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REVIEW

Missing pieces in the Parkinson's disease puzzle

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Parkinson's disease is a neurodegenerative process characterized by numerous motor and nonmotor clinical manifestations for which effective, mechanism-based treatments remain elusive. Here we discuss a series of critical issues that we think researchers need to address to stand a better chance of solving the different challenges posed by this pathology.

Parkinson's disease is often considered to be a simple pathological process that involves selective degeneration of the nigrostriatal pathway and a concomitant reduction in the striatal concentration of dopamine. This model has guided the development of the existing pharmacological treatments for Parkinson's disease and the search for new ones. The supposed simplicity of the underlying pathology has also led to the view that we are close to finding disease-modifying, as opposed to symptom-alleviating, treatments for the disease. However, this optimism has been tempered by the recognition that many nonmotor features of Parkinson's disease relate to the degeneration of nondopaminergic transmitter systems[1]. This observation, together with the fact that drugs such as levodopa—the mainstay of Parkinson's disease therapy—lose efficacy and cause dyskinesias and behavioral abnormalities in many patients, means that many people with Parkinson's disease ultimately develop both motor and nonmotor problems that result in a marked reduction in quality of life.

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Instead of providing a review of the field, which is already available elsewhere[2–6], here we focus on identifying key aspects of the onset and progression of Parkinson's disease and discuss emerging therapeutic options, highlighting what we believe to be critical areas of future research.

Onset and progression—clinical questions

Is Parkinson's disease a single disorder? The traditional view of Parkinson's disease as a single clinical entity is under scrutiny[7,8]. Clinically, the disease is heterogeneous, and subtypes may be recognized on the basis of age of onset, predominant clinical features and progression rate. Two major clinical subtypes exist: a tremor-predominant form that is often observed in younger people, and a type known as "postural imbalance and gait disorder" (PIGD) that is often observed in older people (>70 years old) and is characterized by akinesia, rigidity, and gait and balance impairment. In very general terms, the first subtype leads to a slow decline of motor function, whereas the latter worsens more rapidly[8].

More refined studies that analyze the effect of genetic and environmental factors on clinical presentation may lead to the identification of further subtypes that could allow us to stratify subjects during clinical studies and, eventually, to start thinking about personalized therapies for the disease.

When does Parkinson's disease begin? Although Parkinson's disease is classically diagnosed by the insidious onset of motor manifestations, the concept of premotor Parkinson's disease has gained support[7,9]. There is increasing evidence that olfactory dysfunction, sleep abnormalities, cardiac sympathetic denervation, constipation, depression and pain may antedate the onset of motor signs of Parkinson's disease[10].

It would be very informative to perform longitudinal studies of individuals who do not have motor signs of Parkinson's disease but show the full constellation of premotor signs, as they could be thought of as high-risk candidates to develop the disease. Such longitudinal studies not only will enhance our basic understanding of disease onset and progression but also may provide us with biomarkers that would enable us to start therapeutic intervention much earlier than is currently possible.

