fibrils, and filaments.  $\alpha$ -Synuclein is normally processed and cleaved at its C terminus by unidentified synucleinases (77). Truncation of  $\alpha$ -synuclein appears to correlate with disease severity and with its propensity to oligomerize. It appears that the protofibrils and fibrils are the most toxic forms, and the creation and stabilization of these forms by mutation or cellular milieu may be a central pathologic mechanism (Figure 1).

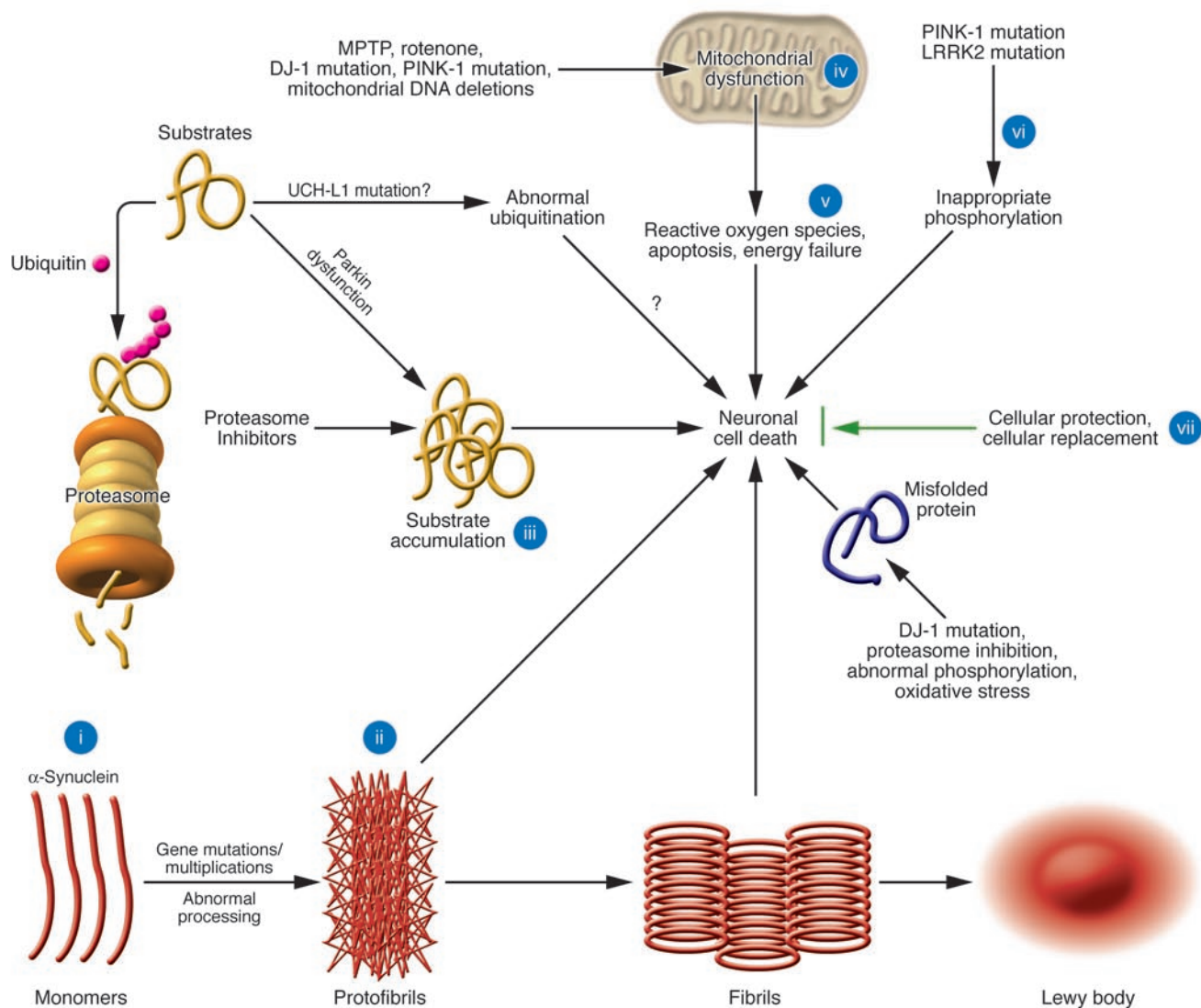
$\alpha$ -Synuclein likely is involved in synaptic vesicle function (78), and its intracellular distribution and metabolism involve axonal transport (79) and degradation via the autophagic and proteasomal systems (80). Recent studies suggest that it may act as a co-chaperone with cysteine-string protein  $\alpha$  in the maintenance of nerve terminals (81). The association of  $\alpha$ -synuclein with vesicles has led to speculation that the oligomerized protein may rupture cellular membranes through a pore-forming mechanism, leading to neurotransmitter leakage and toxicity (82). Other theories suggest that the abnormally aggregated protein may inhibit a range of normal cellular functions, such as axonal transport and protein turnover, via the ubiquitin-proteasomal or chaperone-mediated autophagic systems (83–85). Methods to interrupt synuclein aggregation by reducing its expression, increasing its degradation, impairing the formation of toxic aggregates, or inhibiting its truncation are logical therapeutic targets and are actively being explored (84).

