Published in final edited form as:
Clin Pharmacol Ther. 2010 October ; 88(4): 467–474. doi:10.1038/clpt.2010.138.

Gene–Environment Interactions in Parkinson's Disease: The Importance of Animal Modeling

MP Horowitz[1,2,3] and **JT Greenamyre**[2,3,4]

[1]Medical Scientist Training Program, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[2]Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[3]Department of Neurology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[4]Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Abstract

Parkinson's disease (PD), a late-onset neurodegenerative disorder, occurs most commonly in a "sporadic" (idiopathic) form, without a clearly defined genetic basis and only a vaguely delineated pathogenesis. Together, the various monogenic forms of PD (i.e., those arising from mutations in single genes) account for a minority of PD cases but have provided crucial insights into disease mechanisms. Although it is commonly believed that sporadic PD is caused by a lifetime of environmental exposures that are superimposed on an individual's composite genetic susceptibility, this hypothesis has not been tested adequately. This article reviews genetic and environmental factors that have been associated with PD and attempts to put these into a pathogenic framework. We argue that animal modeling will become increasingly important in attempting to elucidate gene–environment interactions, to define pathogenic mechanisms, and to provide a platform for testing of targeted therapeutic interventions.

Parkinson's disease (PD) is thought to arise from the convergence of genetic susceptibility, environmental exposures, and aging. It is currently believed that PD is largely sporadic, meaning that the disease arises in individuals without a family history of PD. In a minority of patients, the cause of the disease can be ascribed to mutations in single genes that have been convincingly demonstrated to be pathogenic; these patients are said to have monogenic PD. Characterization of these causative genes has begun to lead to important insights into the mechanisms of the disease, but the extent to which these genes are dysregulated in sporadic PD is a matter of debate (and mostly, conjecture).

Numerous large association studies have identified factors that correlate with altered risk for developing PD, and they have implicated both genetic and environmental factors that may play a role in this risk. The view that gene-environment interactions are critical in the development of PD is generally supported by the following observations, (i) Genes are not everything: the penetrance of some monogenic forms of PD is incomplete and variable, suggesting the existence of modifiers, such as environmental factors, that increase or decrease the disease risk associated with a pathogenic mutation, (ii) There is discordance with respect to PD diagnosis in monozygotic twins. Findings such as a substantial

Correspondence: JT Greenamyre (jgreena@pitt.edu).

CONFLICT OF INTEREST
The authors declared no conflict of interest.

discrepancy in age at onset of the disease in monozygotic twins support the argument that there are modifying factors, (iii) In rare instances, a form of parkinsonism that is virtually indistinguishable from idiopathic PD can be caused by environmental toxins, (iv) An individual's risk of disease after toxin exposure is determined in part by genetic factors; this represents another form of “incomplete penetrance.” Despite the general agreement that gene-environment interactions probably play a role in PD pathogenesis, few studies have been able to address this issue directly in an experimental system.

This review briefly summarizes recent advances in our understanding of the genetic and environmental factors that have been associated with PD, highlighting what can be inferred about mechanisms of cellular pathogenesis. We first examine the relatively few proven monogenic forms of PD and discuss the roles of these genes and proteins in disease initiation and progression. We then discuss evidence associating various environmental factors with PD. After a summary of preliminary studies attempting to elucidate gene-environment interactions, we conclude with a discussion of the importance of continued development of accurate animal models, so that it is possible to trace the ways in which an individuals genetic background, types of environmental exposure, and age interact to result in the development of PD.

MONOGENIC FORMS OF PD

The identification of monogenic forms of PD has led to major advances in our understanding of the pathophysiology of this disease. To date, 16 loci (PARK 1–16) have been associated with PD.[1] Of these, mutations in five genes have been confirmed to cause parkinsonian syndromes that resemble PD: the dominantly inherited α-synuclein (*SNCA*) and leucine-rich repeat kinase 2 (*LRRK2*), the recessively inherited parkin, PTEN-induced putative kinase 1 (*PINK1*), and DJ-1. The other 11 loci require further investigation to determine the precise extent of their contribution to PD. Much of what is known about PD pathogenesis came from work investigating monogenic forms of PD *in vitro* and *in vivo*. However, it should be noted that genetic mutations may give rise to clinical parkinsonism that does not necessarily involve the same pathogenic events as sporadic PD. Hardy and colleagues argue that mechanistic insight into PD can be gleaned only from genetic mutations that cause pathology identical to sporadic cases of PD.[2] However, it is clear that multiple mutations may cause the same pathology and that any single mutation may produce multiple pathologies. In this context, it is prudent to exercise caution when extrapolating from monogenic cases of PD to pathogenic mechanisms in sporadic PD.

SNCA

The first monogenic form of PD was discovered in 1997, when missense mutations in *SNCA*, the gene encoding the presynaptic protein α-synuclein (α-syn) were identified in Italian and Greek families.[3] Subsequent examination of the pathological hallmark of PD—the Lewy body—revealed that they contain abundant α-syn.[4] Given that Lewy bodies were, until recently, believed to be an invariable feature of idiopathic PD, this finding placed α-syn at the center of both familial and sporadic PD pathogenesis.

The aggregation properties of α-syn have been investigated, and many factors, in addition to the pathogenic mutations, have been found to enhance aggregation.[5] These include: phosphorylation at serine 129; C-terminal truncations; interactions with metals or certain proteins or lipids; and pesticides. Of particular note, oxidative stress—thought to be a central feature of PD pathogenesis—has been shown to modify α-syn and increase the likelihood of its aggregation. Dopamine has also been shown to interact with α-syn, forming an adduct and resulting in increased levels of α-syn protofibrils, a toxic form of α-syn. Additionally,

aggregation of α-syn is concentration dependent, and α-syn levels increase within dopamine neurons with aging and in PD.

