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New Developments in Genetic Rat Models of Parkinson's Disease

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Abstract

Preclinical research on Parkinson's disease (PD) has relied heavily on mouse and rat animal models. Initially, PD animal models were generated primarily by chemical neurotoxins that induce acute loss of dopaminergic neurons in the substantia nigra. Upon the discovery of genetic mutations causally linked to PD, mice were used more than rats to generate laboratory animals bearing PD-linked mutations because mutagenesis was more difficult in rats. Recent advances in technology for mammalian genome engineering and optimization of viral expression vectors have increased the use of genetic rat models of PD. Emerging research tools include "knockout" rats with disruption of genes in which mutations have been causally linked to PD, including LRRK2, α-synuclein, Parkin, PINK1 and DJ-1. Rats have also been increasingly used for transgenic and viral-mediated overexpression of genes relevant to PD, particularly α-synuclein. It may not be realistic to obtain a single animal model that completely reproduces every feature of a human disease as complex as PD. Nevertheless, compared to mice with the same mutations, many genetic rat animal models of PD better reproduce key aspects of PD including progressive loss of dopaminergic neurons in the substantia nigra, locomotor behavior deficits, and age-dependent formation of abnormal α-synuclein protein aggregates. Here we briefly review new developments in genetic rat models of PD that may have greater potential for identifying underlying mechanisms, for discovering novel therapeutic targets, and for developing greatly needed treatments to slow or halt disease progression.

Keywords

Parkinson's disease; synuclein; LRRK2; Parkin PINK1; DJ-1; transgenic; knockout; viral; neurotoxin; rat; model

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Introduction

Animal models of Parkinson's disease (PD) are important tools to better understand PD etiology and to develop and test more effective treatments. There are many advantages of rodent PD models compared to other animal models commonly used in basic science research. The brains of mice and rats have relatively conserved neuroanatomy compared to human brains, including the function and connectivity of nuclei that are most affected in PD, such as the substantia nigra pars compacta, locus coeruleus, dorsal raphe, and ventral tegmental area. Involvement of other organs and systems including the enteric system, the olfactory system as well as the innate and adaptive immune systems, can easily be studied in mouse and rat models of PD. This is particularly important for studies of the role of inflammation in PD.[1, 2] There is currently no single PD animal model that perfectly recapitulates all the central features of PD. After many years of substantial efforts to develop animal models of PD, there are now good rat models that reproduce individual or multiple aspects of PD, such as loss of dopaminergic neurons in the substantia nigra, reduced dopamine levels in the striatum, L-DOPA induced dyskinesia, formation of protein aggregates that resemble Lewy bodies or Lewy neurites, locomotor behavior deficits, age-dependent onset and age-dependent progression in severity of PD-related symptoms or pathology.

Prior to the identification of genes with mutations causally linked to PD, animal models were generated primarily using neurotoxins, such as 6-hydroxydopmine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that can cause acute neurodegeneration and death of dopaminergic neurons in the substantia nigra upon intracranial 6-OHDA or intraperitoneal MPTP injection.[3, 4] Chronic administration of L-DOPA to 6-OHDA-lesioned rats can create useful animal models to study L-DOPA induced dyskinesia.[5, 6] There continues to be significant preclinical research using PD animal models generated with 6-OHDA and MPTP, along with other neurotoxins such as rotenone.[7] One of the main drawbacks of neurotoxin models of PD is that the onset of neurodegeneration and behavioral deficits is induced and relatively acute rather than spontaneous and gradually progressing in severity, as occurs in PD. Furthermore, the mechanisms of neurodegeneration in neurotoxin PD models may not be physiologically relevant to the mechanisms of neurodegeneration in PD, except for cases of parkinsonism caused by inadvertent exposure to neurotoxins like MPTP.[8] With the exception of rats treated chronically with rotenone, most neurotoxin animal models do not develop α-synuclein immunoreactive intraneuronal protein inclusions resembling pathognomonic Lewy bodies.[7] Since the identification of human mutations linked to familial forms of PD, transgenic animal models have been generated and characterized with the goal of obtaining PD animal models that more closely model human disease physiology and progression.[9, 10]

Homozygous or compound heterozygous loss-of-function mutations in three genes have been causally linked to recessively inherited PD: PINK1, Parkin and DJ-1.[11–13] Mutations in these genes can be modeled by targeted disruption to generate "knockout" (KO) mice and rats. Gain-of-function single point mutations in LRRK2 and α-synuclein have been linked to dominantly inherited PD.[14–17] Also, duplication or triplication of the entire *SNCA* gene on chromosome 4, coding for normal α-synuclein, causes PD with more *SNCA* gene copies

causing earlier onset, more rapid progression and prominent end-stage dementia.[18–20] This has important implications for generating animal models of PD because elevated expression of normal α-synuclein is sufficient to cause PD in rare families with *SNCA* gene multiplication.[21] This enhances the genetic construct validity of transgenic α-synuclein overexpression models. Because many genetic animal models share construct validity with PD patients bearing the same or similar PD-linked mutations, genetic animal models could reveal pathogenic mechanisms and potentially provide better predictive models to expedite discovery and development of more effective therapies.