#### Parkin and the role of the ubiquitin-proteasomal system

Mutations in the gene encoding parkin cause a form of autosomal-recessive, early-onset PD (86). Affected patients, though Parkinsonian and highly responsive to levodopa, do show several less typical features including early age of onset, prominent dystonia, severe motor fluctuation, and a more symmetric onset of symptoms (87, 88). Though initially described as a recessive disorder, there is controversial evidence that possessing a single parkin mutation does make one more likely to develop PD and show evidence of nigrostriatal dysfunction on imaging studies (89–92). Moreover, there are certain missense mutations that seem to be inherited in an autosomal-dominant manner (89).

Mutations in the gene encoding parkin are the most common genetic cause for early-onset PD, with prevalence rates approaching 50% for those with an autosomal-recessive family history and perhaps 18% of all those developing early-onset disease (less than 45 years of age) without a clear family history (93). Patients with parkin-related PD show loss of nigrostriatal and locus ceruleus neurons, but, with rare exception, do not develop classic Lewy bodies (94–96).

Parkin is an E3 ligase that participates in the addition of ubiquitin molecules to target proteins, thereby marking them for proteasomal degradation (97). Loss of normal parkin function is postulated to lead to the abnormal accumulation of toxic substrates and resultant cell death. Numerous putative parkin substrates



**Figure 1**

Model of dopaminergic cell death and possible sites for therapeutic intervention in PD. Studies on inherited forms of PD have led to the identification of genes that, when mutated, lead to dopaminergic cell loss. These genes are involved in a variety of cellular processes that include protein ubiquitination and degradation via the proteasome, response to oxidative stress, protein phosphorylation, mitochondrial function, and protein folding. Potential points of therapeutic intervention are highlighted: gene silencing therapies to reduce synuclein levels (i); inhibitors of synuclein aggregation and/or processing (ii); interventions to downregulate toxic substrates or upregulate parkin or proteasomal function (iii); interventions to enhance mitochondrial function with factors such as CoQ10, DJ-1, or PINK-1 (iv); free radical scavengers and antioxidants (v); kinase inhibitors to block LRRK2 activity or interventions to increase PINK-1 function (vi); and other therapies using trophic factors such as GDNF, survival genes, or fetal/stem cell replacement that would protect or replace susceptible cells (vii).

have been identified (75). The candidate substrates CDCrel-1, CDCrel-2, Pael-R, cyclin E, p38/JTV-1 (also known as AIMP2), and far upstream element-binding protein-1 (FBP-1) appear to accumulate in patients with parkin-associated PD, but only p38/JTV-1 and its interacting partner, FBP-1, accumulate in sporadic PD as well as in parkin knockout mice, suggesting that these proteins are true parkin substrates (98, 99).

Additional evidence for the importance of the ubiquitin-proteasomal system in PD pathogenesis comes from genetic association studies implicating the gene encoding ubiquitin-specific protease 24 (100). Moreover, mutations in ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in 2 individuals from a single

affected German family (101) provide further support for the role of the ubiquitin-proteasome system in PD. The ability of mutations in this gene to cause or increase the risk of PD is highly controversial, and no additional families have been reported (75, 102, 103). Indeed, recent studies suggest that UCH-L1 is not a PD susceptibility gene (75, 102, 103). The exact function of UCH-L1 is unclear, but it does involve ubiquitin hydrolase and ligase activities, and, interestingly, the protein is localized to Lewy bodies (see ref. 75). In addition, UCH-L1 can promote an alternative form of synuclein ubiquitination that uses the lysine 63 (K63) residue of ubiquitin to form polyubiquitin chains (104). This linkage is not directly related to proteasomal



degradation, but may be involved in  $\alpha$ -synuclein inclusion formation as well as in the function of parkin (105). The role of K63 ubiquitination in the development of inclusions and ultimately the pathogenesis of PD and other ubiquitin-positive neurodegenerative diseases is a promising field of study.