Intriguingly, although it was recognized that mutations in the *SNCA* gene cause PD, it was subsequently found that duplications and triplications of the locus containing wild-type (WT) *SNCA* also cause PD.[6] These locus multiplications lead to 1.5- to 2-fold increases in α-syn mRNA and protein levels relative to normal α-syn expression levels.[7] Individuals with *SNCA* gene triplication have an earlier onset of the disease and a more severe phenotype than those with gene duplication.[8] This suggests that there is a dosage effect whereby higher levels of α-syn, whether WT or mutant, are associated with more toxicity. *SNCA* gene multiplications appear to have age-dependent or variable penetrance, in view of the fact that they have been found in older individuals in whom imaging of the dopamine system using single photon-emission computed tomography yielded normal results.

It follows that if excessively high levels of WT α-syn are toxic *per se*, then any event contributing to increases in α-syn levels—e.g., decreased degradation of α-syn and increased transcription/translation of *SNCA*—may be involved in PD pathogenesis. In fact, there is evidence suggesting that both of these mechanisms are involved in PD. Intracellular levels of α-syn protein are elevated in the substantia nigra in normal aging and in PD. Impairment of both the ubiquitin–proteasome system and of chaperone-mediated autophagy have been implicated in α-syn accumulation in PD.

With respect to transcriptional alterations, Gründemann and colleagues investigated the content of α-syn mRNA within nigral dopamine neurons in postmortem human brain using laser-capture microdissection and found that the α-syn mRNA level is significantly increased in surviving nigral dopamine neurons in PD relative to controls.[9] This transcriptional deregulation may be explained by the finding that some common polymorphisms in the *SNCA* promoter enhance α-syn expression and are associated with PD. For example, a dinucleotide repeat polymorphism (Rep1) has been identified in the *SNCA* promoter, leading to increased SNCA expression.[10] Additionally, common variants in the 3′ untranslated region of *SNCA* were recently associated with an increased risk of PD in two large genome-wide association studies: one examining a population of European descent, the other a Japanese population. In another study, a polymorphism in the 3′ untranslated region of *SNCA* was associated with increased risk for disease and was shown to increase expression levels of α-syn in a human dopaminergic cell line.

Transgenic expression of WT or mutated α-syn has been used to model this autosomal dominant form of PD *in vivo*, with varied degrees of success. There are several transgenic mouse models in which WT, mutant (A53T or A30P), or truncated human α-syn is overexpressed ubiquitously, either in an endogenous promoter-restricted pattern or via an inducible promoter. Although many of these mice develop motor impairment, few exhibit protein inclusions similar to Lewy bodies, and even fewer display loss of nigral cells. Efforts have also been made to model PD-relevant α-syn toxicity in *Drosophila* and *Caenorhabditis elegans*, with somewhat better success. For example, Feany and Bender generated a transgenic *Drosophila* model using human α-syn that exhibits adult-onset dopaminergic neuron loss, formation of proteinaceous inclusions, and a concomitant motor deficit.[11]

Although α-syn overexpression models are exciting, conclusions must be drawn with caution, given that supraphysiologic levels of α-syn are being produced. As noted, even in the case of *SNCA* locus duplication or triplication, mRNA and protein levels of α-syn increase less than twofold relative to controls.[7] Bacterial artificial chromosome technology, in which entire genes (complete with their native promoter and regulatory elements) are introduced into organisms, maybe advantageous in that transgene expression levels are

typically more mildly elevated in this approach as compared with other transgenic strategies. Nonetheless, because conventional transgenic models with high expression levels fail to reproduce much of the pathology of PD, it seems unlikely that bacterial artificial chromosome transgenics—with lower expression—will provide better models of PD neuropathology.

LRRK2

Gain-of-function mutations in *LRRK2* have been identified as the most common cause of familial PD (accounting for an estimated 5–10% of instances) as well as a relatively common cause of sporadic PD (an estimated 1–5% of instances).[12,13] One of the *LRRK2* mutations, G2019S, is remarkably common in certain populations. For example, in North African and Ashkenazi Jewish populations, up to 40% of familial and sporadic PD is associated with the G2019S mutation. However, this mutation has an age-dependent and highly variable level of penetrance. It has been estimated that the penetrance of the G2019 mutation is 28% at age 59, 51% at age 69, and 74% at age 79.[14] Therefore, this mutation as well as others in *LRRK2* may function more as genetic risk factors than high-penetrance disease genes in many instances. If so, there must be other factors, perhaps including environmental exposures, that influence risk for developing PD. Patients with *LRRK2* mutations are generally difficult to distinguish clinically from patients with sporadic PD in that they exhibit similar age at onset and similar (if not more benign) signs and symptoms and are generally responsive to L-dihydroxyphenylalanine treatment. In rare instances, *LRRK2* mutations are associated with dementia and a tauopathy that is not seen in typical PD.

