Parkinson’s Disease: Genetics and Pathogenesis

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Abstract
Recent investigation into the mechanisms of Parkinson’s disease (PD) has generated remarkable insight while simultaneously challenging traditional conceptual frameworks. Although the disease remains defined clinically by its cardinal motor manifestations and pathologically by midbrain dopaminergic cell loss in association with Lewy bodies, it is now recognized that PD has substantially more widespread impact, causing a host of nonmotor symptoms and associated pathology in multiple regions throughout the nervous system. Further, the discovery and validation of PD-susceptibility genes contradict the historical view that environmental factors predominate, and blur distinctions between familial and sporadic disease. Genetic advances have also promoted the development of improved animal models, highlighted responsible molecular pathways, and revealed mechanistic overlap with other neurodegenerative disorders. In this review, we synthesize emerging lessons on PD pathogenesis from clinical, pathological, and genetic studies toward a unified concept of the disorder that may accelerate the design and testing of the next generation of PD therapies.
INTRODUCTION

In his 1817 monograph *An Essay on the Shaking Palsy*, the British physician James Parkinson (1) published his observations of six individuals with “paralysis agitans.” Further refinement in our understanding of this syndrome, including tremor, slowed movements, and gait impairment, was subsequently undertaken by Jean-Martin Charcot, who also coined the modern label Parkinson’s disease (PD). In 1912, Friedrich Lewy (2), a German neurologist, described the cardinal neuropathological lesion that would later bear his name: the Lewy body (LB). As we approach the 200-year anniversary of the clinical recognition of this disease and the 100-year anniversary of the key pathological insight, what would Dr. Parkinson and Dr. Lewy think today of the vast field of research that their work has spawned? In some ways, it has become more challenging than ever to answer the deceptively simple question “What is PD?”.

Despite tremendous progress on several fronts—in fact, because of the advances by clinicians, pathologists, geneticists, and others—the answer to this question often depends on one’s particular point of view. For example, does PD encompass the frequent finding of incidental LB pathology in the brain in the absence of apparent clinical manifestations? Conversely, what can we learn from cases that fit the clinical profile of PD but fail to demonstrate typical autopsy findings? Further, how do we integrate into our case definitions the recent wealth of evidence that genetics plays a key role in disease susceptibility when, historically, a positive family history was an exclusionary consideration in diagnostic algorithms? Such questions illustrate how progress can often challenge traditional frameworks for understanding disease. Fortunately, the confusion that accompanies the crest of a rapidly advancing wave of discovery is usually transient, and in its wake comes a deeper understanding. We now know, for example, that the α-synuclein gene (*SNCA*), which encodes the primary constituent of the LB, influences susceptibility for rare familial PD as well as sporadic disease in the general population. This key insight follows nearly a century of investigation into PD, linking the earliest pathological observations of this disorder to findings from the latest genome-wide association (GWA) studies. Given the currently rapid pace of discovery in PD research, there is cause for optimism that many such exciting realizations are imminent.

In this review, we aim to update the reader on progress in our understanding of PD genetics and pathogenesis, as well as to place recent results in the context of the rich history of investigation in this field. Throughout, we highlight examples of discoveries that challenge existing conceptions of PD, and where possible, we try to synthesize lessons from divergent lines of inquiry. We begin with a review of our current clinical and pathological understanding of PD, because a precise definition of the disease entity subsequently guides our exploration of etiology. A coherent answer to the question “What is PD?” will facilitate the next generation of genetic advances and animal models and will hopefully propel translational research, thereby leading to novel neuroprotective and restorative therapeutic approaches.

Epidemiology and Clinical Features

With a prevalence of approximately 1% at age 65, which rises to nearly 5% by age 85, PD is the second most common neurodegenerative disorder after Alzheimer’s disease (3, 4). The mean age of PD diagnosis is in the seventh decade of life, but due to PD’s insidious nature, the onset of symptoms may precede clinical recognition by many years (5). PD can be diagnosed at any age, and an estimated 3% of cases are initially recognized in individuals younger than age 50 (4). PD is typically a chronic and slowly progressive disorder with a mean duration of 15 years from disease recognition until death, although affected individuals can frequently survive two decades or longer (6, 7). Given the aging of the population, the prevalence of PD is anticipated to increase dramatically,
which would lead to increased urgency for the need to identify improved therapies that delay progression and mitigate disability.

The cardinal manifestations of PD include resting tremor, bradykinesia (slowed movements), rigidity (increased muscular tone), postural instability, and gait impairment (7). Together, these manifestations produce the syndrome of parkinsonism, of which PD is the major cause; however, a similar clinical picture can also accompany other neurodegenerative disorders or nondegenerative conditions, including parkinsonism due to cerebrovascular injury and medication-induced parkinsonism. As detailed below, PD motor manifestations are attributable to dopaminergic cell loss within the substantia nigra (SN) pars compacta and resultant dysfunction of the basal ganglia, a cluster of deep nuclei that participate in the initiation and execution of movements (Figure 1) (8). Indeed, motor symptoms respond well to dopamine replacement therapy, which has been the pillar of PD treatment since its introduction in the late 1960s (7). In advanced disease, direct modulation of basal ganglia activity via deep brain stimulators implanted in the subthalamic nucleus can also be effective. Although currently available PD therapies both delay disability and prolong life expectancy, none has been proven to significantly alter the ongoing neurodegenerative process.

Motor features remain the essential criteria for clinical diagnosis of PD and are a major source of disability, but PD has a much broader impact on the nervous system (9, 10). Nonmotor symptoms, including impaired olfaction, disordered sleep, and constipation, are now believed to presage the clinical recognition of bradykinesia, tremor, or gait impairment by as much as 20 years (5). As PD progresses, frequent motor freezing and falls, treatment-related involuntary movements (dyskinesias), pain and sensory complaints, autonomic dysfunction (urinary incontinence and orthostatic intolerance), and neuropsychiatric manifestations (depression, hallucinations, and dementia) become prominent, and these features are probably due to the spread of pathology beyond the basal ganglia (9, 10). Importantly, most nonmotor symptoms show little or no response to dopamine replacement and contribute substantially to overall disability, especially late in disease (11). As detailed below, because the investigation of PD mechanisms, including the development of animal models, has focused largely on the vulnerability of dopaminergic cells in the SN, there remains an unmet need to understand how this disorder spreads beyond the basal ganglia to other brain systems.

Figure 1
Anatomy and physiology of Parkinson’s disease (PD) motor manifestations. A simplified schematic of the neuronal circuits involving the basal ganglia, thalamus, and cortex and their derangement in PD (8). For simplicity, only the direct pathway is shown. It normally functions to facilitate movements (left), but in PD the output is attenuated (right). The midbrain substantia nigra pars compacta (SNC) provides dopaminergic input to the putamen (Pu), which is excitatory to the direct pathway. The putamen inhibits (red) the globus pallidus interna (GPi), which subsequently inhibits the thalamus (Th). The thalamus projects excitatory input (green) to the motor cortex. In PD, degeneration within the SNC leads to net increased inhibition of the thalamocortical projection. The indirect pathway (not shown), including the globus pallidus extrema (GPe) and subthalamic nucleus, is inhibited by SNC dopaminergic input and normally functions to repress movements, but its activity is enhanced in PD.

SN: substantia nigra
In addition to advanced age, male gender, and European ancestry, a number of other epidemiological factors have been proposed to increase PD risk (3). In 1983, a cluster of individuals who developed a PD-like syndrome following abuse of intravenous drugs contaminated with methyl-phenyl-tetrahydropyridine (MPTP) was discovered (12), and the hypothesis that exposure to environmental toxins may increase risk of developing PD has subsequently attracted intense interest. A meta-analysis of 19 studies evaluating the potential impact of pesticide exposure found an estimated doubling of disease risk (13). Potentially consistent with this finding, numerous mitochondrial toxins, including MPTP, the herbicide paraquat, and the pesticide rotenone, cause dopaminergic cell loss in animal models, as reviewed further below. In addition, occupational or other exposure to heavy metals, including manganese and iron, has been suggested to increase PD risk, but epidemiological support for this hypothesis has been lacking (3). Many studies have also evaluated dietary and habitual factors, and both cigarette smoking and coffee consumption are associated with reduced PD susceptibility (14). Importantly, however, epidemiological association does not necessarily imply causation, and the role of the basal ganglia in impulse control and reward mechanisms may predispose individuals with PD to be less susceptible to engaging in addicting behaviors.