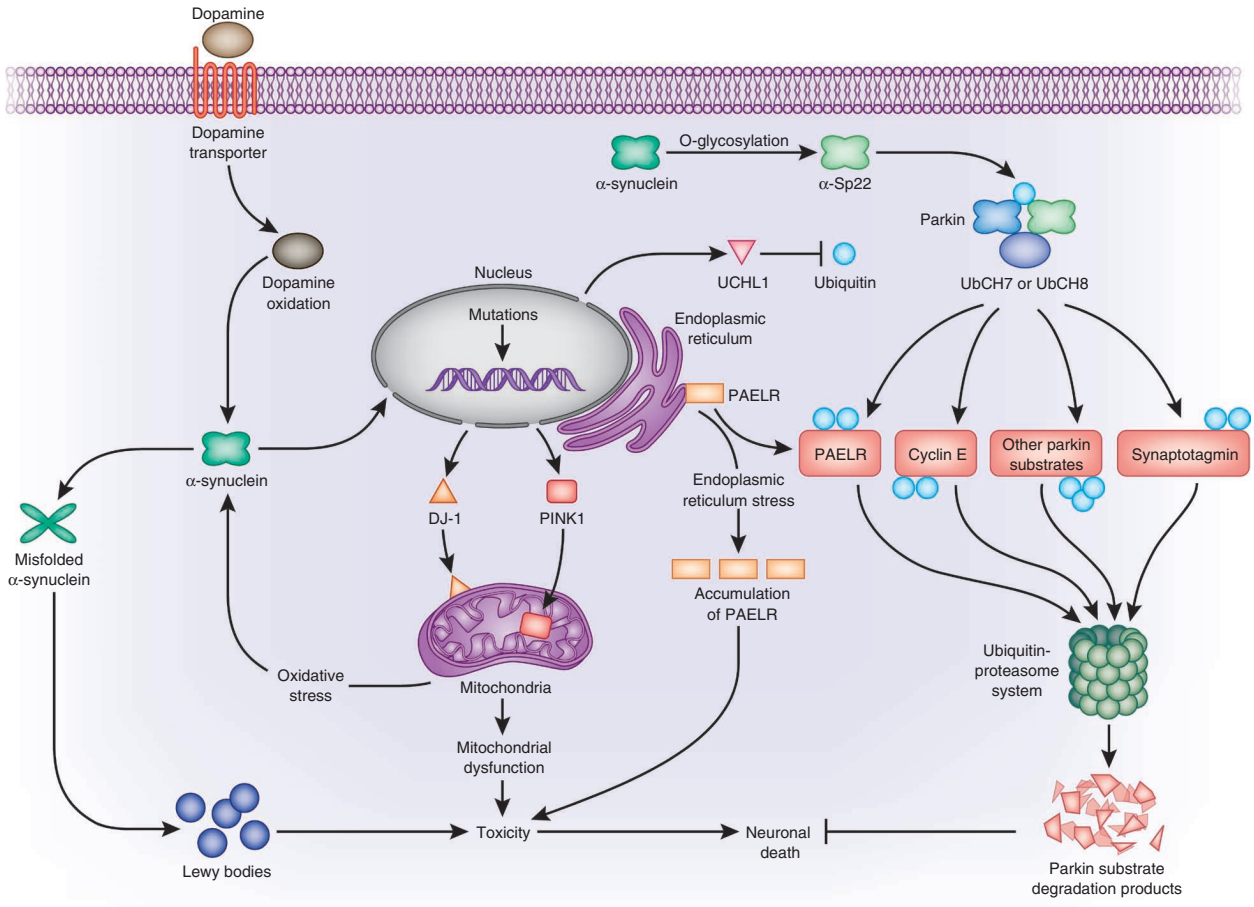

Figure 1 Schematic summary of established etiopathogenic mechanisms and interactions in the dopaminergic cells of the substantia nigra in Parkinson's disease. Cell death may be caused by α-synuclein aggregation, proteosomal and lysosomal (not shown) system dysfunction, and reduced mitochondrial activity. Gene mutations are associated with impairment of one or several of these mechanisms. In addition, secondary changes (not shown) such as excitotoxicity and inflammation are likely to play a relevant role in progressive neuronal degeneration. α-Sp22, a 22-kilodalton glycosylated form of α-synuclein; PAELR, parkin-associated endothelin receptor-like receptor; UbCH7, ubiquitin-conjugating enzyme 7; UbCH8, ubiquitin-conjugating enzyme 8; UCHL1, ubiquitin carboxy-terminal hydrolase L1.

What factors determine disease progression? Long-term longitudinal studies suggest that the rate of decline in motor function in Parkinson's disease is not linear; it is faster in subjects with very mild motor impairment than in those with marked impairment at first evaluation[9,11]. Age is the best predictor of Parkinson's disease progression rate and remains the most prominent risk factor for developing the disease[12]. Cognitive impairment is also more frequent and begins earlier in individuals who are older at symptom presentation (>70 years old)[13]. Although the interplay between aging and Parkinson's disease is confounded by comorbidities that normally occur in the elderly, statistical methods might control for these issues and tease apart the role of normal aging in Parkinson's disease outcomes.

What are the primary factors that cause disability in Parkinson's disease? Dopaminergic drugs and functional neurosurgery largely reverse the classic motor features of Parkinson's disease—tremor, rigidity and akinesia. As a result, most of the disability caused by Parkinson's disease relates to symptoms such as gait dysfunction, loss of balance, swallowing and speech difficulties, autonomic disturbances and cognitive decline, which are less influenced by available therapies (**Box 1**)[11]. Indeed, dementia is considered to be the major long-term cause of disability in people with Parkinson's disease and may be found in 30–80% of affected individuals[13–15]. The shift in focus from the classic motor features to other motor and nonmotor factors has followed from strong evidence that, under chronic dopaminergic treatment, most people with long-term disease remain in stages I–III of the Hoehn and Yahr scale[16], a common staging system to describe the progression of Parkinson's disease. So, whereas progression of motor dysfunction stabilizes in the long term[16], disability increases, shifting from motor to nonmotor features[15]. Studies on the pathological mechanisms of the nonmotor symptoms are urgently required.

Moving forward. As the two major clinical subtypes of Parkinson's disease differ in their rates of progression, the identification of factors that account for these differences is more likely to emerge from studying samples enriched in each subtype. Moreover, as new tools are developed to image transmitter systems, it will become possible to longitudinally examine the involvement of nondopaminergic systems in the nonmotor symptoms of the disease. Recent advances in understanding the relative roles of striatofrontal pathways and hippocampal circuitry[17,18] provide new opportunities to evaluate progressive cognitive decline in Parkinson's disease through neuropsychological, anatomical and neuroimaging methods. A similar

BOX 1 Treatments for Parkinson's disease—2010

CURRENT SYMPTOMATIC THERAPIES

Oral medications

Levodopa + a dopadecarboylase inhibitor ± a catechol-O-methyltransferase inhibitor
Dopamine agonists, including slow-release formulations such as ropinirole, pramipexole
Monoamine oxidase B inhibitors: for example, selegiline, rasagiline
Anticholinergics: for example, trihexyphenidyl
Antiglutamatergics: for example, amantadine