The link between sporadic PD and proteasomal dysfunction is supported by studies showing that parkin is nitrosylated in sporadic PD patients and that this modification impairs parkin's E3 ligase activity (106). Moreover, dopamine also may directly inhibit this activity through covalent modification (107). Additional evidence for the importance of the proteasome comes from pathologic studies that have found decreased proteasomal activity and expression in the SN of PD patients and the intriguing, but controversial, finding that exposure to proteasomal toxins can induce PD-like pathology in animal models (47, 108, 109). Interventions that increase proteasomal function or perhaps induce parkin expression may be a means of neuroprotection in PD.

### DJ-1 and the role of oxidative stress

Mutations in the gene encoding DJ-1 similar to those in parkin lead to early-onset, autosomal-recessive PD (110). Clinically, patients are levodopa responsive with asymmetric onset of symptoms, slow progression, and variable severity. Behavioral, psychiatric, and dystonic features occur in patients with DJ-1 mutations (111). The prevalence of DJ-1 mutations is likely less than 1% in the early-onset patient subgroup (112).

The exact function of the DJ-1 protein is unknown, though evidence suggests it may act as an antioxidant, oxidation/reduction sensor, chaperone, and/or protease (75). Pathologically DJ-1 is not present in Lewy bodies, though it colocalizes with  $\alpha$ -synuclein in other neurodegenerative diseases such as multiple system atrophy and Alzheimer disease (113). Some fraction of the protein is found in mitochondria (114). Oxidized and insoluble forms of the protein accumulate in the brains of patients with sporadic PD (113, 115). Interestingly, DJ-1 can interact with parkin *in vitro* under conditions of oxidative stress (115). In addition, when in a particular oxidized state, DJ-1 interacts with  $\alpha$ -synuclein to prevent formation of fibrils (116). This latter activity suggests that DJ-1 acts as a redox-sensitive chaperone that protects cells from  $\alpha$ -synuclein misfolding and toxicity under conditions of oxidative stress. *In vivo* studies have documented increased susceptibility of mice deficient in DJ-1 to the toxic effects of MPTP, and in culture, neurons from these mice were more sensitive to oxidative stress than those expressing DJ-1 (117). In addition, similar to parkin, DJ-1 is susceptible to protein S-nitrosylation that may be involved in the control of protein activity (118). There is speculation as well that DJ-1 functions as a regulator of apoptosis by modification of PTEN function and through interactions with several apoptosis-regulating proteins (119). The biology of DJ-1 links synuclein and parkin function with the phenomenon of oxidative stress, apoptosis, and the mitochondrion — all of which play a role in the pathogenesis of PD.

### PINK-1 and the role of the mitochondrion

Mutations in the gene encoding PTEN-induced putative kinase 1 (PINK-1) are a cause of early-onset, autosomal-recessive PD (120). The clinical phenotype of point mutations is difficult to distinguish from other forms of early-onset disease, whereas a deletion mutation can induce a broader phenotype including dystonia and cognitive impairment (121). In general, patients with PINK-1

mutations show onset at an average of 35 years, slow progression, and levodopa responsiveness. The phenotype of PINK-1-, parkin-, and DJ-1-associated PD are indistinguishable, and atypical features may be a result of early onset rather than the etiology of disease (122). Heterozygous PINK-1 mutations were found in a cohort of early-onset patients in excess of the number seen in controls (123). This suggests that possessing a single PINK-1 mutation may predispose an individual to PD. In addition, imaging studies have demonstrated altered dopaminergic function in single PINK-1 mutation carriers (124). The prevalence of PINK-1 mutation is between that of parkin and DJ-1 and is present in the homozygous state in 2–3% of early-onset patients (122, 125).