The sizes of the *LRRK2* gene (51 exons) and protein (2,527 amino acids, 285 kDa) have posed technical challenges to characterizing its physiological and pathophysiological roles *in vitro* and *in vivo*. Despite this, it is clear that LRRK2 is a cytosolic serine/threonine kinase that probably acts in a complex with other proteins and is often found in association with membranes (e.g., endoplasmic reticulum, endosomes, and mitochondria) in neuronal cell bodies, axons, and dendrites. LRRK2 is unique in that it is a multidomain protein that contains both a kinase domain and a GTPase domain in the same open reading frame. How these domains interact to influence LRRK2 function under physiological and pathophysiological conditions is still under investigation. PD-causative mutations have been localized to conserved regions throughout several disparate domains, and it is possible that mutations in different functional domains dysregulate LRRK2 in different ways. This may account for the diverse neuropathological features of LRRK2-related PD discovered at the time of autopsy: histopathology that ranges from nigral dopamine cell loss in the absence of Lewy bodies, to nigral degeneration with Lewy body pathology involving the nigra as well as other structures—and even the presence of tau-positive neurofibrillary tangles.[12]

The most prevalent *LRRK2* mutation, G2019S, occurs within the kinase domain and causes an increase in kinase activity.[15] The increased kinase activity of G2019S mutants may induce pathogenic signaling cascades; however, 43 mutations of LRRK2 have been associated with PD to date (eight of which have been confirmed to be pathogenic) and many of these do not alter LRRK2 kinase activity. Nevertheless, kinase-activating mutations have received the most attention, and there is a great amount of effort to identify substrates of LRRK2-mediated phosphorylation. LRRK2 itself, and also moesin and eukaryo-tic initiation factor 4E-binding protein, have been proposed as possible LRRK2 phosphorylation substrates. The role of each of these putative LRRK2 phosphorylation substrates requires confirmation. Also, additional studies are required to determine whether deregulation of their corresponding pathways plays any significant role in PD pathogenesis from G2019S *LRRK2* mutations.

Transgenic *Drosophila* models of LRRK2 degeneration have been created. In two studies, both mutant and WT LRRK2 expression was associated with neurodegeneration; in a third study, only mutant LRRK2 caused neuronal loss. Partly because of the large size of the *LRRK2* gene (51 exons), it has been difficult to generate conventional transgenic mice expressing full-length LRRK2. However, recent efforts using bacterial artificial chromosome technology have yielded lines that express the entire WT or R1441G mutant *LRRK2* gene.[16] The *LRRK2* R1441G mutant mice display no apparent change in the number of nigral dopamine neurons or density of dopaminergic nigro-striatal terminals, but they do show an age-dependent decrease in locomotion (rearing), decreased spontaneous dopamine release, neuritic pathology in the striatum, and a substantial increase in phosphorylated tau in brain homogenates.[16]

Parkin

Mutations in parkin, an E3 ubiquitin ligase, cause a recessive, early-onset, slowly progressive parkinsonism.[17] Mutations in *parkin* account for the majority of early-onset familial PD, and more than 100 distinct mutations have been identified. Point mutations and deletions can present in a homozygous or compound heterozygous fashion, in any combination. Single heterozygous mutations have been found in some patients with PD, but these are difficult to interpret in terms of causality. There are also reports that single-nucleotide polymorphisms in *parkin* may contribute to some cases of sporadic PD. Clinically, parkin-associated PD typically has an early onset (in the 30s rather than in the 50–60s), a good response to L-dihydroxyphenylalanine (L-DOPA), and a benign course. In contrast to typical PD, parkin cases do not generally have Lewy bodies in the substantia nigra (although they have been reported in a few compound heterozygous patients).

As an E3 ubiquitin ligase, parkin catalyzes the transfer of ubiquitin to target proteins to either mark them for degradation by the ubiquitin–proteasome system or for nondegradative signaling purposes. Several putative parkin ubiquitylation substrates have been identified *in vitro*: CDCrel-1, parkin-associated endothelin receptor–like receptor, synphilin-1, aminoacyl-tRNA synthetase-interacting multifunctional protein type 2, α/β tubulin heterodimers, synaptotagmin XI, HSP70, DJ-1, PICK1, and ataxin-2. Only some of these putative parkin targets accumulate in the brains of patients with pathogenic parkin mutations, and none of these proteins has been found to be significantly elevated in parkin-knockout (KO) animals—suggesting E3 ubiquitin ligase redundancy for some substrates. Parkin-KO animals show some mild mitochondrial defects and evidence of oxidative stress, but there is no loss of nigral dopamine neurons; overall, therefore, they fail to recapitulate the typical parkinsonian syndrome found in humans. So far, although additional parkin substrates will probably be identified, accumulation of particular parkin substrates does not appear to be the mechanism by which mutant parkin causes toxicity. This leaves open the possibility that parkin mutations cause neurodegeneration via loss of anondegradative (signaling) function.

Recently, parkin has been implicated in mitochondrial maintenance, during which it may, under some circumstances, play a role in selectively targeting depolarized mitochondria for mitochondria-specific autophagic degradation (mitophagy).[18] Narendra and colleagues have shown *in vitro* that depolarization of mitochondria with an uncoupling agent results in translocation of cytosolic parkin selectively to depolarized mitochondria and that these mitochondria are subsequently removed in an autophagic process. These findings have been replicated by others, and it appears that the presence of PINK1 on depolarized mitochondria is critical for proper translocation of parkin (see below). Once it reaches the mitochondria, the precise process through which parkin seals their fate (degradation) is unresolved at this point, but it may involve ubiquitylation of a key mitochondrial target protein associated with initiating mitophagy. It is important to note that, although these results are intriguing, the

studies have been carried out primarily in immortalized cell lines, and there is as yet little evidence that these events occur in bona fide neurons.

PINK1

Mutations in *PINK1* cause a rare form of autosomal recessive parkinsonism that is both clinically and neuropathologically similar to parkinsonism due to mutations in parkin.[19] PINK1 is a nuclear-encoded, mitochondrial protein kinase. Most mutations occur in or near the kinase domain and consequently disrupt the kinase activity of the protein.