Continuous delivery therapies

Dopamine agonists: subcutaneous or intravenous, such as apomorphine and lisuride
Transdermal patch: for example, rotigotine
Intraduodenal levodopa: for example, Duodopa

Surgical therapies

Deep brain stimulation of the subthalamic nucleus, globus pallidum pars interna
Lesions: for example, subthalamotomy, pallidotomy

FUTURE SYMPTOMATIC THERAPIES, INCLUDING ANTI-DYSKINETICS

Partial dopamine agonists: for example, pardoprunox
Adenosine A2a antagonists
Safinamide—MAOB inhibitor, anti-glutamatergic and sodium-channel blocker
Zonisamide—MAOB inhibitor, glutamate release blocker
mGluR5 antagonists
Alpha-adrenoreceptor antagonists: for example, fipamexole
AMPA antagonists: for example, perampanel, talampanel
5HT2A partial agonists: for example, pimavanserin

PUTATIVE NEUROPROTECTIVE DRUGS IN CLINICAL TRIALS

Pramipexole—dopamine agonist
Coenzyme Q10—respiratory-chain enhancer and antioxidant
Creatine—ATP synthesis enhancer
Green tea polyphenol—antioxidant
Inosine—urate elevator
Isradipine—calcium channel blocker
Cogane—GDNF, BDNF synthesis stimulator

holistic strategy can be feasibly developed to study other nonmotor problems to further define Parkinson's disease subtypes.

Onset and progression—basic questions

Does a unifying mechanism account for neurodegeneration? After decades of research, a single cause for Parkinson's disease has not been found and is unlikely to emerge. Whereas some forms of Parkinson's disease are genetic, most cases are idiopathic, and the underlying environmental causes (if any) remain to be discovered. Intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and postencephalitic parkinsonism are the only examples of neuronal degeneration in the dopaminergic substantia nigra pars compacta (SNc) that are clearly induced by environmental factors, but neither one fully reproduces the clinical and pathological features of true Parkinson's disease. Moreover, causative factors may differ among individuals with different clinical subtypes of the disease.

An emerging concept is that SNc homeostasis is vulnerable to different genetic, cellular and environmental factors that independently or concomitantly cause cell death over time[19,20]. These factors may lead to mitochondrial dysfunction and oxidative stress, to abnormal protein degradation due to alterations in the ubiquitin system or in chaperone-mediated autophagy, and to other forms of subcellular dysfunction (**Fig. 1**). Combined, these alterations can precipitate cell death. Which (if any) of these mechanisms is more important to disease pathogenesis is not known.

Epidemiological data showing that consumption of coffee, tobacco and nonsteroidal anti-inflammatory drugs reduces the risk of Parkinson's disease are intriguing[21]. Combined with better stratification of Parkinson's disease subjects in clinical studies, as indicated above, epidemiological observations[22,23] may provide insights into the causal mechanisms that trigger the disease.

How do mutations help us understand the disease? There is a growing list of mutations linked to Parkinson's disease. They account for 2–3% of the late-onset cases and ~50% of early-onset forms[24,25]. Typical, late-onset Parkinson's disease with Lewy body pathology is linked to mutations in three genes: *SNCA* (encoding α-synuclein), *LRRK2* (encoding leucine-rich repeat kinase 2) and *EIF4G1* (elongation initiation factor 4G1; M.F., unpublished data). Missense mutations in *SNCA* were first linked to familial parkinsonism with late onset[26], and subsequent *SNCA* duplications were found in kindreds in which age of onset, progression and associated comorbidities relate to gene dosage[27,28]. In particular, the development of nonmotor features correlates with α-synuclein gene copy number as well as gene and protein expression[29]. These studies suggest that increased neuronal α-synuclein protein levels are a primary factor in the disease. The causes and consequences of α-synuclein aggregation in neurons are not yet fully understood, despite a large number of molecular studies[30]. Mutations in and overexpression of α-synuclein seem to be especially toxic to dopaminergic neurons, as dopamine-synuclein adducts may inhibit chaperone-mediated autophagy[31]. Even with the limited mechanistic insight currently available, reduction of α-synuclein expression may represent a potential therapeutic approach[32].

Mutations in *LRRK2* represent the highest risk of familial and, seemingly, sporadic Parkinson's disease[33,34]. Among mutation carriers, disease penetrance markedly increases as a function of age[35]. LRRK2 is a large protein and contains both Rab GTPase and kinase enzymatic activities, as well as other domains suggestive of a multimeric protein scaffold[36]. In model organisms, it has numerous roles within the secretory pathway, and it may contribute to adult neurogenesis, remodeling of cytoskeletal architecture and membrane dynamics, and dopaminergic signaling[36].