PINK-1 is a mitochondrial protein kinase whose substrates are unknown (S1). PINK-1 is induced by PTEN, the same protein whose activity is suppressed by DJ-1. PINK-1 mutation may lead to mitochondrial dysfunction and increased sensitivity to cellular stress through a defect in the apoptosis pathway (S2). PINK-1 appears to play an important role in mitochondrial function, as *Drosophila* lacking PINK-1 have substantial mitochondrial defects resulting in apoptotic muscle degeneration and male sterility. Interestingly, parkin rescues the PINK-1 loss-of-function phenotype, suggesting that parkin and PINK-1 function in a common biochemical pathway (S3–S5).

The presence of PINK-1 and DJ-1 in the mitochondrion underscores the role that this cellular organelle plays in PD pathogenesis. A common feature of sporadic PD is evidence of complex I mitochondrial dysfunction (119). This component of the electron transport chain also is affected by rotenone and MPTP, 2 toxins whose effects can model PD (S6). Rotenone, when given chronically to animals by infusion, produces SN cell and dopaminergic fiber loss with  $\alpha$ -synuclein accumulation and the formation of Lewy body-like inclusions (S7). Similarly, MPTP exposure in both patients and animal models leads to nigral cell loss and Parkinsonian symptoms (S6) and under conditions of chronic administration leads to the formation of  $\alpha$ -synuclein-containing inclusions (S8). Furthermore, animals deficient in  $\alpha$ -synuclein are resistant to MPTP, implying the participation of this protein in the pathogenic mechanism of MPTP (71). Additionally, the toxins 6-hydroxydopamine and paraquat produce dopaminergic cell damage by inducing oxidative stress that may mimic the mitochondrial toxicity seen with rotenone and MPTP (S9).

Two recent studies provide a clue as to how oxidative stress may lead to cellular dysfunction in PD. These studies showed that in PD, SN neurons accumulate mitochondrial DNA deletion mutations at an abnormally high rate (S10, S11). The deletion in each individual cell appears clonal and likely results from a single mutation event and subsequent expansion of the affected mitochondrion. The authors suggest that this mutation load is sufficient to cause impaired cellular respiration, as determined by the loss of cytochrome *c* oxidase staining. These findings suggest a possible mechanism that begins with increased oxidative stress in the SN and leads to mitochondrial DNA mutation and subsequent failure of mitochondrial function. The accumulation of impaired mitochondria within a cell in turn leads to respiratory chain deficiency and SN cellular pathology. Interestingly, a mutation in the polymerase responsible for mitochondrial DNA replication has been associated with the accumulation of deletions in mitochondrial DNA, SN cell loss, and early-onset Parkinsonism (S12). The role that mitochondrial dysfunction and oxidative stress play in PD patho-



genesis has led to trials of antioxidant and promitochondrial compounds, including coenzyme Q10 (CoQ10), vitamin E, and creatine, as possible neuroprotectants in the disease.

### LRRK2 and phosphorylation

Mutation in the gene for LRRK2 (also called dardarin) was identified in 2004 as a cause of autosomal-dominant PD (S13, S14). Most cases of LRRK2-related disease show typical late and asymmetric onset of symptoms, levodopa responsiveness, and other features that are indistinguishable from sporadic disease, although rarely, early onset, amyotrophy, gaze palsy, dementia, and psychiatric symptoms are observed (S13–S15). The prevalence of LRRK2 mutations in familial and sporadic PD is highly dependent on the particular study population and the nature of the mutation. In a Spanish cohort, 5.3% of all PD patients (9.6% of those with a family history) tested positive for the mutation, with G2019S being most common (4.3% of all patients; 6.4% of PD patients with a family history) (S15). The R1441G mutation was found in 8% of PD patients from the Basque region of Spain (S14), but only 0.7% of a more diverse Spanish cohort (S15). Remarkably, the G2019S mutation was found in 18.3% of all PD cases from an Ashkenazi Jewish cohort and in 30% of those with a family history (37). The lack of a family history in many of these patients suggested an estimated penetrance rate of 32%. Even more striking is the finding that in a North African Arab PD cohort, the prevalence of the G2019S mutation was approximately 40% in both sporadic and familial cases, again suggesting possible reduced penetrance (38). Mirroring sporadic PD, the prevalence rate of the G2019S mutation increases with age, reaching 25% by age 55 and 85% by age 70 (S16). Autopsy material from the majority of affected patients demonstrates typical PD pathology, including SN and locus ceruleus cell loss with Lewy bodies and Lewy neurites. Atypical pathology also occurs and likely is more common with particular mutants such as R1441C (S13, S17). This pathology has included the absence of Lewy bodies and the presence of synuclein-negative, ubiquitin-positive inclusions, LRRK2-filled neurites, and abnormal tau pathology, the latter of which is suggestive more of progressive supranuclear palsy than of PD (S13). Additionally the Y1699C mutation may lead to abnormal pathology including features similar to motor neuron disease, though a subsequent report found typical PD pathology in a patient carrying the same mutation from another family (S13, S18).