PINK1-KO flies exhibit a phenotype of mitochondrial defects that is strikingly similar to that of *parkin*-KO flies; and genetic rescue experiments demonstrated that parkin overexpression can rescue the *PINK1*-KO phenotype but that the converse does not occur.[20,21] This indicates that PINK1 not only operates in the same genetic pathway as parkin but also acts upstream of parkin. On the basis of this finding and the possibility that parkin plays a role in targeting dysfunctional mitochondria for mitophagy (see above), recent efforts have been made to further dissect the interaction between PINK1 and parkin. Narendra and colleagues have demonstrated that, when mitochondria are polarized, PINK1 undergoes cleavage and inactivation; they propose that this proteolysis is catalyzed by an as yet unidentified voltage-dependent protease.[22] When mitochondria are depolarized, the protease is inactive, and PINK1 is able to accumulate on the mitochondrial outer membrane and recruit parkin to these mitochondria in a kinase-dependent fashion.[22] PINK1 does not appear to directly phosphorylate parkin, although it may start a signaling cascade that results in parkin recruitment.[22] There is evidence that, once parkin is recruited to depolarized mitochondria, it ubiquitylates VDAC1, leading to the recruitment of the adaptor protein p62, which is known to tether ubiquitylated proteins to nascent autophagic vacuoles. More work is required—particularly in neurons—to define the biochemical pathway responsible for PINK1/parkin-dependent mitophagy in response to mitochondrial membrane impairment, but the studies conducted to date have enhanced our understanding of PINK1 and parkin dysfunction in genetic forms of PD, and have further implicated mitochondrial dysfunction in the pathogenesis of PD.

DJ-1

Homozygous and compound heterozygous mutations in *DJ-1* are extremely rare causes of early-onset recessive parkinsonism,[23] the neuropathology of which is unknown. DJ-1 is a redox-active protein expressed predominantly in astrocytes; this protein senses oxidative stress through modification of a critical cysteine and is subsequently translocated to mitochondria to protect the cell against oxidative stress.[24] There is no consensus as to the precise mechanism through which DJ-1 orchestrates such protection, given that the protein has been proposed to have a broad range of functions—e.g., in RNA binding, in stabilization of antioxidant transcription factors, and in acting as a cysteine protease. Hypothesizing a role for DJ-1 in signaling may explain these disparate observations, but details are still lacking. Despite these uncertainties, DJ-1 represents the third “mitochondrial” protein (after parkin and PINK1) whose loss of function results in a parkinsonian syndrome, thereby helping to reinforce the concept that mitochondrial dysfunction is central to PD pathogenesis.

ENVIRONMENTAL FACTORS AND PD

The term “environmental factor” refers to any influence that originates from outside the genome. Environmental factors include compounds in the air we breathe, the substances we ingest, and certain metabolic changes induced by activities we perform. When considering chemical toxicants, there is a tendency to focus on man-made synthetic compounds, but

humans are exposed on a daily and chronic basis to a very large number of naturally occurring compounds in the atmosphere and in food and water supplies. Fruits and vegetables contain many beneficial compounds, but they may also contain some harmful substances, many of which have yet to be characterized. If environmental factors influence PD pathogenesis or progression, they may do so either through direct action on the cells that die in PD or through indirect actions—for example, by altering the metabolism of other substances, permeabilizing the blood–brain barrier, overstimulating the immune system, or altering hormonal signaling.

The list of environmental factors associated with PD continues to grow in number and diversity. Unfortunately, scientific support for many of the factors posited to contribute to the risk of PD is sometimes limited to retrospective (case–control) studies of low sample size that might be biased by the subjects' limited ability to recall past exposures or activities. These studies can, at best, uncover associations, but they cannot prove causality. Demonstrating a causative role for any of these factors requires the use of *in vitro* and *in vivo* disease modeling.

Age and gender

Among the factors most commonly associated with risk of developing PD are increased age and male gender. The risk for sporadic PD increases in an age-dependent manner across all populations studied to date. The penetrance of certain monogenic forms of PD has also been shown to increase with age. For example, the penetrance of the G2019S mutation in *LRRK2* is incomplete, but increases from an estimated 28% at 59 years of age to 74% at 79 years of age. The odds ratio for risk of developing PD for men as compared with women is consistently found to be ~ 1.5–2.0. It is not known whether the reasons underlying this difference between the genders with respect to risk for PD are biological (e.g., hormonal), sociological (e.g., occupational), or both.

Lifestyle factors

Lifestyle and dietary habits also seem to exert an influence over one's risk of developing PD. There are numerous reports of an inverse, dose-dependent association between tobacco use and PD. Whether this is due to a component of tobacco or to a feature related to tobacco users themselves remains to be determined; however, there is evidence that nicotine alters various components of dopaminergic systems and may protect against dopaminergic cell death.25 Consumption of caffeine—from coffee and tea alike—is also associated with a dose-dependent decrease in the risk of developing PD, according to the results of some studies.[26]

In addition to dietary factors, various medical conditions—obesity, diabetes mellitus type 2, premorbid levels of physical activity, cholesterol levels, and cholesterol-lowering medications (reviewed in Tanner[27])—have also been reported to be associated with altered risk of developing PD; however, many of these studies require replication. Interestingly, serum or plasma levels of urate were found to inversely correlate with risk of PD,[28] perhaps further implicating oxidative stress as a factor in the disease.