PATHOLOGY

The cardinal neuropathological feature of PD is dopaminergic cell loss within the SN in association with the development of intracytoplasmic, protein-rich inclusions termed LBs (15). As shown in Figure 2, the classic LB is spherical and eosinophilic, and it stains strongly for α-synuclein protein, which aggregates to form the fibrillar core (16). On autopsy, the brains of individuals with PD are additionally characterized by α-synuclein-positive accumulations within neuronal processes, termed Lewy neurites, as well as by neurons that show more diffuse or granular perikaryal staining patterns. Because dopaminergic cells contain melanin, cell loss within the SN is accompanied by depigmentation of the midbrain that is readily visible in gross material postmortem.

Dopaminergic neurons in the SN project primarily to the striatum, which is composed of the caudate and putamen nuclei; therefore, α-synuclein pathology and associated nigral cell loss result in the depletion of striatal dopamine. As illustrated in Figure 1, decreased nigrostriatal input leads to a net increase of inhibitory output from the globus pallidus interna to the thalamus and, indirectly, to the cortex, thereby repressing the initiation of movements and leading to the characteristic motor manifestations of PD (8). Indeed, the severity of bradykinesia and rigidity in PD patients proximate to death correlates with nigral cell loss and reduced striatal dopamine levels found at autopsy (17). By the time PD motor symptoms are clinically recognized, 60% of dopaminergic SN cells are lost, resulting in a concomitant 80% depletion of striatal dopamine. The progression of these changes can also be demonstrated in living patients by using nuclear imaging to measure radiolabeled dopamine uptake in the striatum or by using tracer ligands that bind to the dopamine transporter (18).

In parallel with the growing appreciation that, clinically, PD causes a host of nonmotor manifestations, it has been recognized that α-synuclein pathology ranges beyond the SN into much of the neuraxis (19, 20). α-Synuclein pathology has been described in the peripheral cutaneous nerves, autonomic nervous system, enteric nervous system, spinal cord, lower brainstem (dorsal motor nucleus of the vagus), limbic structures (amygdala and hippocampus), and neocortex. Importantly, the vulnerable cell populations include numerous nondopaminergic cell types, such as noradrenergic neurons of the locus coeruleus, serotonergic projections from raphe nuclei, and acetylcholinergic cells of the basal forebrain in the nucleus of Meynert. On the basis of a careful, postmortem analysis, Braak and coworkers (20) proposed a staging system for PD pathology (Figure 3). Accordingly, the
Pathology of Parkinson’s disease. (a) Low-power view of the substantia nigra showing marked depletion of dopaminergic neurons (arrow, remaining neuron), reactive gliosis, and neuromelanin present in phagocytic cells (arrowheads). (b) Typical brainstem-type Lewy body (LB; arrow) in a pigmented dopaminergic neuron. The LB has a characteristic dense eosinophilic core and surrounding paler halo. (c) Brainstem-type LB showing staining for α-synuclein (arrow). (d) Lewy neurites (arrows). (e) Cortical-type LBs (arrows) are indistinct with standard histological hematoxylin and eosin staining. (f) Cortical-type LB stain with antibodies against α-synuclein (arrow).

earliest Lewy pathology affects the enteric and peripheral autonomic nervous system as well as the olfactory bulb, and it subsequently spreads in a stereotyped, caudal-to-rostral wave from the lower brainstem (stage 1) to diffuse involvement of the neocortical ribbon (stage 6). In the Braak paradigm, α-synuclein pathology is not observed in the midbrain SN until stage 3, consistent with the building consensus that a substantial prodromal syndrome precedes the development of PD motor symptoms and the subsequent clinical recognition of the disorder (5). Further, the anatomic pattern of the earlier Braak stages fits remarkably well with proposed premotor disease manifestations, including hyposmia (olfactory bulb), constipation (enteric nervous system), and sleep disorder (brainstem reticular formation) (19). In later stages, widespread cortical involvement with LBs correlates with the frequent occurrence of cognitive impairment in long-standing PD.

Although it is now widely acknowledged that Lewy pathology can be found throughout the nervous system, the Braak proposal of a stereotyped caudal-to-rostral spread of pathology remains an attractive hypothesis and awaits definitive confirmation (17). Although a number of postmortem studies have validated the Braak sequence, others have identified a substantial minority of cases with patterns of pathology that diverge from the expectations of the model (21, 22). Regardless, data from large cohort studies show that as many as 20% of brains have evidence of LB pathology on autopsy; this figure is approximately tenfold higher than the known prevalence of PD. The finding of LBs on autopsy in the absence of clinical evidence of neurologic disease
Figure 3
Progression of Parkinson’s disease (PD) pathology. Schematic outlining the major stages of PD pathology, as proposed by Braak et al. (20). In stages 1–2, Lewy neurites and Lewy bodies are found within the medulla and pons. Only in stage 3 does aggregated α-synuclein affect the substantia nigra. Later stages (4–6) involve the supratentorial compartment in a graded fashion, which ultimately leads to substantial neocortical pathology in stage 6.

**Stages 1–2**
- Cingulate cortex (C)
- Temporal cortex (T)
- Frontal cortex (F)
- Parietal cortex
- Occipital cortex

**Stage 3**
- Amygdala (A)
- Nucleus of Meynert
- Hippocampus

**Stage 4**
- Substantia nigra pars compacta (SN)

**Stages 5–6**
- Dorsal motor nucleus of vagus (DM)
- Raphe nucleus (RN)
- Locus coeruleus

DLB: dementia with Lewy bodies
MSA: multiple system atrophy

proximate to death has been termed incidental LB disease, and this population may represent preclinical PD. In support of this hypothesis, case series with incidental LB pathology were found to have decreased nigral cell counts and reduced striatal dopaminergic nerve terminals, intermediate between brains from subjects with known PD and controls without α-synuclein pathology (23). Additional large clinical and pathological studies are needed to further address the intriguing hypothesis that incidental LBs may represent the earliest stages of PD; ideally, such studies should include prospective assessment of premotor disease features along with neuroimaging studies of striatal dopamine uptake.

On the basis of the preceding discussion, PD can be clinically and pathologically defined as a progressive disorder comprising (a) a core of motor manifestations attributable to LB pathology and degenerative cell loss in the SN and (b) a halo of nonmotor features, many of which precede the motor symptoms and probably result from more widespread Lewy pathology throughout the peripheral and central nervous systems. However, PD is the major, but not the only, cause of parkinsonism. We briefly consider the other parkinsonian disorders here, as the marked clinical and, in some cases, pathological similarities may hold important clues to understanding PD pathogenesis. Some diseases, including dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), are also associated with α-synuclein pathology and are therefore classified along with PD as α-synucleinopathies. DLB is characterized clinically by parkinsonism and early development of dementia, often accompanied by prominent visual hallucinations and fluctuations in arousal (24). Pathologically, DLB shows widespread α-synuclein deposition, prominently involving the neocortex; examples of cortical LBs are shown in Figure 2e,f. MSA is a more rapidly progressive disorder consisting of prominent failure of the autonomic nervous system, including urinary incontinence and orthostatic intolerance, accompanied by parkinsonism and/or cerebellar dysfunction and associated with glial α-synuclein inclusion pathology (Figure 4a).

In addition to the α-synucleinopathies, several other disorders frequently associated with parkinsonism are characterized by Tau
Pathology of other disorders that cause parkinsonism. (a) Typical oligodendroglial inclusion (arrow) of multiple system atrophy stained with an antibody to α-synuclein. (b) The substantia nigra from a patient with progressive supranuclear palsy shows depletion of dopaminergic neurons, reactive gliosis, and neuromelanin present in phagocytic cells (arrowheads). A remaining neuron contains an indistinct round inclusion (arrow). (c) Immunostaining for Tau reveals a globose neurofibrillary tangle (arrow) that is typical of progressive supranuclear palsy and corticobasal degeneration. (d) Neuronal inclusion (arrow) from a patient with frontotemporal dementia, stained with an antibody against ubiquitin. (e) The pathology of neurodegeneration with brain iron accumulation includes spheroids (arrow) and iron pigment deposition (arrowhead). (f) A well-organized infarct shows loss of brain parenchyma with central cavitation (arrow) surrounded by reactive astrocytes (arrowheads).

pathology and are collectively referred to as tauopathies. Tau is a neuronal microtubule-associated protein, and like α-synuclein, it can aggregate to form intracytoplasmic pathologic inclusions associated with neurodegeneration (25); examples are shown in Figure 4b,c. Corticobasal ganglionic degeneration (CBD) and progressive supranuclear palsy (PSP) are two tauopathies that can be difficult to clinically distinguish from PD at onset; however, symptoms generally respond poorly to dopamine replacement, and as the disease progresses additional characteristic features develop. Parkinsonism can also be observed in association with the frontotemporal dementias, which are a group of disorders characterized primarily by profound and heterogeneous cognitive manifestations (26). At autopsy, frontotemporal dementia cases generally reveal either Tau pathology or intranuclear inclusions that stain positive for ubiquitin and the TAR-DNA-binding protein TDP-43; an example is shown in Figure 4d. Tau pathology is perhaps best known for its association with Alzheimer’s disease, in which Tau aggregates to form neurofibrillary tangles concomitant with the development of amyloid plaques. Parkinsonism is also common in Alzheimer’s disease, and in some cases, motor manifestations have been associated with Tau pathology in the SN (27). Finally, there are at least two examples of tauopathy and parkinsonism that result from environmental
triggers. A major cause of PD-like illness in the early twentieth century was postencephalitic parkinsonism, in which nigral neurofibrillary tangles and neurodegeneration followed influenza infection (28). Similarly, the Guam parkinsonism and dementia complex, a rare indigenous parkinsonian disorder, has been linked to a neurotoxin in the cycad fruit (29).