Recently identified point mutations affecting eIF4G1 act in a dominant-negative fashion to perturb complex assembly, eIF4E or eIF3e binding, and subsequent recruitment of the 40S ribosome for 5′ cap–dependent mRNA translation (M.C. Chartier-Harlin *et al.*, unpublished data presented at the XVIII WFN World Congress on Parkinson's Disease and Related Disorders, Miami, Florida, USA, December 2009). Pathogenic eIF4G1 mutations are rare but can affect families with late-onset Lewy body disease within many

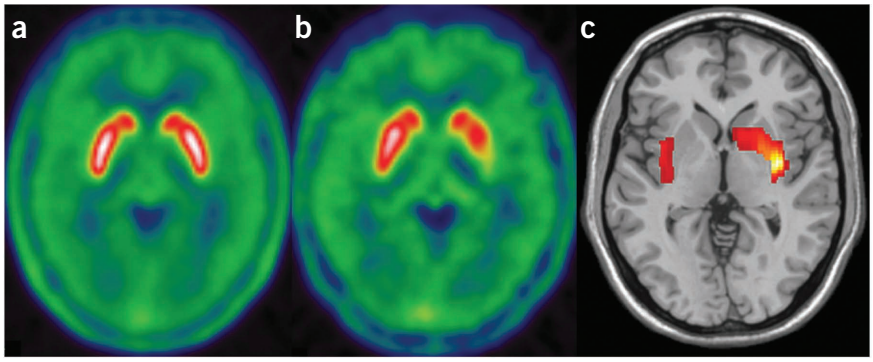

Figure 2 Striatal dopamine innervation assessed by ^{18}F-dopa positron emission tomography. (**a**) Mean control values for eight control subjects shows high uptake (highest value in white) in the striatum. (**b**) Subject with Parkinson's disease (right) featuring slowness and rigidity on the right limbs but minimal signs on the left limbs. Uptake is markedly reduced (70% below normal) in the left posterior putamen and reduced to a minor extent in the anterior putamen and caudate of the left hemisphere. (**c**) SPM2-based analysis (yellow represents the largest statistical difference and red the smallest one), showing the difference in uptake between **a** and **b** to highlight the caudorostral pattern of denervation. The statistical map is rendered over the MRI for anatomical localization.

populations. eIF4G1 normally links mTOR-dependent nutrient sensing to regulation of protein translation and cell proliferation; loss of function downregulates mitochondrial biogenesis and enhances autophagy[37].

Recently, heterozygous mutations in *GBA* (encoding glucocerebroside and famously linked to Gaucher disease) have been associated with a typical phenotype of Parkinson's disease and Lewy body pathology[38,39]. It is now clear that this heterozygous loss-of-function mutation also leads to a >5-fold–increased risk of Parkinson's disease in all populations as well as to earlier disease onset (typically in the early 50s)[40,41]. The pathogenic mechanism is unclear[41], and possibilities such as lysosomal dysfunction, interference with the helical binding of α-synuclein to lipid membranes or decreased ceramide metabolism are under scrutiny.

Additional mutations linked to early-onset Parkinson's disease are found in affected individuals under the age of 45 years and account for about 1% of cases of all types of Parkinson's disease. They are recessive loss-of-function mutations in the genes encoding parkin, PINK1 and DJ-1. DJ-1 mutations, the most uncommon, affect a protein implicated in redox sensing[42]. PINK1 is a mitochondrial protein kinase, and parkin was originally considered to be a ubiquitin E3-protein ligase, required for the proteosomal degradation of target substrates. It now appears that both PINK1 and parkin are functionally linked, as their expression induces mitochondrial fission[43] and the survival of nigrostriatal neurons. Parkin is recruited to dysfunctional mitochondria to promote their autophagic degradation and rescues degeneration in PINK1-null flies[44]. However, the relevance of the findings from animal models to the human disease is uncertain, as aged parkin/DJ-1/PINK1 triple-knockout mice fail to develop nigral neurodegeneration[45], and the impact of these proteins in sporadic Parkinson's disease seems to be low[46]. Other gene mutations (such as those that encode the recessive loss of function of tyrosine hydroxylase, ATP13A2 and PANK2 proteins) have been linked to early-onset Parkinson's disease but often with atypical symptoms and no Lewy bodies or loss of dopaminergic nigral neurons. Last, mutations affecting UCH-L1, FGF20, Omi/HTRA2 and GIGYF2 may be linked to Parkinson's disease, but the data remain equivocal[47].

Recently, genome-wide association studies (GWASs) have provided evidence for a contribution of common genetic variability in α-synuclein and microtubule-associated protein tau (MAPT) to Parkinson's disease[48–50]. Array-based GWASs have limitations in that they only test the hypothesis that common variants (or SNPs in linkage disequilibrium) cause common disease and only consider those variants that are present in the arrays. In the coming decade, massively parallel sequencing methods, which can comprehensively survey the entire genome, may provide far more genetic insight than previous GWASs or linkage studies.

In sum, mutations may help define the molecular pathways underlying neurodegeneration in Parkinson's disease[47]. Ideally, genetic studies should identify critical pathways (such as mTOR[37] or ceramide metabolism[41]), which may be affected by mutations in several of their components. Genetic studies could also help clarify some clinical findings, such as a possible association between the *MAPT* locus and dementia in Parkinson's disease. However, despite extensive experimental scrutiny, the process by which mutations in the genes we have mentioned lead to SNc cell death and Lewy body formation is not understood[51–53].