Environmental toxins

Initial investigation into the relationship between pesticides and PD began after several young intravenous drug users residing in the same area of California developed acute onset of severe parkinsonism that proved to be irreversible.[29] It was discovered that they had mistakenly injected 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP), a potent neurotoxin that easily crosses the blood–brain barrier into the brain, where its toxic metabolite selectively poisons dopaminergic neurons. This work provided the first proof of

principle that an “environmental” toxin could produce parkinsonism in humans. Because MPTP acts by inhibiting mitochondrial function, this work also provided the first clue that mitochondrial impairment might be important in PD pathogenesis. Furthermore, a structural similarity between MPTP and the commonly used herbicide paraquat was noted (although more recent studies have shown that they have different mechanisms of toxicity). Nevertheless, partly on the basis of this structural similarity, subsequent epidemiological studies found an association between agricultural pesticide use and death from PD.[30]

An increasing number of studies have reported an association between pesticides and PD; however, several issues make such studies difficult to perform and hard to interpret unambiguously. First, they often rely on reports from individual study subjects on the duration, amount, and type of pesticide exposure; thus, there maybe reporter (recall) bias. Second, the accuracy of clinical diagnosis of PD is variable and depends in large part on the training and experience of the investigators. Even experienced, specialized neurologists make the wrong diagnosis 10–15% of the time, and this rate of inaccuracy is higher at earlier stages of the disease. Third, professionals who work with pesticides are often exposed to several pesticides over time rather than to a single agent. Finally, the degree of exposure can vary depending on the duration of use, the concentrations handled, and the safety precautions taken by pesticide handlers. Nonetheless, meta-analyses have found an increased incidence of PD among subjects who have a history of exposure to pesticides. In one such meta-analysis, the odds ratio for an association between professional pesticide use and PD development was 1.9 (95% confidence interval = 1.5–2.5).[31] Living in rural areas and drinking water from wells are factors that have been associated with an increased risk of PD in some studies, and these associations are thought to be due to pesticide exposure.

The identification of individual pesticides that are associated with PD has been challenging for the reasons mentioned, but recent well-designed studies have succeeded in this attempt.[32,33] In a case–control study involving 368 patients with PD and 341 control subjects from the same area, Costello and colleagues used state records of pesticide use over a 25-year period and corresponding land maps of areas these pesticides were used so as to eliminate recall bias.[32] They found a 75% increase in relative risk for PD (95% confidence interval = 1.13–2.73) in subjects who were exposed to paraquat and maneb (a fungicide that is often used concomitantly with paraquat).[32] Subjects <60 years of age at the time of exposure who had been exposed to either of these agents alone had a significantly higher risk of PD, with an odds ratio of 2.27 (95% confidence interval = 0.91–5.70).[32] This risk was significantly elevated when the same age group had been exposed to both paraquat and maneb (odds ratio = 4.17, 95% confidence interval = 1.15–15.16).[32] In another report, Kamel and colleagues used data from the Agricultural Health Study, a large self-report study examining pesticide exposure and PD, and found an increased risk of PD associated with application of four individual pesticides: dieldrin, maneb, paraquat, and rotenone.[33] Levels of the pesticide dieldrin have been found to be elevated in the caudate and substantia nigra of patients with PD as compared with controls.

In order to determine whether certain pesticides can cause a PD-like phenotype, a variety of animal models have been used, including fruit flies, worms, rodents, and nonhuman primates. For example, subchronic administration of rotenone to rats reproducibly induces many of the hallmark behavioral, neurochemical, and neuropathological features of PD, as well as several of the nonmotor neuropathological features seen in PD in humans, including gastrointestinal dysfunction.[34,35] Rotenone also causes α-syn accumulation/aggregation and oxidation as well as mitochondrial translocation of DJ-1. Similarly, treatment of rodents with the herbicide paraquat, the fungicide maneb, or both has also been used to model features of PD.[36] Dieldrin has been used to model aspects of PD *in vivo* as well. Of course, when evaluating *in vivo* pesticide models of PD, it is important to remember that the

chronicity, route of administration, and metabolism of the toxin often differ markedly from occupation-related pesticide exposure in humans. Nevertheless, at a minimum, such models can often provide proof of concept that certain compounds can cause a parkinsonian syndrome.

Aside from pesticides, exposure to several occupational chemicals has been associated with increased risk for PD, but there are often discrepancies between study results, with some of the findings being inconsistent. Associations between metal exposure and PD (e.g., from mining or welding) has been suggested by data from some studies; however, this work has not been consistently replicated, and the clinical and neuropathological features in those patients may have differed in important respects from those in patients with PD. Other chemicals that may be associated with PD include polychlorinated biphenyls and hydrocarbon solvents. Recently, a cluster of PD cases was associated with industrial exposure to the common solvent trichloroethylene, and this compound was subsequently found to cause selective loss of nigrostriatal dopamine neurons and accumulation of α-syn in rats.[37]

Common mechanisms?

Although information regarding specific compounds that increase the risk of PD is limited, it is possible to begin to look for commonalities in their mechanisms of toxicity. For example, although rotenone, paraquat, maneb, and dieldrin are structurally dissimilar, they are all associated with marked oxidative stress. Moreover, rotenone and trichloroethylene, like MPTP, are inhibitors of mitochondrial complex I. Such oxidative and mitochondrial mechanisms of action are consistent with what is known about certain monogenic causes of PD, including *parkin*, *PINK1*, and *DJ-1*. Therefore, to some extent, there may be convergence of mechanisms of action of environmental and genetic risk factors in PD.