In some cases of neurodegenerative parkinsonism, nigral cell loss and gliosis are observed without any other specific pathology, including the absence of α-synuclein, Tau, or other protein inclusions, and this pattern is typical for certain Mendelian disorders, as detailed below. Lastly, parkinsonism can also be observed in nondegenerative conditions, and one common cause is cerebrovascular disease. Pathologically, vascular parkinsonism is associated with ischemia-related cell loss and gliosis involving the basal ganglia (Figure 4f). Understanding how nonsynuclein pathologies contribute to parkinsonian syndromes may provide key insights into the mechanisms of cell vulnerability within the SN that could ultimately be translated into improved understanding and treatment of PD. In addition, because autopsy studies in aging populations commonly show mixed pathologies, it will be important to determine whether α-synuclein pathology may interact with other neuronal insults to modify the onset and progression of PD in an individual patient.

GENETICS
The past decade has witnessed a remarkable transformation in our understanding of the role of genetics in PD pathogenesis. PD has historically been considered a sporadic disorder in which environmental triggers played a dominant etiologic role; this view was influenced in part by the 1980s outbreak of MPTP-induced parkinsonism (12). The traditional tools of genetic epidemiology, twin studies and familial aggregation, have produced conflicting results in PD, adding to the skepticism that genes had significant influence (3). However, such studies may be ill-equipped to detect genetic effects in some complex traits, such as PD in which genetic heterogeneity and incomplete penetrance may predominate. Further, given the strong influence of aging, underestimates of heritability probably come from either the failure to follow subjects to a sufficiently advanced age or the subjects’ premature death before PD symptoms manifest. Indeed, heritability estimates in PD substantially increase when young-onset disease is considered, which probably magnifies the effects of more penetrant loci. Among several more recent epidemiological studies, 10–30% of PD subjects reported a positive family history, and first-degree relatives of subjects with PD were estimated to have a twofold- to sevenfold-increased relative risk of PD (30, 31). In a study of PD concordance in monozygotic versus dizygotic twins, heritability was estimated to vary from zero to one, depending on whether older- or younger-onset disease was considered, respectively (32). On the basis of the empirical findings of genetic studies in PD, discussed below, it appears that epidemiological methods have substantially underestimated the genetic contribution to PD. For example, familial PD, in which there is a known family history, has traditionally been differentiated from sporadic disease, where genetics is assumed to play a minor role. However, such distinctions are becoming increasingly blurred, as several examples of gene variants were initially identified in PD families and subsequently found in substantial proportions of apparently sporadic PD cases.

Two primary methods have been used to search for PD genes. The first, linkage analysis, tracks the segregation of chromosomal regions in pedigrees with multiple affected family members. This approach is most effective at identifying rare genetic variants that have highly penetrant effects and are characterized by Mendelian patterns of disease inheritance (33). The second method, association analysis, uses a simple comparison of case and control subjects to identify distortions in the frequency of genetic variants in each group. This method has greater statistical power for the discovery of common variants of modest effect sizes and incomplete penetrance, as
illustrated by the discovery of many such susceptibility loci in a variety of common but genetically complex disorders (34). The full potential of this approach was realized by the availability of high-density single-nucleotide polymorphism (SNP) genotyping arrays that interrogate the contribution of common sequence variation (frequency >5%) across the genome in an unbiased manner.

The challenges of discovering PD-susceptibility genes are similar to those encountered by epidemiological studies evaluating for evidence of heritability. One potential confounder is etiologic heterogeneity: Parkinsonism is a common feature of other neurodegenerative and nonneurodegenerative disorders, and such entities may be difficult to distinguish clinically from PD without autopsy confirmation. Perhaps an even greater potential problem is the growing evidence that PD motor symptoms may develop only after years of ongoing neurodegenerative cell loss in the SN and elsewhere in the nervous system (5). The tempo of pathological progression and the ultimate age of PD symptom onset may be manifestations of variable disease expressivity. As mentioned above, the prevalence of incidental Lewy pathology in individuals without recognized PD proximate to death is as high as 20% (17). If genetic variants act to increase α-synuclein pathology, which subsequently produces the clinical manifestations of disease, significant numbers of control subjects with substantial pathology and subclinical disease might significantly dilute the power of association analyses.

Fortunately, despite these potential hurdles, linkage analyses have successfully identified a number of highly penetrant genetic variants (33), and recent GWA studies have discovered additional susceptibility loci of more modest effect sizes that meet rigorous statistical thresholds and have been independently replicated (35, 36). Remarkably, in several cases, these two approaches have identified both high- and low-penetrant genetic variants affecting the same loci, linking the etiology of Mendelian PD in rare families with the genetics of sporadic PD in the general population. Therefore, the convergence of findings from traditional approaches (linkage analysis) and more modern, complex genetic methods (GWA analysis) reveals a continuum of disease risk from sporadic to familial disease, upon which age, environment, and still-unknown genetic factors probably interact to manifest disease (Figure 5).

As with the question “What is PD?”, nearly as much controversy is engendered by the related question “What is a PD gene?”. We adopt the guiding principles that a PD gene must cause a syndrome with substantial clinical overlap with PD, as delineated above, and that the resultant disorder must be characterized predominantly by α-synuclein pathology on autopsy. In applying these criteria, we have tried to determine whether both a neurologist and a pathologist, absent knowledge of the family history and genotyping, might diagnose these cases as PD (Table 1). Following our discussion of these PD genes, we discuss a larger group of loci, collectively termed parkinsonism genes, that cause parkinsonian syndromes but with additional clinical features atypical for PD, and/or with distinct pathologies, or in some cases, where the pathology is not yet known. A similar scheme of classifying the growing list of genes relevant to PD has been suggested by others (15, 37). Notably, the naming of PD loci has been largely historical and defers little to such clinical and pathologic criteria. Therefore, some of the PARK genes cause syndromes with only limited resemblance to PD, and other parkinsonism genes have been subsumed under alternative naming schemes, such as those used for spinocerebellar ataxias or dystonias (38).

A systematic definition of PD, including clinical, pathological, and genetic dimensions, is important for the field. Although knowledge of a specific genetic etiology is not likely to immediately impact patient treatment, in the near future genotyping might realistically find its way into clinical practice, for example, in the form of diagnostic and prognostic algorithms, or for risk stratification of patients with known family histories. In addition, identifying a population of subjects with increased genetic risk
Known genetic variants

Familial Sporadic

Environment and unknown genetic variants

Age

Disease risk

Disease threshold

Figure 5

The emerging genetic architecture of Parkinson’s disease (PD). A model summarizing the relative effect sizes of known PD-susceptibility genes and illustrating possible interactions with age; potential environmental risk factors; and other, unknown genetic modifiers. The discovered genetic variants include both highly penetrant Mendelian alleles that cause familial PD and polymorphisms with modest effects on disease risk that contribute mainly to sporadic disease. In several cases, high- and low-penetrant variants were identified in the same loci. The known genetic variants form a continuum of risk for PD that, coupled with the effects of age, environment, and other genes, blurs the distinction between familial and sporadic disease.