Why are SNc cells especially vulnerable? Dopamine metabolism[19] is considered to be critical for the preferential susceptibility of ventrolateral SNc cells to damage in Parkinson's disease. Dopamine metabolism produces highly reactive species that oxidize lipids and other compounds, increase oxidative stress and impair mitochondrial function[19,53,54]. At neutral pH, dopamine can auto-oxidize. Therefore, reduced sequestration of dopamine into synaptic vesicles, where the pH is lower and dopamine cannot auto-oxidize, may represent a vulnerability factor for neurons. Accordingly, dopamine neurons with low dopamine transporter activity in the cell membrane are less sensitive to oxidative stress induced by dopamine or neurotoxins[55] and are also less affected in Parkinson's disease[56]. Interestingly, dopamine toxicity in the SNc is reduced in α-synuclein–knockout mice, thus suggesting a critical interaction between the cellular concentrations of α-synuclein and dopamine and the inhibition (by dopamine) of chaperone-mediated autophagy in SNc neurons[31]. The dopamine toxicity hypothesis is appealing, but it is supported only by indirect evidence, as differences in dopamine metabolism in the most vulnerable ventrolateral neurons are not readily apparent. The most obvious difference in relation to the regional pattern of cell loss in the SNc occurs in the neuromelanin-containing neurons, which are more susceptible than neuromelanin-free dopamine neurons[57]. However, vulnerability within the ventrolateral SNc is unrelated to the amount of neuromelanin per neuron[57].

Other factors that may selectively affect SNc neurons compared to other catecholaminergic cells include differences in their handling of ionic fluxes—less capacity for calcium buffering[58] and increased reliance on L-type calcium channels[59]—and in their expression of specific transcription factors that regulate cell fate and survival[60]. Emphasis has recently been placed on calcium-mediated toxicity in SNc neurons through Cav1.3 channels[3,59], as compared to neurons of the ventral tegmental area, which use sodium channels for pacemaking activity. Nevertheless, pacemaking is not a feature of the

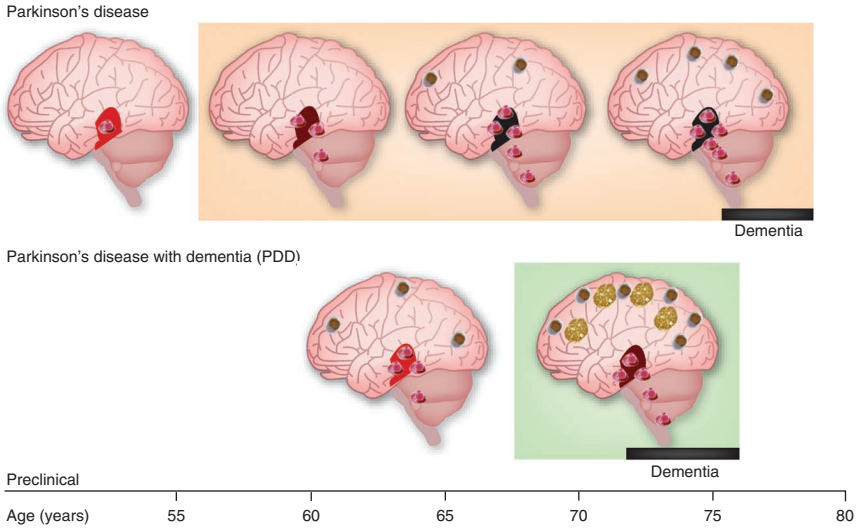

Figure 3 Distribution of Lewy bodies in Parkinson's disease. Diagrammatic representation of pathological data from longitudinally studied cases showing the severity of midbrain dopamine cell loss and Lewy body infiltration over time in an average individual who develops symptoms around 55 years of age versus one who develops symptoms after the age of 70. The severity of dopamine cell loss is related to the duration of symptoms, with those with longer durations having greater cell loss (represented as progressively darker color). The infiltration of Lewy bodies appears more marked in late-onset disease, and in many instances, individuals with late-onset disease have additional age-related pathologies (represented as cortical plaques). Dementia, as indicated in the lower bar, occurs earlier in the disease in older-onset individuals, consistent with the greater pathology observed.

SNc in awake primates, and the experimental levels of dopamine required for toxicity are much higher than those seen under normal physiological conditions[61]. Otherwise, SNc degeneration would be extremely common.

Important questions remain regarding how the levels of α-synuclein and dopamine are modified and maintained in SNc neurons, how this might change with age to influence SNc vulnerability, and whether there are cellular differences among SNc neurons in features such as the number of synaptic contacts and the degree of neuronal activity and energy consumption[62] that explain the degeneration pattern observed in the disease.

Does neurodegeneration begin in the SNc? In people with Parkinson's disease who die after a relatively short disease (<5 years), early cell loss occurs in two places—the SNc and the presupplementary motor cortex[63]. The time course of cell loss has been studied in the SNc, where most of the cell loss occurs in a 5–10-year preclinical period during which the ventrolateral tier appears most vulnerable[63,64]. Such a pattern of cell loss differs substantially from the pattern of abnormal α-synuclein deposition (see below).