GENE-ENVIRONMENT INTERACTIONS: IMPORTANCE OF ANIMAL MODELS

It is widely assumed that PD arises from a combination of an individual's composite genetic susceptibilities and a lifetime of environmental “hits,” in the context of aging. However, there is little experimental evidence that directly supports this idea. In part, this lack of evidence is attributable to the current limitations of experimental systems and techniques. From the environmental perspective, given the differences in lifespan and xenobiotic metabolism alone, it is difficult to model accurately in an animal model the duration, route, and overall burden of exposure to a putative neurotoxicant. From the genetic standpoint, an accurate model of a dominant form of PD would express one mutant allele and one WT allele at endogenous levels under control of the endogenous promoter and regulatory elements; a recessive form might be modeled by either homozygous (or compound heterozygous) mutant alleles or, in some cases, by KO of the gene. Unfortunately, by necessity, most models of dominant PD use conventional transgenic technology and massive overexpression of the mutant gene under the control of an “irrelevant” promoter. To date, genetically faithful models have not been exposed to putative toxicants in a manner that is clearly relevant to human exposures. Instead, most studies have used conventional genetically modified animals and convenient—rather than accurate—dosing paradigms. In most cases, “environmental” exposures have involved administration of MPTP, paraquat, or rotenone, and, as discussed below, for any given PD gene of interest the results have been contradictory.

SNCA

Studies of gene–environment interactions have been performed primarily using transgenic models in which human α-syn (WT or mutant) is overexpressed ubiquitously or in a restricted cellular pattern under a pan-neuronal or catecholaminergic promoter. As noted, these models vary greatly in their behavioral, biochemical, and neuropathological features, and, unfortunately, depending on the specific mutation (A53T, A30P, or WT), the promoter used, and the administration protocol, toxin-induced neuropathology was exacerbated, attenuated, or unchanged by the presence of mutant α-syn.

In studies on the effect of MPTP in mice expressing the A30P mutation, one group reported no exacerbation of MPTP toxicity in mutant mice in which α-syn expression was driven by the Thy-1 or tyrosine hydroxylase promoter, whereas another group reported a clear worsening of toxicity when the transgene was driven by the prion protein promoter.[38,39] Because α-syn gene multiplications also cause PD, transgenic mice overexpressing WT α-syn have been examined. It has been reported that, relative to littermate controls, transgenic mice showed no worsening of MPTP damage, and primary neurons derived from these animals even appeared to be protected against the toxin.[40] In contrast, another group described the presence of ultrastructural damage after MPTP that was not seen in controls.[41]

Paraquat (with or without maneb) has also been investigated in several studies involving transgenic α-syn mice. Treatment of A53T transgenic mice with paraquat and maneb reportedly resulted in widespread α-syn pathology that was not present in littermate controls.[42] Another group found that transgenic mice overexpressing either WT or mutant A53T α-syn under the tyrosine hydroxylase promoter were protected against paraquat-induced loss of nigral dopamine neurons as compared with nontransgenic controls.[43] A third group found that the same paraquat regimen produced no differences in toxicity between transgenic mice overexpressing WT α-syn under the Thy-1 promoter and their littermate controls.[44]

LRRK2

Transgenic LRRK2 animal models have been developed in *Drosophila*,[45,46] *C. elegans*,[47] and mice[16] and are beginning to be utilized in studies of gene–environment interactions. In *C. elegans*, both mutant and WT LRRK2 protected against rotenone and paraquat, and silencing of LRRK2 was associated with increased neurodegeneration after toxin administration. In contrast, in *Drosophila*, expression of either mutant or WT LRRK2 has been associated with spontaneous neurodegeneration and increased sensitivity to rotenone.[46] To date, there have been no reports of gene—environment interactions using transgenic mice overexpressing WT or mutant LRRK2.

Parkin

Because recessively inherited cases of PD caused by mutations in parkin are the result of loss of parkin function, animal modeling of *parkin*-related PD has relied largely on the use of *parkin*-KO animals. *Parkin*-KO mice display no nigrostriatal degeneration but do display subtle defects in mitochondrial respiration and decreases in proteins related to mitochondrial and antioxidant functions.[48] After treatment with MPTP, transgenic and WT littermates reportedly showed equivalent levels of dopaminergic neurodegeneration; however, cultured midbrain neurons from KO animals had an enhanced sensitivity to rotenone.[49] In transgenic *Drosophila* lines that express PD-related missense *parkin* mutations, flies expressing the R275W mutation developed degeneration of dopaminergic neurons, motor impairment, and ultrastructural abnormalities in mitochondria; these features were exacerbated by exposure to rotenone.[50]

PINK1

PINK1-KO mice undergo no changes in the number of nigral dopamine neurons or striatal dopamine content.[51] However, viral-mediated overexpression of WT PINK1 in dopaminergic neurons provides partial protection against MPTP.[52] Interestingly, both the WT and a cytosolic form of PINK1 (lacking a mitochondrial targeting sequence) were protective, whereas a mutant form associated with human PD and a "kinase-dead" form of the protein both failed to protect against the toxin. These data suggest that PINK1 confers protection against an exogenous toxin. Whether the protection is mediated primarily by a mitochondrial form of the protein or a cytosolic form remains to be determined.