Table 1 Parkinson’s disease–susceptibility loci

<table>
<thead>
<tr>
<th>Locia</th>
<th>Chromosome</th>
<th>Genetic variants (penetrance)</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>SNCA (PARK1/4)</td>
<td>4q21</td>
<td>A53T, A30P, E46K duplication, triplication</td>
<td>REP1, rs2736990, rs11931074</td>
</tr>
<tr>
<td>LRRK2 (PARK8)</td>
<td>12p12</td>
<td>R1441C/G/H, I2020T, Y1699C, G2019S</td>
<td>R1628P, G2385R, rs1994090</td>
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<tr>
<td>GBA</td>
<td>1q21</td>
<td>—</td>
<td>N370S, L444P, others</td>
</tr>
<tr>
<td>MAPT</td>
<td>17q21</td>
<td>—</td>
<td>H1 haplotype, rs393152</td>
</tr>
<tr>
<td>BST1</td>
<td>4p15</td>
<td>—</td>
<td>rs4538475</td>
</tr>
<tr>
<td>PARK16c</td>
<td>1q32</td>
<td>—</td>
<td>rs947211, rs823128</td>
</tr>
</tbody>
</table>

aThe susceptibility loci Parkinson’s disease genes, have been identified as significant results of linkage studies but await validation and identification of the causal genes.
bMany of these polymorphisms are probably markers for the true causal variants, which remain to be defined.
cThe PARK16 locus contains five gene candidates.

The α-Synuclein Gene

There is overwhelming evidence that SNCA, which maps to chromosome 4q21 and was initially identified as the PARK1 locus, causes PD both in families with rare Mendelian forms of the disease and in sporadic cases. In 1997, linkage analysis of an Italian family with autosomal
dominant PD led to the identification of the A53T mutation in SNCA, and subsequent studies have identified two additional mutations in other families (A30P and E46K) (39–41). Clinically, SNCA mutations cause disease onset during the fourth or fifth decade of life, with initially asymmetric development of bradykinesia and rigidity and an excellent response to dopamine replacement therapy that is similar to that of idiopathic PD (42). However, SNCA mutations are associated with a relatively rapid progressive course and, frequently, the early development of dementia similar to DLB. At autopsy, these cases demonstrate widespread α-synuclein pathology involving the brainstem, limbic areas, and neocortex, as observed in later–Braak stage PD cases, with associated dopaminergic cell loss in the SN. Shortly after the SNCA mutations were found, α-synuclein protein was first demonstrated to be a major component of the LB (43). This finding established a key link between the cause of rare familial disease and the defining neuropathological lesion of PD in the general population.

Subsequent to the discovery of SNCA mutations, additional families believed to harbor dominantly inherited mutations in a separate disease-causing locus, PARK4, were found to have genomic duplication or triplication of the SNCA gene, which led to a corresponding 50% or 100% increase in the dose of α-synuclein messenger RNA and protein (44–46). SNCA locus multiplication causes a heterogeneous clinical syndrome that ranges from a late-onset presentation indistinguishable from idiopathic PD to early-onset parkinsonism in association with dementia and/or autonomic dysfunction; these syndromes are typical of DLB and MSA, respectively (47). In addition, as has been reported for SNCA mutation carriers, gene multiplication can also lead to the manifestation of nonmotor PD symptoms, including abnormal olfaction and sleep disturbance (42, 48). As expected, autopsy studies of SNCA locus multiplication cases demonstrate widespread LB pathology similar to that observed in PD, as well as in DLB and MSA.

Given its involvement in familial PD, the SNCA gene became an excellent candidate susceptibility locus for PD in the general population. Association studies soon suggested that variation in the length of a dinucleotide repeat near the SNCA gene promoter (REPI) both augments α-synuclein expression (49) and modestly increases the risk of disease (50–52). In a large meta-analysis in subjects of European ancestry, including more than 2,500 PD cases and a similar number of controls, the extended 263-bp REPI allele was associated with an odds ratio of 1.4 for PD (53). REPI is present in approximately 5–10% of the population studied and was therefore estimated to account for nearly 3% of the risk for PD in individuals of European ancestry. Whereas initial investigations of the REPI allele were hypothesis driven, the SNCA gene was recently validated as a PD-susceptibility locus in several GWAS studies (35, 36, 54, 55). In the largest study in subjects of European ancestry to date, involving more than 5,000 cases and 8,000 control subjects, multiple SNPs at the SNCA locus showed highly significant associations with PD (36). The best SNP in this study, rs2736990, was associated with only a modest increased risk of disease (odds ratio = 1.2, \( P = 2.2 \times 10^{-16} \)); however, the risk allele at this marker is present in nearly half of the Caucasian population. In an independent study conducted in Japanese subjects, including more than 2,000 cases and 18,000 controls, common variation at SNCA was also implicated in susceptibility to PD (35). The discovered association signals localize near the 3′ region of the SNCA gene and show partial linkage disequilibrium with REPI, which raises the possibility that these variants may be different surrogate markers of a single causal variant, possibly affecting gene expression.

α-Synuclein is a small, 140-amino acid protein that contains an amphipathic aminoterminal domain, a hydrophobic central core [the nonamyloid component (NAC) region], and a negatively charged C terminus (56). It is widely expressed throughout the nervous system and is enriched at presynaptic nerve
Leucine-Rich Repeat Kinase 2

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) on chromosome 12, originally designated as the PARK8 locus, were first identified after linkage analysis and positional cloning methods were applied to isolated families with autosomal dominant parkinsonism (60, 61), but variants in this gene were subsequently discovered to be a common contributor to PD risk in both sporadic and familial cases (62–67). To date, nearly 50 different LRRK2 variants, mostly missense mutations, have been reported to be associated with disease, and additional sequencing of this locus in large numbers of cases and controls will be required to comprehensively define the allelic spectrum and understand the full impact of this locus on the overall burden of PD. Of the large number of described variants, broad consensus has thus far been reached on the pathogenicity of a subset including R1441C/G/H, I2020T, Y1699C, and G2019S, which occur at highly conserved residues and appear to have functional consequences, but the list of validated causative alleles is likely to grow (63). The genetic architecture of the LRRK2 locus is complicated; the contribution of individual risk alleles is strongly dependent on the ancestry of the population studied. For example, the most common and best-studied variant, G2019S, is found at a frequency of 1% to 4% in PD patients of European descent (68). However, whereas this allele is virtually absent in Chinese and Japanese populations, it is a major cause of disease in Portuguese, Ashkenazi Jewish, and North African Arab patients, in whom it is found at frequencies of 6%, 15%, and 40% of PD patient cohorts, respectively (69–71). Frequency estimates are even higher when PD cases with known family history are selectively considered; however, as alluded to above, this distinction between sporadic and familial disease is probably misleading. The data strongly suggest that LRRK2 variants are inherited ancestral polymorphisms, as opposed to de novo mutations (72, 73); therefore, the absence of family history in apparently sporadic cases is probably due to a number of confounders, including incomplete reporting, unrecognized or subclinical disease, premature death from other causes, the strong effect of age on disease manifestation, and the probable impact of environmental and other genetic modifiers.

In large patient cohorts in which common LRRK2 mutations (mostly G2019S) have been discovered, the presentation and course of disease is consistent with idiopathic PD. LRRK2-associated disease has a mean onset age approaching 60 years and may be associated with a slightly more benign, tremor-predominant disease course (68). In one of the initial reports in the Basque population, the LRRK2 gene was named dardarin, which is derived from the Basque word for tremor (60). Nonmotor manifestations, including impaired olfaction, urinary symptoms, constipation, and
depression, are commonly described in LRRK2-associated disease (66, 68). The initially described rare familial variants and the more common G2019S polymorphism behave genetically as highly penetrant risk alleles; however, age appears to be a key factor in disease manifestation, suggesting the possibility of variable expressivity. In a large study of G2019S-associated disease, penetrance increased from 30% at age 60 to nearly 75% by age 80 (68). This mutation is extremely uncommon in older control populations; however, isolated cases of asymptomatic G2019S carriers as old as 80 have been described (74). The predominant pathology of LRRK2-associated disease is consistent with that of idiopathic PD and includes LB formation and associated neurodegeneration within the SN and other vulnerable brain regions (65, 75). Notably, there have been isolated reports of alternate pathologies associated with LRRK2 mutations, including tauopathy, ubiquitin-positive pathology, and nonspecific nigral degeneration in the absence of inclusions (61, 76, 77). Further study will be required to understand the full clinical and pathologic spectrum of LRRK2-associated neurodegenerative disease.

The allelic spectrum within the LRRK2 gene extends beyond less common and highly penetrant variants; association analyses have found that this locus also harbors susceptibility alleles with more modest effects on PD risk. The best-validated examples include the R1628P and G2385R variants in Chinese and Japanese populations (78–80). These two variants, present in approximately 5–9% of PD cases of East Asian descent (compared to ~3% of controls), are each associated with an approximately twofold-increased risk of disease; together, these variants are estimated to explain ~10% of the risk of sporadic PD in this population (33). In addition, a large GWA study conducted in Japanese subjects recently identified a highly significant association between SNPs in the LRRK2 genomic region with risk of PD (rs1994090, odds ratio = 1.4, \( P = 2.7 \times 10^{-8} \)) (35). The G2385R and R1628P variants are not found in subjects of European descent, and initial candidate association studies to investigate the contribution of more common LRRK2 variants in this population were equivocal (81). However, in a recent large GWA study (36), polymorphisms within the LRRK2 locus displayed suggestive evidence of association with susceptibility to PD in individuals of European ancestry (rs1491923, odds ratio = 1.1, \( P = 1.6 \times 10^{-7} \)). Additional genotyping in even larger population samples will therefore be required to validate this finding and to identify the causal variant(s) within LRRK2 responsible for this association.