Which mechanisms underlie progressive SNc cell loss? The motor features of Parkinson's disease are mainly related to the loss of striatal dopamine secondary to degeneration of dopamine neurons in the SNc[65]. The typically focal, somatotopic progression of motor features and the characteristic rostrocaudal gradient of ^{18}F-dopa uptake (**Fig. 2**) are consistent with a self-expanding process[66]. Indeed, the rate of decline of striatal dopamine innervation is faster in the initial years of disease evolution, when it fits an exponential pattern, as measured in one post-mortem study[65] and by imaging studies of ^{18}F-dopa uptake in individuals with Parkinson's[67]. This is compatible with the idea of an acute or subacute disease onset followed by a slow, nonlinear progression[66,68] that may involve mechanisms including inflammatory response, glutamate-mediated excitoxicity and reduced trophic support.

What underlies the nondopaminergic features of the disease? The clinical development of nonmotor symptoms and the underlying mechanisms involving nondopamine neurons are still hotly debated. Affected nondopamine neurons include monoaminergic cells in the locus coeruleus[69] and raphe nuclei, cholinergic cells in the nucleus basalis of Meynert (associated with cognitive deficits)[70] and the pedunculopontine tegmental nucleus (which may be related to gait problems)[71], and hypocretin cells in the hypothalamus (which likely mediate the sleep disorders seen in Parkinson's disease)[72]. Approximately 30–50% of these nondopamine cells have been lost by end-stage Parkinson's disease. The pathological model proposed by Braak and colleagues[73] reflects the stepwise progression of Lewy body pathology in the brain. These authors have suggested a caudo-rostral gradient of Lewy body formation from the lower brainstem to the neocortex[74]. This suggests that once the disease has started, there is a single progression wave, and that the onset of dementia, generally late in the course of Parkinson's disease, is due to cortical Lewy bodies[75]. Indeed, in people with slow disease progression, dementia usually occurs late, and individuals with dementia show a high incidence of limbic and neocortical Lewy bodies as predicted by Braak's Parkinson's disease staging[75,76].

However, substantial cortical Lewy bodies can be found in persons affected with Parkinson's without frank dementia, whereas Lewy body density in the medial temporal lobe is related to visual hallucinations[77,78], which usually precede dementia in Parkinson's disease. In these individuals without dementia, stereological counting of neurons in the temporal lobe reveals their overall preservation[79,80]. In Parkinson's disease of the PIGD subtype, the severity of cortical Lewy body formation and other age-related pathologies are enhanced[81,82], as are non-dopamine symptoms, which correlate poorly with Braak's stages[81,83].

Because of this clinical heterogeneity, there is some doubt about the significance of Lewy bodies and neurites as direct determinants of clinical progression for all symptoms in all types of individuals with Parkinson's disease[82,83]. Until we develop better phenotyping of Parkinson's disease, the two firmest conclusions we can draw are that Lewy body accumulation relates to symptoms for some Parkinson's disease subtypes and that Lewy bodies in the medial temporal lobe are associated with hallucinations but not necessarily with dementia.

Overall, the current data support the existence of two phenomena that affect disease progression—one associated with cell loss as disease progresses and the other associated with an increase in the abnormal accumulation of Lewy bodies (**Fig. 3**). The second mechanism seems to be dominant in patients with late-onset disease.

What do Lewy bodies in transplanted cells reveal about disease progression? Recently, postmortem studies on patients with advanced

Parkinson's disease who died 13–16 years after transplantation of fetal nigral cells into the striatum revealed inclusion bodies that appear identical in morphology and staining to the Lewy bodies found in host dopamine neurons in the SNc[84–88]. These Lewy bodies occurred in about 5–8% of the grafted neurons (similar to the proportion found in SNc neurons in cases of Parkinson's disease) and stained for α-synuclein, ubiquitin and thioflavin S—common Lewy body markers. In a few cases, the transplanted cells showed phenotypic alterations, such as loss of dopamine transporters. Similar findings had not been encountered in Parkinson's-affected individuals who survived for less than 10 years after similar transplants[89]. It is therefore likely that these otherwise healthy and 'young' dopamine cells experienced a pathological or toxic process in the striatum.

One suggestion to account for these results is that α-synuclein pathology spreads by a prion-like process[90]. Accordingly, extracellular α-synuclein is taken up by neighboring neurons through endocytosis, leading to aggregation and intracellular inclusions[91], and α-synuclein can be transmitted from affected neurons to engrafted neuronal precursor cells in a transgenic model[92]. Other mechanisms have been considered for the presence of Lewy bodies in transplanted cells[93], but we still lack a definitive interpretation of their significance. Interestingly, secretion of both monomeric and aggregated α-synuclein is elevated in response to proteasomal and mitochondrial dysfunction, cellular defects found in Parkinson's disease[91,94,95]. Clearly, the findings in grafted cells raise some important points about the origin of Lewy bodies and about neurodegeneration in Parkinson's disease[90,94].