LRRK2 is a large protein that includes Roc (Ras in complex protein), COR (C-terminal of Roc), leucine-rich repeat, mixed lineage kinase, WD40 (a putative protein-protein interaction domain terminating in a tryptophan-aspartic acid dipeptide), and ankryin domains (S13, S14, S19). Function and localization data for this protein is limited, but there is evidence that it is cytoplasmic, associated with mitochondria, and capable of autophosphorylation (S20). Recent data suggests that an increase in LRRK2 kinase activity plays a role in the pathogenesis of at least 3 of the known mutations (S20, S21). In addition, LRRK2 binds to parkin and leads to increased protein aggregation and ubiquitination in a cell culture model, and disease-associated LRRK2 mutants are toxic to SH-SY5Y cells and cortical neurons (S22). Furthermore, LRRK2 may, as do other WD40 proteins, participate in mitochondrial fission or interact with the cytoskeleton, suggesting a role in membrane, vesicular, or protein trafficking (S23).

The biology of LRRK2 and PINK-1 adds to other data suggesting that protein phosphorylation plays a vital role in PD pathogenesis. Clearly if abnormal kinase activity is responsible for LRRK2-mediated

disease, kinase inhibitors, particularly LRRK2 kinase inhibitors, may have therapeutic relevance. Protein phosphorylation involving the mixed lineage kinase pathway may play a role in PD and remains an important therapeutic target despite the recent failure of the kinase inhibitor CEP-1347 in clinical trials (S24). In addition, abnormal phosphorylation of proteins such as  $\alpha$ -synuclein is seen in PD and in models of the disease (S25), suggesting that inhibitors of  $\alpha$ -synuclein phosphorylation may be therapeutic. Finally, the neurotrophic effects of glial cell line-derived neurotrophic factor (GDNF) on dopaminergic neurons involve the activation of the protein kinase Ret and a myriad of downstream targets including Akt that are important in a cell's response to oxidative stress and to DJ-1 function (S26, S27). These data suggest that other inducers of Akt signaling may be therapeutic.

### Symptomatic therapy

Current medical and surgical therapies for PD are symptomatic and lack significant disease-modifying effect. Indeed the most effective medical therapy continues to be levodopa mixed with a peripheral decarboxylase inhibitor (S28–S30). The most recent advances in available symptomatic medical therapies in the United States have revolved around prolonging the effect of levodopa through the use of COMT inhibition and by changing the availability and formulation of older medications such as selegiline and apomorphine. The latter, given subcutaneously with an oral antiemetic, can temporarily rescue patients during disabling motor “off” time (time spent with reduced levodopa effect) (S31). Looking beyond dopaminergic therapy, other symptomatic medical therapies showing promise in published clinical trials include istradefylline, an adenosine A2 antagonist that reduces “off” time (S32), and sarizotan, a serotonin agonist that reduces levodopa-induced dyskinesias (S33, S34).

There are data suggesting that deferring levodopa therapy in favor of dopamine agonists such as ropinerole or pramipexole may delay motor complications such as dyskinesias (S29, S30). The dopamine agonists are less effective than levodopa in symptomatic relief, and nearly all patients with advancing PD will require levodopa at some point. The reduced risk of dyskinesias with the dopamine agonists may be related to their longer half-life, and thus more stable receptor stimulation, relative to levodopa. Supporting these data are studies showing that continuous infusion of levodopa or an agonist has benefit over oral interval dosing (S35–S37). These findings have led to the development of experimental therapies including the transdermal patch (for delivery of rotigotine) and infusion delivery systems (for Duodopa) that reduce pulsatile drug delivery. In addition, this concept has given rise to the theory that COMT inhibitors given with levodopa may reduce the risk of future dyskinesias by prolonging brain exposure to a given levodopa dose (S38).