DJ-1

Drosophila has two orthologs of DJ-1: DJ-1α and DJ-1β. When both are knocked out, the flies show an extreme sensitivity to paraquat and rotenone but not to reducing agents (dithiothreitol or β-mercaptoethanol) or to the proteasome inhibitor MG132.[53] Mice lacking DJ-1 display no nigrostriatal pathology, but it has been reported that they show extraordinary sensitivity to MPTP, an effect that can be rescued by reintroducing WT DJ-1 (by viral-mediated gene transfer) prior to toxin treatment.[54] Manning-Bog and colleagues have suggested that the enhanced sensitivity to MPTP caused by *DJ-1* KO could be related to an upregulation of the plasma membrane dopamine transporter in striatum, which would increase the accumulation of toxin by nigrostriatal dopamine terminals, thereby increasing their vulnerability to MPTP.[55]

Experimental gene–environment interactions: current limitations and future directions—In less than 15 years, we have gained profound insights into the genetic underpinnings of PD. On the heels of these discoveries, we are beginning to understand the environmental factors that influence the risk of developing PD. In addition, there are now good reasons to believe that gene–environment interactions are major determinants of an individual's chances of developing the disease. As is apparent from the preceding discussion, however, current strategies for modeling such interactions are somewhat rudimentary and have led to contradictory results that are difficult to reconcile or interpret. Whether these unexpected and inconsistent effects reported from studies in animal models are relevant to PD in humans is unclear. It certainly seems logical that the presence of a mutation that predisposes to spontaneous neurodegeneration would lower the threshold or increase the toxicity of exogenous neurotoxicants.

To some extent, the discrepant, counterintuitive results of these studies may reflect the limitations of the current genetic models. As noted, massive overexpression of atransgene may yield misleading results—for example, by promoting protein–protein interactions that would not occur in nature or by inducing compensatory (protective) responses that would not be seen in the disease. A more accurate but technically challenging approach would involve "knocking in" a mutation—inserting a disease-causing mutation into the native genome of the experimental animal. Of course, all models are a compromise between accuracy and expediency—and knock-in animals may have a phenotype that develops too slowly or subtly to be useful.

Similarly, the methods of administering putative neurotoxicants that are commonly used bear little relevance to presumed routes and durations of exposure in humans. Whereas human exposures may occur at low levels over decades, experimental exposures are typically acute. In some cases, experimental toxins are administered over several hours; at best, they are administered over a period of weeks. Of course, the shorter lifespan of experimental animals (especially genetically tractable organisms like *Drosophila* and *C. elegans*) forces the use of unrealistic toxin-exposure paradigms. Furthermore, just as

supraphysiological levels of a transgene may cause spurious results, excessive toxin doses may induce pathogenic mechanisms that are unrelated to human disease.

Against this background, there is a clear and pressing need for refining the models used in such studies. Scientists with expertise in creating accurate, state-of-the-art genetic models of disease need to work closely with environmental neurotoxicolo-gists and neuroscientists. Toxicologists must seek out the most sophisticated and accurate genetic models with which to test putative environmental toxins and must use the most relevant rigorous methods of toxin exposure. Best practices from each discipline must be combined if we are to understand how environmental factors interact with an individual's genetic makeup to produce this devastating illness.

Acknowledgments

This work was supported by National Institutes of Health grants 1P01NS059806 and 1RC1ES018058 and the American Parkinson Disease Association Advanced Center for Parkinson Research at the University of Pittsburgh. We apologize to colleagues whose important work we were unable to cite because of journal format requirements.

References

1. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum. Mol. Genet. 2009; 18:R48–R59. [PubMed: 19297401]
2. Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C. The genetics of Parkinson's syndromes: a critical review. Curr. Opin. Genet. Dev. 2009; 19:254–265. [PubMed: 19419854]
3. Polymeropoulos MH, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997; 276:2045–2047. [PubMed: 9197268]
4. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997; 388:839–840. [PubMed: 9278044]
5. Lundvig D, Lindersson E, Jensen PH. Pathogenic effects of alpha-synuclein aggregation. Brain Res. Mol. Brain Res. 2005; 134:3–17. [PubMed: 15790525]
6. Singleton AB, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003; 302:841. [PubMed: 14593171]
7. Miller DW, et al. Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. Neurology. 2004; 62:1835–1838. [PubMed: 15159488]
8. Ross OA, et al. Genomic investigation of alpha-synuclein multiplication and parkinsonism. Ann. Neurol. 2008; 63:743–750. [PubMed: 18571778]
9. Gründemann J, Schlaudraff F, Haeckel O, Liss B. Elevated alpha-synuclein mRNA levels in individual UV-laser-microdissected dopaminergic substantia nigra neurons in idiopathic Parkinson's disease. Nucleic Acids Res. 2008; 36:e38. [PubMed: 18332041]
10. Chiba-Falek O, Nussbaum RL. Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. Hum. Mol. Genet. 2001; 10:3101–3109. [PubMed: 11751692]
11. Feany MB, Bender WW. A Drosophila model of Parkinson's disease. Nature. 2000; 404:394–398. [PubMed: 10746727]
12. Zimprich A, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004; 44:601–607. [PubMed: 15541309]
13. Paisán-Ruíz C, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 2004; 44:595–600. [PubMed: 15541308]
14. Healy DG, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008; 7:583–590. [PubMed: 18539534]
15. West AB, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc. Natl. Acad. Sci. USA. 2005; 102:16842–16847. [PubMed: 16269541]
16. Li Y, et al. Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat. Neurosci. 2009; 12:826–828. [PubMed: 19503083]