Beyond the question of their propagation, a major problem persists in understanding the meaning of Lewy bodies to the pathophysiology of Parkinson's disease: the lack of experimental models. Cell culture systems and animal models expressing Lewy body-like inclusions would be useful to address this problem. The development of methods for labeling Lewy bodies *in vivo* to establish more accurate clinicopathological correlations would also be a welcome development.

Moving forward. Our current understanding of Parkinson's disease points to a multifactorial cause for cell death in the SNc. Genetic studies have revealed proteins involved in the initiation of some forms of Parkinson's disease, and establishing their relative roles and their interactions should be a priority. Molecular and cellular abnormalities (**Fig. 1**) occur to different degrees in the SNc of individuals with Parkinson's disease[95–97]. Dopamine metabolism seems to interact with and enhance these abnormalities, but a specific sequence of events has not been defined.

Understanding the vulnerability of the SNc and the mechanism whereby pathology becomes widespread are primary objectives of basic and clinical research in Parkinson's disease. In this context, *in vivo* monitoring of nondopaminergic pathways and their correlation with impairment of dopamine pathways and symptom progression in the different Parkinson's subtypes should be a priority, in order to better assess degree and pattern of cell loss throughout disease progression.

A corollary of this discussion is that Parkinson's disease does not result from a single cause but from many interacting factors. Recognizing this heterogeneity would help explain many clinical observations as well as the plethora of biochemical abnormalities that have been identified in individuals and experimental systems.

New therapeutic avenues

Before levodopa (**Box 1**), Parkinson's disease was essentially a motor disorder. After the arrival of levodopa, the development of motor complications and psychiatric manifestations—such as hallucinations and delirium—came to the fore and became the prevailing clinical problems in Parkinson's disease for the next two decades. More judicious use of levodopa, the introduction of dopamine agonists and of atypical neuroleptics (clozapine, quetiapine) and the possibility of treating severely affected individuals with surgery have reduced the urgency of these problems. Indeed, there is general agreement that new Parkinson's treatments should tackle two unresolved problems: moving from symptom-alleviating to disease-modifying therapies, and reducing the growing prevalence of nonmotor disease symptoms such as loss of balance, autonomic dysfunction and cognitive impairment, which are the real causes of disability in long-term Parkinson's disease.

Is there a role for transplants? Starting in the 1980s, many people with Parkinson's disease received striatal grafts from various sources (fetal tissues, porcine fetal SNc neurons, carotid body cells and immature retinal cells). Despite evidence for a beneficial effect of mesencephalic fetal grafts in some open-label studies, two double-blind, placebo-controlled trials failed to show clinical improvement[98,99]. The profound effect on the placebo arm (sham surgery) in these studies may be a confounding factor[100]. In any case, the best result of any transplant study in Parkinson's disease does not surpass the clinical benefits of either deep-brain stimulation (DBS; **Box 1**) of the subthalamic nucleus or parenteral delivery of levodopa and apomorphine[101].

To date, all cell-replacement studies have used fetal ventral mesencephalic tissue or paraneural dopamine cells. Recently, there has been great interest in human stem cells, which have been shown to survive, innervate to some extent, and reverse motor dysfunction in rodent and monkey models of Parkinson's disease[102–104]. It is clear that human embryonic stem cells are the easiest to manipulate, but they can form teratomas[102] and have raised ethical concerns. The possible application of induced pluripotent stem cells (iPS cells) to treat Parkinson's disease and other disorders[105,106] may address the ethical concerns and provide a means to deliver cells of autologous origin, eliminating immunological reactions after transplantation. However, there is no evidence yet that iPS cells will be more efficacious than DBS. We would therefore argue that the high hopes for cell-replacement therapy need to be tempered until more experimental data are available.

What can we learn from gene therapy? Currently, there are four clinical trials testing different gene therapy approaches against Parkinson's disease. One finding common to all of these studies is that no serious adverse events have yet been reported for any of the procedures.

One approach uses adeno-associated viral vector serotype 2 (AAV2) to deliver aromatic amino acid decarboxylase (AADC)—the enzyme that converts levodopa to dopamine. The idea is to make this conversion more efficient, allowing for optimal therapeutic benefit with lower levodopa doses and avoiding treatment-related side effects. This procedure has been through a successful phase 1 trial[107] and is currently in phase 2. However, it is difficult to see how this technique will avoid the tendency of levodopa to induce motor complications.

A second approach uses AAV2 to deliver glutamic acid decarboxylase (GAD) to the subthalamic nucleus[108]. As GAD synthesizes γ-aminobutyrate, the main inhibitory neurotransmitter in the nervous system, the underlying idea is that delivering this enzyme will increase inhibitory tone. In a sense, this approach is a gene therapy version of DBS, and the advantages of this gene therapy–based approach over DBS are unclear.

The third approach[109], which is in phase 1 trials, involves a tricistronic vector encoding tyrosine hydroxylase, AADC and GTP

cyclohydrolase hydroxylase—the last one of which is an enzyme necessary for tetrahydrobiopterin synthesis, an essential cofactor for AADC.