Surgical DBS is perhaps the most influential development in symptomatic PD therapy since levodopa and is reviewed in detail elsewhere (S39). In this procedure, an electrode is inserted through the skull to reach and stimulate the globus pallidus, subthalamic nucleus (STN), or ventral intermediate thalamus. A pacemaker-like device is implanted and connected to the electrode through wires buried beneath the skin. The results of this therapy can be quite marked and include the reduction of “off” time, increased “on” time without dyskinesias, reduction of levodopa dose, and improved tremor. The STN has recently emerged as the preferential target due to improved symptom control and a reduced energy



requirement (S40). Currently this procedure is largely reserved for advanced cases of PD in which motor complications and/or medication intolerance have led to an unacceptable decline in quality of life. In addition, disabling tremor that is not responsive to medical therapy may respond well to DBS. Very encouraging are the recent, albeit small, studies showing the benefits of electrode implantation into the pedunculopontine nucleus with resultant improvement in bradykinesia, gait freezing, and postural stability (S41).

A newer, experimental field of study is the use of transcranial magnetic stimulation (TMS). In this process a current is passed through a coil to generate a magnetic field. The coil is placed near the head to induce stimulation in the nearby brain structures. TMS studies show that PD patients have a measurable abnormality in the inhibitory control of the cortex that results in a shortened "silent period." Also seen are inconsistently measurable changes in motor threshold and abnormal activation during voluntary input (S42). Some of these abnormalities normalize with medical and surgical PD therapies. The effect of repetitive TMS (rTMS) is now being explored as a possible therapeutic intervention. A meta-analysis of available data has shown a small but significant effect of rTMS (approximately 20% improvement on the motor Unified Parkinson's Disease Rating Scale [UPDRS]). Other large studies have failed, prompting concern regarding the standardization of technique. In addition, a measurable placebo effect, as detected by PET scan, can be seen with rTMS that may be confounding study results (S43). A well-defined protocol including rTMS applied while in the "on" state, inclusion of a placebo control, and specific parameters for intensity and frequency of stimulation was used in a recent study, showing improvement of limb bradykinesia and gait for at least one month after a course of rTMS therapy (S44). The magnitude of improvement was thought to be similar to the effect of a single levodopa dose. These results require duplication, and the cumulative effect and clinical significance of this therapy must be subjected to further study before rTMS can be recommended for routine treatment. The paucity of adverse effects of rTMS does make the optimization of this treatment an attractive field of study.

A review of symptomatic PD therapies would not be complete without consideration of progress made in the treatment of non-motor sequelae. Given the good response of motor symptoms to medical and surgical therapies, it is often poor balance, sleep interruption, cognitive impairments, anxiety, depression, and drooling that become most disabling (S45). Indeed the recognition of widespread pathology in PD suggests that these affected areas lying outside of the dopaminergic motor pathway are contributing to patients' symptoms. Specifically, abnormalities in the noradrenergic and serotonin nuclei may lead to anxiety and depression as well as the autonomic, sleep, and visual disturbances seen in PD, while changes in the neocortex, limbic system, and cholinergic nucleus basalis may be involved in cognitive decline later in the disease. Treatment of these symptoms can be rewarding and involves interventions including agents such as midodrine and pyridostigmine for blood pressure support (S46), atropine drops for symptomatic control of salivation (S47), cholinesterase antagonists for cognitive decline (S48), antidepressants (serotonin selective reuptake inhibitors and possibly others) for treatment of depression (S49), and atypical antipsychotics (most commonly clozapine and quetiapine) for treatment of psychosis (S50). A recent study by Ondo et al. has cast some doubt on the effectiveness of

quetiapine for the treatment of psychosis, underscoring the need for more rigorous trials of therapies aimed at the treatment of nonmotor PD symptoms (S51).

### Neuroprotection and future therapies

The search for compounds that can slow or halt the progression of PD is an active area of clinical research. The neuroprotective properties of selegiline, an MAO-B inhibitor, continue to be debated, with studies showing that even after years of treatment, individuals receiving selegiline appear to demonstrate slower PD progression (S52, S53). Rasagiline is an irreversible MAO-B inhibitor that likely works via several pathways to provide symptomatic relief, but may also slow disease progression (S54, S55). Like selegiline, rasagiline's symptomatic effect has made it difficult to convincingly extract neuroprotective data, and the magnitude of this effect will likely rely on further observation. The protective effect of both selegiline and rasagiline may occur through prevention of the GAPDH cell death cascade by blocking the S-nitrosylation of GAPDH, the binding of GAPDH to Siah (a protein E3 ligase that aids in the translocation of GAPDH to the nucleus), and the subsequent Siah-mediated degradation of nuclear proteins that leads to cell death (S56).