Additional methodologies for generating PD models include viral mediated expression of transgenes in various brain regions upon stereotaxic intracranial injection and, more recently, intracranial injection of monomer and fibril forms of α-synuclein, which is the major protein component of Lewy bodies and Lewy neurites.[22] All of these methods continue to be widely used because each PD animal model has advantages and disadvantages, depending upon the outcome measures and the specific aspects of PD being studied (e.g. neuroprotection, regeneration, dyskinesia, progression, etc.). The recent advance in transgenic technology has enabled greater use of rats for genetic PD animal models. We focus this review on genetic rat models of PD, including rats injected with proteins or virally expressed genes with mutations linked to PD. Mouse and neurotoxin animal models of PD remain important research tools described in more detail by many comprehensive and focused reviews.[10, 23–28]

Genetic Rat Models of PD

The identification of human mutations causally linked to inherited forms of PD prompted the generation of genetic PD animal models with the hope of reproducing the spontaneous adult-onset and progressive neurodegeneration characteristic of PD. Most of the genetic PD animal models were initially generated in mice because this was the most feasible system. Surprisingly, genetic mouse models of PD largely fail to develop greater than 50% loss of nigral dopaminergic neurons except when genes are overexpressed or disrupted exclusively in dopaminergic neurons.[29, 30] In contrast to mice, some genetic rat models of PD better reproduce the significant (greater than 50%) nigral cell loss that presumably occurs in humans before the onset of PD motor symptoms.[31] The reasons for the different phenotypes of mice and rats bearing similar gene mutations are not clear. In addition to many behavioral, physiological and biochemical differences between mice and rats, there are substantial differences in gene sequence and expression. For example, rats have significantly lower expression of LRRK2 in the substantia nigra compared to mice.[32]

At the level of individual protein domains (Figure 1), PD-linked mutations appear in highly conserved domains, consistent with the evolutionarily conserved sequences having functional importance. One noteworthy difference between human and rodent proteins is the sequence of α-synuclein at amino acid position 53 (circled in Figure 1). The mutation of alanine 53 to threonine (A53T) in humans is causally linked to familial PD.[14] This presents a conundrum of how best to model the human α-synuclein A53T mutation in rodents because rats and mice already have a threonine at position 53. In fact, position 53 is threonine in α-synuclein from fish, birds, reptiles and all other mammals except primates.[33] The human A53T mutations that appears “naturally” in most other species does not appear

to be detrimental, even in species that live as long or longer than humans.[33] Perhaps humans are uniquely susceptible to A53T due to changes at other amino acid positions in α-synuclein, due to differences in other genes, or due to physiological differences.

PINK1 KO rats

Loss-of-function mutations in the mitochondrial kinase PTEN-induced kinase 1 (PINK1) are causally linked to early onset recessive PD.[11] PINK1 missense, nonsense, and frameshift mutations as well as large deletions spanning multiple exons account for about 4–9% of early onset (~24–47 years) PD in Asian populations but only 2–4% in Caucasian poulations.[34] *In vitro* studies suggest that PINK1 functions as a sensor of mitochondria with low membrane potential and as an activator of Parkin (discussed below) to promote sequestration and degradation of defective mitochondria.[35, 36] In contrast to PINK1 KO mice,[37, 38] PINK1 KO rats exhibit age-dependent loss of nigral dopaminergic neurons beginning at age 6–8 months (corresponding to age 30–40 years in humans).[31, 39] Locomotor behavior deficits appear as early as age 4 months and include reduced rearing frequency and distance traveled in an open field apparatus; reduced hind limb grip strength, and significantly increased foot slips and traversal time on a tapered balance beam apparatus.[31, 40] Non-motor behavioral abnormalities have yet to be fully characterized, however, PINK1 KO rats have significant age-dependent vocalization deficits (reduced intensity by age 2 months, reduced bandwidth by age 4 months, and reduced peak frequency by age 8 months).[40] PINK1 KO rats also exhibit mitochondrial respiration defects and proteinase K resistant α-synuclein aggregates in various brain regions.[39, 40] Although the synuclein aggregates in PINK1 KO rats do not have the same appearance as Lewy bodies in PD brains, it is noteworthy that postmortem examinations of PD patients bearing PINK1 mutations have found Lewy body pathology,[41, 42] which is not always the case for familial forms of PD.[16, 43] The spontaneous nigral neuron loss and the spontaneous appearance of synuclein aggregates are phenotypes not found in most other genetic animal models. Therefore, this recently developed PD animal model may be particularly useful for studying the role of synuclein aggregation in PD pathogenesis and for testing methods to protect against age-dependent loss of nigral dopaminergic neurons.