Last, AAV2-mediated delivery of neurturin, a functional analog of glial cell–derived neurotrophic factor (GDNF), aims to provide neuroprotective benefits in addition to symptomatic improvement. Neurturin provides robust neuroprotection and upregulation of dopamine function in a variety of rodent[110] and nonhuman primate models[111], and has completed a successful phase 1 clinical trial[112]. However, it is now known (J. Siffert, W.J. Marks Jr., P.A. Starr, M.A. Stacy, N.M. Boulis *et al.*, unpublished data) that neurturin failed in phase 2 clinical testing. Postmortem evidence (J.H.K., unpublished observations) from two people who died from events unrelated to neurturin therapy indicates that gene delivery was suboptimal. New trials are in the planning stages.

Gene therapy remains a viable and apparently safe procedure, particularly if aiming to deliver neuroprotective molecules. However, its actual clinical value is unknown, and further research is required to draw firmer conclusions.

What are the prospects for neuroprotective treatments? Several molecules have been proposed as potential neuroprotective agents against Parkinson's disease. Molecules that reduce dopamine cell death include monoamine oxidase-B inhibitors (selegiline, rasagiline), anti-apoptotic agents (TCH346, CEP-1347), glutamate antagonists, promitochondrial drugs (coenzyme Q10, creatine), calcium channel blockers (isradipine) and growth factors (GDNF)[113]. However, none of these molecules has definitively shown neuroprotective effects in clinical trials[114]. This may indicate the ineffectiveness of these compounds, but may also be a consequence of the limitations of clinical-trial design[115]—use of the wrong dose, recruitment of too broad a patient population or selection of inappropriate endpoints[115,116].

Despite these limitations, some Parkinson's disease neuroprotection trials—pramipexole and ropinirole versus levodopa[117,118], coenzyme Q10 (ref. 119) and selegiline[120]—have had positive outcomes in terms of reducing the progression of motor deficits in early Parkinson's disease. However, the interpretation of these trials is confounded by potential drug modulation of therapeutic endpoints, symptomatic (as opposed to true disease-modifying) effects or trial size[116,121]. The results of the ADAGIO trial using rasagiline in individuals with Parkinson's disease are relevant to some of these problems. This large (>1,000 subjects), randomized, placebo-controlled, delayed-start trial showed that those receiving 1 mg (but not 2 mg) of rasagiline, as compared to placebo, had slower motor progression over 9 months and improved motor outcome after 18 months than those who started the drug later[122]. The ADAGIO design was intended to avoid confounding symptomatic effects on the primary clinical endpoints, but conclusions about rasagiline's real long-term impact and putative mechanism of action remain premature[123].

Concluding remarks

Will it be possible to solve the Parkinson's disease puzzle? To find a definitive solution to Parkinson's disease, several unresolved areas remain. We have highlighted some of the gaps in knowledge that need attention and speculated on their relevance, hoping that these ideas influence Parkinson's disease research. Further definition of clinical phenotypes of and their correlation with prediagnostic manifestations could be crucial but only if associated with genetic and biochemical markers. The dissociation between cell death and Lewy bodies outside the catecholaminergic nuclei is also critical, as is the role of these inclusions in the progression of the cognitive and other nonmotor disease manifestations.

Is a unitary model for Parkinson's disease possible? Mutations of several genes leading to various abnormalities of cellular signaling pathways, infections and toxins[124] are associated with neuronal loss in the ventrolateral SNc. Currently, there is no definitive explanation for why these abnormalities affect dopaminergic neurons earlier and more profoundly than other cell types. One major common theme for most mutations and toxins is the impairment of mechanisms related to cellular energy production leading to oxidative stress. It may be that the selective vulnerability of nigrostriatal cells is determined by their profuse arborization, which may result in high levels of energy consumption. It has been estimated that nigrostriatal dopaminergic neurons can form as many as 40,000 synapses, whereas neurons in the ventral tegmental area only make up to 3,000 contacts[125]. It is known that aging decreases energetic cellular efficiency as well as the synthesis and activity of neuronal growth factors. This could explain the enhanced vulnerability with aging and even the trend toward reduction of regional vulnerability. Dietary habits and lifestyles associated with reduced risk of Parkinson's disease (for instance, smoking, coffee drinking, and diets high in uric acid[126] or greater levels of physical activity in midlife[127]) could somehow modify the cellular processes affected in the pathology and reduce the risk to develop Parkinson's disease[124].

Can we be optimistic? We must appreciate that management of Parkinson's disease has improved considerably in the past two decades thanks to new therapies and better use of old ones. Most affected individuals now have a relatively good quality of life for most of the natural history of their disease. Nevertheless, a better understanding of the biochemical pathogenesis of Parkinson's disease is the best route to lead us to new disease-modifying therapies. A breakthrough has remained elusive, but there is increasing information about the mechanisms underlying neuronal death and regional vulnerability. Finally, the shortcomings of current clinical trials, such as the placebo effect, are now better recognized. We are confident that these developments will lead to substantive advances in Parkinson's disease treatment, but hopes for a cure in the short term may be somewhat unjustified.

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