Another neuroprotective candidate is CoQ10. It was hypothesized that this antioxidant and electron transport chain component might correct the mitochondrial complex I dysfunction and CoQ10 deficiency seen in PD patients. In a pilot study CoQ10 appeared to slow disease progression at the highest dose of 1,200 mg/d, although the subject numbers were small and the results await confirmation (S57). Based on a futility study design, the antiinflammatory, anticaspase drug minocycline and the mitochondrial compound creatine were both recently deemed worthy of future consideration as neuroprotectants in PD (S58).

Mixed results have been obtained in looking at the disease-modifying effects of GDNF. This potent dopaminergic neuron survival factor was infused into PD patients in 3 recent studies. A double-blind, placebo-controlled study failed to demonstrate clinical improvement and was discontinued due to lack of efficacy, the development of antibodies in patients, and safety concerns raised over the development of cerebellar toxicity in a group of exposed nonhuman primates (S59). Two prior open-label studies showed a beneficial effect of infusion, and the reason behind the disparity needs further investigation. In addition, GDNF and related compounds are being examined for efficacy using alternative means of delivery including implantation of capsules, engineered cells, and viral vectors.

One step beyond neuroprotection is cell replacement therapy, wherein cells lost in PD are replaced. There have been mixed results in clinical trials attempting this strategy: some open-label studies showed benefit, while 2 double-blind, placebo-controlled studies failed to meet their primary endpoints and noted the worrisome development of dyskinesias (S60–S63). In these studies, ventral midbrain tissue was isolated from fetal human tissue and ectopically transplanted into the striatum of PD patients. Autopsy and imaging studies do verify that the transplanted tissue can survive and functionally integrate. Studies continue to explore the reasons for variable clinical response with possible explanations involving surgical technique, level of immunosuppression, cell preparation and survivability, and patient selection (S64). In addition, these cells lack normal synaptic input since they are not properly localized to the SN. A fully integrated graft may need



to be placed in the SN and recapitulate functional connectivity to the striatum. Until these problems are addressed and better understood, fetal cell transplantation cannot be recommended as a routine therapeutic option.

The isolation of human embryonic stem cells has provided a potential source for transplantation material. Over the past few years a good deal of effort has gone into developing protocols to induce the proper dopaminergic characteristics in these undifferentiated cells to make them suitable candidates for transplant. Several protocols exist that can induce a dopaminergic phenotype, but poor cell survival after transplant into animal models has led to disappointing results (S65). Several other roadblocks stand in the way of stem cell therapy, including concerns regarding exposing cells to xenogenic factors during the expansion and differentiation phases, the possibility of tumor formation if cells are not properly differentiated, the possibility of tissue rejection, and purification of the transplanted cells. In addition, there is the concern that even if ample numbers of the appropriate cells are produced, these cells may suffer from the same difficulties seen with fetal cell transplants. Finally, there is little reason to believe that transplantation of dopaminergic cells will alleviate the symptoms related to the degeneration seen in non-nigrostriatal brain areas and with other neurotransmitter types. Despite these caveats, if a way can be found to reliably reproduce the symptomatic benefit seen in some fetal transplant studies, the availability of large numbers of differentiated stem cells may one day make such transplantation a very attractive therapy.

### Conclusions

Just as landmark discoveries that identified PD as a disease of dopamine deficiency led to the development of rational symptomatic therapies such as levodopa and dopamine agonists, so

will the understanding of disease mechanisms spurred on by the study of genetics lead to novel neuroprotective and restorative therapies. The identification of monogenetic forms of PD has uncovered a role for proteasomal and mitochondrial dysfunction, oxidative stress, protein misfolding, and aberrant phosphorylation in the pathophysiology of PD. While the interplay and temporal relationship among these pathologic processes are unclear at present, further research to determine which factors are most proximate to the cell death process and which are most amenable to pharmaceutical intervention are the challenges for the future.

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