LRRK2 is a complex locus that comprises 51 exons and encodes a large and structurally unusual 2,257–amino acid protein that includes leucine-rich repeats, a Ras-like GTPase domain (ROC), C-terminal of ROC domain (COR), kinase domain, and WD40 motif (60, 61, 63). The LRRK2 protein is widely expressed in both neuronal and nonneuronal tissues, and a substantial fraction of protein appears to be membrane associated (82). Confirmed pathogenic missense mutations in LRRK2 affect the GTPase (R1441C/G/H), COR (Y1699C), kinase domain, and WD40 motif (60, 61, 63). The LRRK2 protein is widely expressed in both neuronal and nonneuronal tissues, and a substantial fraction of protein appears to be membrane associated (82). Confirmed pathogenic missense mutations in LRRK2 affect the GTPase (R1441C/G/H), COR (Y1699C), and kinase domains (G2019S, I2020T), and several reports suggest that these substitutions impact enzymatic activity (63). Importantly, the most common and best-studied variant, G2019S, increased kinase activity in a number of assays (83), consistent with a toxic, gain-of-function mechanism for LRRK2-associated PD for this allele. LRRK2 protein is capable of autophosphorylation, and several other potential substrates have been nominated on the basis of a variety of experimental approaches (63). Additional study will be required to identify the in vivo LRRK2 substrate(s) relevant to health and disease; some early insights from animal models are described below.

Glucocerebrosidase

Gaucher’s disease is an autosomal recessive, lysosomal storage disorder caused by loss of function of the glucocerebrosidase gene (GBA) on chromosome 1; nearly 300 distinct mutations have been identified to date (84). GBA
catalyzes the conversion of the glycolipid glucocerebroside to glucose and ceramide, and the absence of this enzyme leads to a multisystem disorder that in some cases includes neurologic manifestations. A connection between GBA and PD was first suggested by observations that patients with Gaucher’s disease can develop parkinsonian features (85, 86) and that relatives of Gaucher’s patients who are carriers for disease-causing mutations appeared to have increased susceptibility for PD (87). As these observations became more widely recognized, many reports emerged on the unexpectedly frequent discovery of GBA mutations in clinic populations of sporadic PD patients (88–91); these findings culminated in the recent publication of a large, collaborative case/control comparison that involved more than 5,000 PD patients and a similar number of controls (92). Because of a founder effect, GBA mutations are more common in Ashkenazi Jewish individuals, and two common mutations, N370S and L444P, were discovered in 15% of PD patients compared with 3% of controls. In non-Ashkenazi subjects, the same mutations were disproportionately discovered in 3% of patients and less than 1% of controls. However, full sequencing of the GBA gene led to the identification of many more polymorphisms, increasing the allele frequency to 7% among non-Ashkenazi patients. The study-wide odds ratio for any GBA allele was nearly 6.5 (92), and other reports suggest that less common, more highly penetrant variants may be associated with a greater-than-tenfold-increased risk of disease (91). Importantly, only a minority of PD patients with GBA alleles (24%) reported a known family history of disease, again challenging the distinction between familial and sporadic disease. Clinically and pathologically, the PD phenotype in GBA-variant carriers closely matches that found in patients with idiopathic disease (93), although the age of onset is earlier, with a mean of 55 years in the largest study (92).

Based on the discovery of GBA risk alleles in a small but appreciable number of asymptomatic controls, current evidence supports either a strong susceptibility locus or a Mendelian dominant locus with incomplete penetrance. However, the challenge in precisely determining the penetrance of GBA, as well as of other susceptibility loci, is to establish large control cohorts of sufficiently advanced age; doing so requires a careful and thorough assessment for parkinsonian signs and ideally includes screening for premotor features of PD. Ultimately, it will also be important to evaluate for risk alleles in large numbers of controls with autopsy data to determine whether there is an association with LB pathology. It remains possible that GBA, and other susceptibility loci with apparently incomplete penetrance, will show increased penetrance when considering a pathological definition of disease, inclusive of incidental Lewy pathology.

The example of the Ashkenazi Jewish population underscores how transformative genetics has been in our understanding of PD etiology. We now recognize that in cohorts of Ashkenazi Jewish PD patients, approximately 20% have GBA mutations (91, 92) and at least 15% have LRRK2 mutations (68, 71). Interestingly, in cohorts where both GBA and LRRK2 were genotyped, disease-causing variants rarely occurred together in the same subjects—significantly fewer than would be expected by chance (91, 94). Therefore, in this population, more than one-third of PD cases appear to be accounted for by genetic causes that are potentially compatible with a Mendelian, monogenic inheritance model of disease. Given that only a few years ago, the existence of substantial heritability in PD was widely doubted, this observation should caution against reaching a premature conclusion on the ultimate genetic architecture of PD in Ashkenazi Jewish or other populations.

Microtubule-Associated Protein Tau

The microtubule-associated protein Tau gene (MAPT) has long been of interest in neurodegenerative disease, given that the encoded protein Tau aggregates to form neurofibrillary tangles, a pathological hallmark of Alzheimer’s disease, and also forms filamentous pathological inclusions that characterize
several other neurodegenerative disorders, collectively termed tauopathies (25, 95). As described above, parkinsonism is a common clinical accompaniment to tauopathy, and in 1998, mutations in \textit{MAPT} were identified in one such disorder: familial frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (96). This finding prompted efforts to determine whether genetic variation at the \textit{MAPT} locus might impact other neurodegenerative disorders with similar clinical and/or pathological features. An extended haplotype at the \textit{MAPT} locus, termed \textit{H1}, was demonstrated to increase risk for both PSP and CBD, and subsequently, many groups reported suggestive associations in PD case/control cohorts (95, 97). In a recent, large collaborative study that involved nearly 2,000 cases and a similar number of controls, homozygosity for the \textit{H1} haplotype was found to increase disease risk by nearly 50% (98). Given that \textit{H1} is the common haplotype in populations of European ancestry, it may contribute to a substantial burden of PD. Recently, several GWA studies in PD subjects of European descent validated the association between the \textit{MAPT} locus and PD risk (36, 54, 55). In the largest and best-powered analysis (36), several SNPs in linkage disequilibrium with the \textit{H1} haplotype were discovered with highly significant association statistics ($r^2$93152, odds ratio $= 0.8$, $P = 2.0 \times 10^{-16}$). Interestingly, this association signal was not observed in a similarly powered GWA analysis in Japanese subjects, which further suggests that there is genetic heterogeneity in PD pathogenesis across different populations (35).

Although the mechanism of the \textit{H1} haploypic association with PD remains unknown, several studies suggest that the risk haplotype may increase gene expression at the \textit{MAPT} locus and may also lead to differential expression of alternative transcripts (95, 99, 100). However, because the implicated region on chromosome 17 is large (900 kb) and may affect genes in addition to \textit{MAPT}, future studies of larger cohorts should aim to further refine the association signal and unambiguously identify the causal gene and variant(s). Because mutations in \textit{MAPT} cause a syndrome, FTDP-17, that is clinically and pathologically distinct from PD, how variation at one locus might trigger two such divergent disorders is unknown. Nevertheless, the involvement of \textit{MAPT} in PD adds to the accumulating evidence of a mechanistic link with Alzheimer’s disease and other neurodegenerative tauopathies.