17. Kitada T, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998; 392:605–608. [PubMed: 9560156]
18. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. 2008; 183:795–803. [PubMed: 19029340]
19. Valente EM, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 2004; 304:1158–1160. [PubMed: 15087508]
20. Park J, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature. 2006; 441:1157–1161. [PubMed: 16672980]
21. Clark IE, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature. 2006; 441:1162–1166. [PubMed: 16672981]
22. Narendra DP, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. 2010; 8:e1000298. [PubMed: 20126261]
23. Bonifati V, et al. Mutation sin the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science. 2003; 299:256–259. [PubMed: 12446870]
24. Canet-Avilés RM, et al. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc. Natl. Acad. Sci. USA. 2004; 101:9103–9108. [PubMed: 15181200]
25. Quik M, O'Leary K, Tanner CM. Nicotine and Parkinson's disease: implications for therapy. Mov. Disord. 2008; 23:1641–1652. [PubMed: 18683238]
26. Ascherio A, et al. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 2001; 50:56–63. [PubMed: 11456310]
27. Tanner CM. Advances in environmental epidemiology. Mov. Disord. 2010; 25(suppl.1):S58–S62. [PubMed: 20187243]
28. Weisskopf MG, O'Reilly E, Chen H, Schwarzschild MA, Ascherio A. Plasma urate and risk of Parkinson's disease. Am. J. Epidemiol. 2007; 166:561–567. [PubMed: 17584757]
29. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 1983; 219:979–980. [PubMed: 6823561]
30. Ritz B, Yu F. Parkinson's disease mortality and pesticide exposure in California 1984-1994. Int. J. Epidemiol. 2000; 29:323–329. [PubMed: 10817132]
31. Priyadarshi A, Khuder SA, Schaub EA, Shrivastava S. A meta-analysis of Parkinson's disease and exposure to pesticides. Neurotoxicology. 2000; 21:435–440. [PubMed: 11022853]
32. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am. J. Epidemiol. 2009; 169:919–926. [PubMed: 19270050]
33. Kamel F, et al. Pesticide exposure and self-reported Parkinson's disease in the agricultural health Study. Am. J. Epidemiol. 2007; 165:364–374. [PubMed: 17116648]
34. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 2000; 3:1301–1306. [PubMed: 11100151]
35. Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. Trends Pharmacol. Sci. 2010; 31:141–142. author reply 142. [PubMed: 20096940]
36. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and α-synuclein. J. Biol. Chem. 2002; 277:1641–1644. [PubMed: 11707429]
37. Gash DM, et al. Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity. Ann. Neurol. 2008; 63:184–192. [PubMed: 18157908]
38. Rathke-Hartlieb S, et al. Sensitivity to MPTP is not increased in Parkinson's disease-associated mutant alpha-synuclein transgenic mice. J. Neurochem. 2001; 77:1181–1184. [PubMed: 11359883]
39. Nieto M, et al. Increased sensitivity to MPTP in human alpha-synuclein A30P transgenic mice. Neurobiol. Aging. 2006; 27:848–856. [PubMed: 16006012]

40. Pérez-Sánchez F, et al. Prosurvival effect of human wild-type alpha-synuclein on MPTP-induced toxicity to central but not peripheral catecholaminergic neurons isolated from transgenic mice. Neuroscience. 2010; 167:261–276. [PubMed: 20156526]
41. Song DD, Shults CW, Sisk A, Rockenstein E, Masliah E. Enhanced substantia nigra mitochondrial pathology in human alpha-synuclein transgenic mice after treatment with MPTP. Exp. Neurol. 2004; 186:158–172. [PubMed: 15026254]
42. Norris EH, Uryu K, Leight S, Giasson BI, Trojanowski JQ, Lee VM. Pesticide exposure exacerbates α-synucleinopathy in an A53T transgenic mouse model. Am. J. Pathol. 2007; 170:658–666. [PubMed: 17255333]
43. Manning-Bog AB, McCormack AL, Purisai MG, Bolin LM, Di Monte DA. Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. J. Neurosci. 2003; 23:3095–3099. [PubMed: 12716914]
44. Fernagut PO, et al. Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein over-expression. Synapse. 2007; 61:991–1001. [PubMed: 17879265]
45. Venderova K, et al. Leucine-Rich Repeat Kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease. Hum. Mol. Genet. 2009; 18:4390–4404. [PubMed: 19692353]
46. Ng CH, et al. Parkin protects against LRRK2 G2019S mutant-induced dopaminergic neurodegeneration in Drosophila. J. Neurosci. 2009; 29:11257–11262. [PubMed: 19741132]
47. Saha S, et al. LRRL2 modulates vulnerability to mitochondrial dysfunctionin *Caenorhabditis elegans*. J. Neurosci. 2009; 29:9210–9218. [PubMed: 19625511]
48. Palacino JJ, et al. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J. Biol. Chem. 2004; 279:18614–18622. [PubMed: 14985362]
49. Casarejos MJ, Menéndez J, Solano RM, Rodríguez-Navarro JA, García de Yébenes J, Mena MA. Susceptibility to rotenone is increased in neurons from parkin null mice and is reduced by minocycline. J. Neurochem. 2006; 97:934–946. [PubMed: 16573651]
50. Wang C, et al. Drosophila overexpressing parkin R275W mutant exhibits dopaminergic neuron degeneration and mitochondrial abnormalities. J. Neurosci. 2007; 27:8563–8570. [PubMed: 17687034]
51. Kitada T, et al. Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc. Natl. Acad. Sci. USA. 2007; 104:11441–11446. [PubMed: 17563363]
52. Haque ME, et al. Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. Proc. Natl. Acad. Sci. USA. 2008; 105:1716–1721. [PubMed: 18218782]
53. Meulener M, et al. Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. Curr. Biol. 2005; 15:1572–1577. [PubMed: 16139213]
54. Kim RH, et al. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc. Natl. Acad. Sci. USA. 2005; 102:5215–5220. [PubMed: 15784737]
55. Manning-Bog AB, et al. Increased vulnerability of nigrostriatal terminals in DJ-1-deficient mice is mediated by the dopamine transporter. Neurobiol. Dis. 2007; 27:141–150. [PubMed: 17560790]