**Emerging Loci from Genome-Wide Association Studies**

As mentioned above, GWA studies provide a powerful method to scan the genome in an unbiased fashion to identify common variants underlying disease susceptibility, and this approach has been successfully used to survey the genetic architecture of numerous complex genetic disorders (34). The first PD GWA studies were small (101, 102), including several hundred cases and controls, and therefore lacked statistical power to identify susceptibility loci of modest effect sizes. Nevertheless, these studies were among the earliest GWA studies performed in any neurodegenerative disorder, and more recently, the data have been incorporated into larger meta-analyses (54, 103). In 2009, two large PD GWA studies were reported, each including several thousand cases and controls, making them the best-powered studies to date (35, 36). The rediscovery of \textit{SNCA}, \textit{MAPT}, and \textit{LRRK2} among the top association signals both validates and extends our understanding of the role of these loci in determining susceptibility for PD (Table 1). In addition, these findings highlight PD genetics as an example in which previously known Mendelian loci or candidate genes anticipated the findings of GWA studies; more commonly, the genome-wide approach has identified completely unexpected and novel susceptibility genes. Two new putative PD-susceptibility loci, \textit{PARK16} and \textit{BST1}, were also discovered by these recent GWA studies. The \textit{PARK16} locus, which was discovered in the Japanese PD cohort ($r^2$947211, odds ratio $= 1.3$, $P = 1.5 \times 10^{-12}$) (35) and independently validated in subjects of European ancestry (36), encompasses five genes—\textit{SLC45A3}, \textit{NUCKS1},


**AR-JP:** autosomal recessive juvenile parkinsonism

*RAB7L1, SLC41A1, and PM20D1—and future work should aim to refine this association signal to identify the causal locus. The second novel PD gene, *BST1*, also demonstrated a significant association in the Japanese study (rs4538475, odds ratio = 1.2, *P* = 3.9 × 10⁻⁶) (35). Interestingly, this locus was not associated with PD susceptibility in the European population; however, a comparatively reduced allele frequency may have limited statistical power for replication (36). Little is known about the function of *BST1*, but it appears to encode an enzyme that catalyzes the formation of cyclic ADP-ribose, which can regulate intracellular calcium stores (35). This hypothesis is potentially interesting because calcium dysregulation has previously been implicated in mechanisms of dopaminergic cell loss in PD (104).

Despite the recent success, implementation of GWA in PD research remains behind the curve compared with studies of other complex genetic diseases, which have reported analyses involving tens of thousands of subjects. Similar large-scale collaborative efforts will be essential to achieve the necessary statistical power to define the full complement of common variants, with an allele frequency >5%, that impact susceptibility for PD in the general population. As alluded to above, future GWA studies in PD might additionally boost power through assessment of prodromal nonmotor manifestations or, alternatively, by leveraging the precision of a neuropathologically defined phenotype in autopsy cohorts. Ultimately, the advent of low-cost, full-genome sequencing will usher the future of PD genetics, allowing full cataloguing of both common and rare variants that either cause or modify risk for disease.

**Other Genetic Forms of Parkinsonism**

In neurology diagnostic algorithms, lesion localization within the nervous system traditionally takes precedence over specific etiology. Thus, a patient presenting with parkinsonism indicates a basal ganglia disorder, of which PD is only one of many potential causes. Over the past decade, a number of Mendelian forms of parkinsonism have been enumerated with variable degrees of clinical and pathological overlap with PD (33, 38). This class of disorders is exemplified by autosomal recessive juvenile parkinsonism (AR-JP), of which the best-studied and most important cause is loss of function for *parkin* (*PARK2*). AR-JP was initially described in Japanese families with a chromosome 6 linkage signal, and in 1998, causative mutations were identified in *parkin* (105). This gene encodes a ubiquitously expressed 465-amino acid protein with structural and functional homology to ubiquitin ligases, which target cytoplasmic proteins for degradation by the proteasome. The results of a large case series suggest that *parkin* is a major cause of young-onset parkinsonism (106, 107). In 73 families with an autosomal recessive inheritance pattern and at least one affected member with disease onset prior to age 45, nearly half of the PD cases were due to *parkin* mutations (106). Further, in a cohort of 246 young-onset, sporadic cases, 15% were due to *parkin*, and this fraction increased to 70% in the subset of cases with onset before age 20 (107). In addition, the phenotype of *parkin*-associated disease, including both the typical motor manifestations and an excellent response to dopamine replacement therapy, is often clinically indistinguishable from that of idiopathic PD.

By a number of criteria, however, *parkin*-associated AR-JP diverges from both idiopathic PD and the genetic forms of PD defined in the preceding section, suggesting that it may represent a distinct disease entity. First, the mean age of onset for *parkin* disease (30 years) is substantially younger than for sporadic PD. Second, *parkin* often causes clinical features that are atypical for PD, including symmetric onset of motor symptoms, early prominent dystonia, diurnal symptomatic fluctuations, reflex changes, and an overall slow and benign course of disease (108). Third, individuals with AR-JP due to *parkin* mutations do not appear to develop characteristic nonmotor manifestations of PD, including loss of olfaction or late cognitive decline and dementia, suggesting a more restricted pathology (109). Finally, the
predominant neuropathology of parkin-associated disease at autopsy is bland nigrostriatal degeneration, which consists of dopaminergic cell loss in the SN in the absence of LBs (110, 111), although LB pathology has infrequently been described (112, 113).

PTEN-induced kinase 1 (PINK1) is the second most common cause of AR-JP (114). Clinically, PINK1 (also known as PARK6) causes a syndrome similar to parkin-associated disease, and although in some cases it can be difficult to distinguish from early-onset PD, atypical features are often observed (115). PINK1 encodes a widely expressed 581-amino acid serine-threonine kinase that is localized to mitochondria. No autopsies of PINK1 disease have yet been reported. As detailed below, data from animal models suggest that PARK1 and Parkin proteins may function coordinately. DJ-1, also known as PARK7, is a rare cause of AR-JP and, on the basis of the small number of clinical descriptions, appears to cause a similar syndrome as parkin and PINK1 (116). The pathology of DJ-1 disease is also unknown. DJ-1 encodes a ubiquitously expressed, conserved 189-amino acid protein of still-uncertain function; however, it has been reported to translocate to mitochondria in response to oxidative stress and may thus participate in a protective response, together with the Parkin and PINK1 proteins (117).

Table 2 summarizes the parkinsonism genes, including all of the currently validated PARK loci (except for those already presented in Table 1), and additionally includes examples of some of the more diverse genetic disorders that are also associated with significant parkinsonism (37, 38). Due to space limitations, we are unable to discuss many of these genes in more detail, and the table necessarily omits several other syndromes that, less frequently, can cause parkinsonism. Some of the disorders associated with the parkinsonism genes, such as AR-JP caused by parkin and PINK1, can be clinically indistinguishable from young-onset PD unless atypical features emerge, genetic testing is pursued, or subjects eventually come to autopsy. In other cases, parkinsonism is a minor component of a more heterogeneous neurological syndrome or multisystem disorder that can be readily differentiated from idiopathic PD (38). On the basis of the clinical and pathological criteria outlined above, the disorders caused by the parkinsonism genes can be differentiated from idiopathic PD. Nevertheless, lessons from the study of these syndromes are applicable to our understanding of PD pathogenesis. An attractive hypothesis is that other heritable parkinsonian disorders may provide insights into mechanisms of dopaminergic cell vulnerability in the SN. Other data, such as (a) the finding that α-synuclein can be directly ubiquitinated by Parkin (118) or (b) evidence for potential interactions in animal models (discussed below) raise the possibility of an even more direct functional link between the mechanisms of PD and those of other genetic forms of parkinsonism. In such a model, the parkinsonism genes might additionally function as susceptibility loci in idiopathic PD (33, 119). This hypothesis has been most directly tested in the case of the AR-JP genes, particularly parkin and PINK1. Interestingly, some asymptomatic heterozygous carriers of parkin mutations show evidence of decreased striatal dopamine on nuclear imaging and, on careful examination, show signs of mild parkinsonism (112, 113, 120). However, although these findings are consistent with a haplo-insufficient, subclinical basal ganglionic disorder in some mutation carriers, it has not yet been proven that these individuals progress to develop PD more frequently than the background population rate. In addition, association studies that evaluated for parkin or PINK1 mutations in sporadic PD cases versus controls have not consistently found significant differences in carrier frequencies; however, these investigations have thus far been relatively small and potentially underpowered (119, 121–123). Similarly, targeted efforts to identify more common polymorphisms in these genes have been equivocal (124, 125), and neither parkin nor PINK1 has been identified among the most promising signals from the largest GWA studies performed to date (35, 36). Therefore, although the preponderance of
Table 2  Selected additional genetic causes of parkinsonism

<table>
<thead>
<tr>
<th>Gene a</th>
<th>Chromosome</th>
<th>Protein function</th>
<th>Clinical phenotype b</th>
<th>Pathology</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>parkin (PARK2)</td>
<td>6q25</td>
<td>E3 ubiquitin protein ligase</td>
<td>AR-JP, often indistinguishable from PD, but also with dystonia, reflex changes</td>
<td>Nigrostriatal degeneration, no inclusions</td>
<td>105</td>
</tr>
<tr>
<td>PINK1 (PARK6)</td>
<td>1p36</td>
<td>Mitochondrial serine-threonine kinase</td>
<td>AR-JP, similar to parkin</td>
<td>Unknown</td>
<td>114</td>
</tr>
<tr>
<td>DJ-1 (PARK7)</td>
<td>1p36</td>
<td>Unknown, possible role in stress response</td>
<td>AR-JP, similar to parkin</td>
<td>Unknown</td>
<td>116</td>
</tr>
<tr>
<td>ATP13A2 (PARK9)</td>
<td>22q13</td>
<td>Lysosomal cation transporter ATPase</td>
<td>Kufor-Rakeb disease (AR), parkinsonism and dementia</td>
<td>Unknown</td>
<td>163</td>
</tr>
<tr>
<td>FBXO7 (PARK15)</td>
<td>22q12</td>
<td>E3 ubiquitin protein ligase</td>
<td>Pallido-pyramidal syndrome (AR), dystonia and parkinsonism</td>
<td>Unknown</td>
<td>164</td>
</tr>
<tr>
<td>PLA2G6 (PARK14)</td>
<td>22q13</td>
<td>Phospholipase A2</td>
<td>NBIA-2 (AR), dystonia, parkinsonism, and dementia</td>
<td>Neuroaxonal dystrophy, iron accumulation</td>
<td>165</td>
</tr>
<tr>
<td>PANK2</td>
<td>20p13</td>
<td>Pantothenate kinase</td>
<td>NBIA-1 (AR), dystonia, parkinsonism, and dementia</td>
<td>Neuroaxonal dystrophy, iron accumulation</td>
<td>166</td>
</tr>
<tr>
<td>ATP7B</td>
<td>13q14</td>
<td>Copper transporter ATPase</td>
<td>Wilson’s disease (AR), parkinsonism, liver failure, neuropsychiatric symptoms</td>
<td>Basal ganglia copper accumulation and degeneration</td>
<td>167</td>
</tr>
<tr>
<td>GRN</td>
<td>17q21</td>
<td>Progranulin, growth factor</td>
<td>Frontotemporal dementia (AD), dementia with or without parkinsonism</td>
<td>Ubiquitin/TDP-43 inclusions</td>
<td>168, 169</td>
</tr>
<tr>
<td>ATXN2 (SCA2)</td>
<td>12q24</td>
<td>Unknown, enriched in Golgi apparatus</td>
<td>Spinocerebellar ataxia (AD), with or without parkinsonism</td>
<td>Polyl glutamine-repeat nuclear inclusions</td>
<td>170</td>
</tr>
<tr>
<td>GCH1 (DYT5)</td>
<td>14q22</td>
<td>GTP cyclohydrolase I, dopamine biosynthesis</td>
<td>Dopa-responsive dystonia (AD), occasionally phenocopied by parkin</td>
<td>No nigral degeneration, no inclusions</td>
<td>171</td>
</tr>
</tbody>
</table>

1 All validated PARK loci are presented first, followed by selected causes of other heritable parkinsonian disorders. Of the PARK loci, the original reports of pathogenicity for PARK5 (UCHL1) and PARK13 (Omi/HTR2) have been questioned, and these loci have not been independently replicated (33, 37).

2 Abbreviations: AD, autosomal dominant; AR, autosomal recessive; AR-JP, autosomal recessive juvenile parkinsonism; NBIA, neurodegeneration with brain iron accumulation.

evidence does not yet support a major role for the parkinsonism genes in increasing PD risk, larger studies will be necessary to definitively assess the possibility of more modest effects.

LESSONS FROM ANIMAL MODELS OF PARKINSON’S DISEASE

Experimental animal models have been an important part of PD research for many years. One widely used model is based on the aforementioned outbreak of MPTP-induced parkinsonism in drug users (12). Subsequent work demonstrated that the active metabolite of MPTP, MPP+, poisons the electron-transport chain through inhibition of mitochondrial complex I, leading to a selective loss of dopaminergic neurons. Recognition of the mechanisms of MPTP poisoning has motivated many studies of mitochondrial function in PD, a theme that has been reinforced by recent studies of Parkin and PINK1, as described below. Other toxins, including 6-hydroxydopamine (126), rotenone (127), and paraquat (128), can also produce relatively selective loss of dopaminergic neurons. Although toxin-based models have been instrumental for the study of mechanisms and consequences of dopaminergic neuronal loss, their relevance to...
the mechanisms of pathogenesis in PD continues to be debated.

Not surprisingly, genetic revelations in PD and related parkinsonian disorders have spurred the development of new animal models. The initial key insight was the discovery of SNCA mutations in familial PD and the finding that α-synuclein is the major constituent of LBs. Several groups attempted to model PD by overexpressing wild-type and mutant versions of human α-synuclein in model organisms based on a postulated toxic gain-of-function mechanism. In mice, transgenic expression of wild-type human α-synuclein produces a reduction in striatal dopaminergic nerve terminals, concomitant decreased motor performance, and formation of α-synuclein-rich neuronal cytoplasmic and nuclear inclusions (129). However, such mice do not display loss of dopaminergic neurons. Models based on overexpression of α-synuclein that carry the disease-causing A53T mutation show marked aggregation of α-synuclein in the brainstem and spinal cord, along with severe axonopathy and related motor deficits (130, 131). Again, dopaminergic neurons are largely spared, despite substantial expression of α-synuclein in the SN. Thus, loss of SN dopaminergic neurons, which constitute a clinically relevant cell population, has not been a striking feature of the α-synuclein transgenic mouse models developed to date. However, these models may still provide important tools to explore the in vivo mechanisms regulating α-synuclein aggregation as well as toxicity to nondopaminergic cell populations, as occurs in PD and other α-synucleinopathies.

Nonetheless, because many of the clinically defining symptoms of PD derive from loss of SN dopaminergic neurons, additional strategies for modeling α-synuclein aggregation and neurotoxicity have been pursued. Injection of adeno-associated virus that expresses wild-type or A53T mutant α-synuclein into rat SN produced a 30–80% loss of dopaminergic neurons within eight weeks (132), and similar toxicity was observed with adeno-associated virus–mediated expression of A30P α-synuclein (133). Lentiviral–mediated expression of α-synuclein also produced significant dopaminergic cell death in rats (134), mice (135), and non-human primates (136). Additionally, numerous α-synuclein-containing aggregates in neuronal cell bodies and neurites were observed following viral–mediated expression of α-synuclein in rat (132, 134). Interestingly, loss of SN neurons in this system was rescued by expression of Parkin (137). Because inclusion body formation and neurodegeneration are independent of Parkin in α-synuclein transgenic mice (138), the findings in the rat viral transduction model raise the intriguing possibility that dopaminergic neuron–specific interactions connect α-synuclein and Parkin mechanistically.

Given the power of simple model organisms to dissect fundamental biological pathways, a number of groups have also modeled PD in more tractable genetic systems. Expression of human α-synuclein is toxic in yeast (139, 140), nematodes (141), and fruit flies (142). Many of these models replicate key biochemical and cell biological features of PD. Expression of wild-type or PD-linked mutant forms of human α-synuclein in Drosophila leads to age-dependent degeneration of dopaminergic neurons, progressive locomotor dysfunction, and formation of α-synuclein-rich neuronal cytoplasmic and neuritic aggregates that resemble authentic LBs. Although mutant forms of α-synuclein were modestly more toxic than wild-type α-synuclein, overall similar effects were observed, which is consistent with a central role for α-synuclein in both familial and sporadic forms of the disorder, as suggested by human genetic evidence.

Work in simple model organisms has also contributed substantially to our understanding of the relationship between α-synuclein aggregation and PD pathogenesis. Soon after the development of the α-synuclein transgenic fly model of PD, expression of the human chaperone protein HSP70 was reported to ameliorate neurotoxicity (143), which is consistent with the hypothesis that abnormal protein folding plays a key role in disease pathogenesis. A number of sequence motifs, including the central NAC region and both serine and tyrosine
phosphorylation sites in the C terminus, influence the ability of α-synuclein to form fibrillar aggregates. Targeted mutagenesis of these sequences has provided strong evidence that aggregation of α-synuclein is critical for neurotoxicity (144). Interestingly, however, dopaminergic cell loss appeared to correlate with smaller oligomeric structures of α-synuclein rather than with higher-order fibrils (145, 146); therefore, larger macromolecular aggregates, including the LB, may serve a neuroprotective function by forming a sink for smaller toxic species of α-synuclein. In complementary experiments, a series of mutant forms of α-synuclein were assayed for their propensity to form oligomers and fibrils in vitro and were subsequently evaluated for in vivo neurotoxicity in the Drosophila and Caenorhabditis elegans systems (147). These results also implicated oligomeric species of α-synuclein as toxic, while suggesting that aggregation into higher-order fibrillar species is neuroprotective. Generally, experiments in mammalian models are also consistent with a role for aggregation in mediating α-synuclein neurotoxicity, although it is more challenging to single out a particular α-synuclein species as responsible (148, 149).

A number of laboratories have used the conventional loss-of-function approach in model organisms to investigate the biological roles of genes that cause AR-JP. Mutations in Drosophila parkin cause prominent mitochondrial pathology, including abnormal morphology and resultant cellular toxicity (150). A similar phenotype was subsequently observed in PINK1 mutant flies, and genetic epistasis experiments powerfully demonstrated that parkin and PINK1 act sequentially to regulate mitochondrial function (151, 152). More recently, genetic manipulation of the well-conserved mitochondrial fission and fusion machinery was found to strongly interact with parkin and PINK1 mutants to modify cellular toxicity, which implicates these genes in the regulation of mitochondrial dynamics (153–155). Despite these striking findings in Drosophila, mice lacking parkin and PINK1 individually, or together in double knockouts, fail to show dramatic mitochondrial phenotypes or dopaminergic cell loss, even when DJ-1 is also removed (156). These observations suggest the possibility of additional functional redundancy in the murine system.

Because the genetic link between LRRK2 and PD is a relatively recent one, less work has been reported for animal models based on the manipulation of LRRK2. However, recent experiments in transgenic mice are consistent with an association between gain-of-function kinase activity and neurotoxicity (157). An intriguing recent report suggests that increased levels of LRRK2 may enhance α-synuclein aggregation and neurotoxicity, whereas reducing LRRK2 gene dosage suppresses α-synuclein neuropathology (158). These findings correlate well with the presence of LB pathology in patients with LRRK2-associated PD, and they may provide an experimental system in which to further dissect the possible connection among LRRK2 activity, α-synuclein aggregation, and subsequent neurodegeneration.

A MODEL OF PARKINSON’S DISEASE PATHOGENESIS

Figure 6 presents a synthesis of our current knowledge on the mechanisms of PD pathogenesis, with an emphasis on recent genetic discoveries. In our model, SNCA gene expression and aggregation of α-synuclein protein constitute the central pathway that leads to neurotoxicity and neurodegeneration in PD. Although LB formation is the outcome of this cascade, it remains to be determined which α-synuclein species is harmful, and as suggested above, several studies implicate an oligomeric form. SNCA mutations promoting fibrillation or increased gene expression, via rare locus multiplication or more common promoter polymorphisms, most directly trigger this toxic pathway. Other recently identified PD-susceptibility genes, including LRRK2, GBA, and MAPT, probably influence this central cascade, although understanding the detailed mechanisms remains an important goal. The involvement of GBA implicates the
Figure 6
A model of Parkinson’s disease (PD) pathogenesis. Aggregation of α-synuclein is proposed as the central pathway leading to neurotoxicity in PD. Other PD-susceptibility genes, including the microtubule-associated protein Tau gene (MAPT), the glucocerebrosidase gene (GBA), and the leucine-rich repeat kinase 2 gene (LRRK2), may participate in or modify the progression of this cascade in a still-undefined fashion. The pathway culminates with the formation of α-synuclein fibrils and deposition into Lewy bodies; however, α-synuclein oligomers may be the toxic species, although the mechanisms are still unknown. parkin, the PTEN-induced kinase 1 gene (PINK1), and DJ-1 may coordinately influence mitochondrial dynamics and the response to oxidative stress, thereby contributing to neuronal survival, particularly in substantia nigra dopaminergic cells. The point of intersection between the α-synuclein cascade and the parkin/PINK1 pathway remains to be determined.
lysosomal degradation machinery as potentially important, and the participation of Tau illustrates a possible link between PD and the pathogenesis of other neurodegenerative diseases, including Alzheimer’s disease and other tauopathies. In addition to genetic factors, aging is a potent determinant of PD susceptibility, and additional environmental triggers probably contribute. Some of these exogenous risk factors, such as the potential role of pesticides and toxins, may target mitochondria, leading to the production of damaging reactive oxygen species. Finally, several parkinsonism genes, notably parkin, PINK1, and DJ-1, have been functionally linked to mitochondria and have been proposed to mediate a protective cellular response. A key question remains how directly these genes interact with the central cascade of α-synuclein aggregation and toxicity in PD, or whether they alternatively constitute a parallel pathway that is important to neuronal health and survival, particularly for dopaminergic cells.

**CODA: WHAT IS PARKINSON’S DISEASE?**

Although PD was initially recognized and described as a purely clinical syndrome, recent progress has splintered the unitary conception of this disease into a number of alternate views. Some have suggested embracing everything that behaves clinically as PD under a single diagnostic umbrella. Others have argued in favor of abandoning PD as a single clinicopathologic entity, instead enumerating many subtypes on the basis of varying clinical features, familiality, and autopsy findings. Although we acknowledge the cross currents that motivate these divergent approaches, we believe that a critical survey of the field suggests an alternate tack. We propose that PD is a precisely definable and distinct disease entity with a particular clinical, pathological, and genetic signature. Clinically, PD begins insidiously with a cluster of nonmotor symptoms that anticipate the development of motor manifestations by many years, and late-stage disease nearly universally causes cognitive decline and other disabling complications. Pathologically, PD is characterized by α-synuclein pathology and neurodegeneration of a range of vulnerable cell types throughout the nervous system. Although LB formation in the midbrain SN and associated dopaminergic cell loss remain the accepted pathologic criteria for definitive diagnosis, they may develop relatively late in the overall disease course. Whereas the manifestation of PD is strongly influenced by aging and possibly other environmental factors, genetic susceptibility may be necessary for all cases, and a growing list of genetic variants appear sufficient to cause disease in a substantial minority of cases, especially in certain ethnic populations. The notion of idiopathic or sporadic PD needs to be reconsidered: All PD is probably genetically influenced, although the number and identity of risk alleles vary from case to case (Figure 5). Finally, as with most complex human phenotypes, there is substantial heterogeneity in PD presentations, and there are also great mimics. At present, making this distinction may not always have a significant impact on patient care. However, in the foreseeable future, we anticipate that answering the question “What is PD?” will become routine in clinical practice, enabling us to identify patients with either prodromal or increased susceptibility for disease and to nominate them for therapies targeting the central mechanisms of pathogenesis.

**SUMMARY POINTS**

1. PD is clinically defined by the development of tremor, bradykinesia, rigidity, and postural instability, and it is also accompanied by a host of nonmotor manifestations, including constipation, urinary symptoms, sleep disorder, and dementia. A PD prodrome of nonmotor features may precede the development of cardinal motor symptoms by many years.
2. PD is pathologically defined by neurodegeneration of SN dopaminergic cells in association with α-synuclein pathology; however, Lewy pathology is additionally manifested in vulnerable cell populations throughout the peripheral and central nervous systems.

3. Several PD-susceptibility genes have been validated, and the identified genetic variants define a broad spectrum of disease risk, blurring the distinction between familial and sporadic disease. Some genes, including SNCA and LRRK2, harbor rare, highly penetrant Mendelian alleles in addition to common polymorphisms that have a more modest effect on disease susceptibility.

4. The discovery of other genetic causes of parkinsonism, including parkin, PINK1, and DJ-1, has defined an important cellular response pathway for oxidative stress that may partly explain the relatively selective vulnerability of SN dopaminergic cells in PD.

5. Genetic advances in PD and related disorders have spurred the development of improved animal models and have defined the core mechanisms underlying the disease. The aggregation of α-synuclein probably comprises a central cascade that leads to neurotoxicity and neurodegeneration.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITEd


43. Reports the important discovery that α-synuclein is a constituent of LBs, establishing a link between rare forms of Mendelian PD and the more common sporadic form of disease.

44. Finds that SNCA locus triplication causes Mendelian PD, suggesting that increased expression of wild-type α-synuclein is pathogenic—a potential mechanism for sporadic disease.


60, 61. Report the important discovery of the LRRK2 gene as a cause of autosomal dominant PD; LRRK2 variants were subsequently shown to be common in sporadic disease.

68. Demonstrates that LRRK2 variants are an important cause of PD in both familial and sporadic disease.


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92. Demonstrates that GBA variants are common in several geographic populations and have a substantial
impact on disease susceptibility.


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**NOTE ADDED IN PROOF**

After this review went to press, another large and important PD GWAS study (see below) was published. This study confirmed the previously known associations at the *SNCA* and *MAPT* loci and identified two additional loci with genome-wide significant associations, including *GAK* and *HLA-DRA*. Suggestive association between the *GAK* locus and PD was previously detected by another study (55).

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Errata

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