DJ-1 KO rats

DJ-1 homozygous deletion and point mutations have been linked to autosomal recessive Parkinson's disease.[13] The exact function of DJ-1 remains unclear, but it may be involved in sensing or protecting against oxidative stress.[44, 45] DJ-1 KO mice show mild locomotor behavioral deficits and altered nigrostriatal synaptic physiology without dopaminergic neuron loss, even though they are more susceptible to MPTP-induced neuron loss.[46–48] By contrast, DJ-1 KO rats show significant (~50%) age-dependent nigral dopaminergic neuron loss between 6 and 8 months of age.[31] This is accompanied by locomotor impairments and altered radioligand binding to dopamine receptors in the striatum.[31, 49] Mitochondria isolated from the striatum of DJ-1 KO rats showed significantly altered respiration compared to mitochondria isolated from wild-type control rats.[50] These PD-related abnormalities in DJ-1 KO rats may be useful to study pathogenic mechanisms of familial and possibly idiopathic PD. Although DJ-1 KO rats are relatively new, they have already been used

successfully to test neuroprotective compounds; an HDAC inhibitor was reported to alleviate locomotor behavior impairments and apoptosis in DJ-1 KO rats.[51]

Parkin KO rats

Deletion, truncation and missense mutations in the Parkin gene account for about half of all cases of early onset PD (before age 30) but are rarely found in PD cases with onset of symptoms after age 30.[12, 52] Over 150 pathogenic mutations have been found in all exons and domains, with a clear recessive mode of inheritance and apparent 100% penetrance in homozygous or compound heterozygous individuals.[34, 53] About one third of reported Parkin mutations are point mutations, while deletions or duplications of one or more exons are the most common.[53] Parkin is an E3 ubiquitin ligase that is activated by PINK1 and targets dysfunctional mitochondria for degradation by autophagy, which further implicates mitochondrial dysfunction as a cause of PD.[54, 55] Augmenting Parkin activity or enhancing mitochondrial quality control are potential therapeutic strategies.[56] Overexpressing Parkin can prevent neurodegeneration in rat substantia nigra induced by 6-OHDA or α-synuclein overexpression.[57–60] Parkin KO mice have nigrostriatal mitochondrial respiration defects and increased brain markers of oxidative damage, but no loss of dopaminergic neurons, even when crossed with mice deficient for antioxidant enzymes.[61–64] Unlike PINK1 KO and DJ-1 KO rats, initial characterization of Parkin KO rats shows no significant behavioral deficits or age-dependent nigral dopaminergic neuron loss.[31] Parkin KO rats also lack Lewy bodies or any other pathology immunoreactive to α-synuclein, consistent with the absence of Lewy bodies in most published neuropathology reports of Parkin-linked PD.[65] Nevertheless, Parkin KO rats have proven useful for proteomic studies and for identifying substrates of Parkin, thereby furthering research on cellular and molecular mechanisms of PD.[50, 66]

α-synuclein Transgenic and KO Rats

SNCA, the gene that codes for α-synuclein, was the first gene in which point mutations were causally linked to PD.[14] α-synuclein is also the major protein component of Lewy bodies and Lewy neurites, which are pathological hallmarks of PD.[67] α-Synuclein is abundantly expressed in brain and localizes predominately to presynaptic terminals of neurons, where it facilitates the exocytosis of synaptic vesicles.[68] Because α-synuclein mutations are dominantly inherited, and because duplication and triplication mutations of the wild-type human *SNCA* gene are also causally linked to PD,[18, 19] many laboratories have generated mice and rats overexpressing wild-type and mutant α-synuclein to generate PD animal models.[10, 26] Surprisingly, most lines of transgenic mice overexpressing wild-type or mutant α-synuclein fail to develop significant loss of dopaminergic neurons in the substantia nigra or significant synuclein-immunoreactive pathology similar to Lewy bodies, which are the hallmarks of PD. α-Synuclein knockout mice are neuropathologically normal and α-synuclein knockout rats recently developed using CRISPR/Cas9 genome targeting have yet to be characterized.[69, 70]

In contrast to α-synuclein transgenic mice, several lines of transgenic rats overexpressing human α-synuclein have phenotypes useful for modeling aspects of PD. Bacterial artificial chromosome (BAC) transgenic rats expressing the PD-linked E46K mutant human α-

synuclein at a level 2–3 fold greater than endogenous α-synuclein develop α-synuclein protein aggregates in nigral dopamine neurons with no loss of dopamine neurons and no overt motor deficits at age 12 months, but increased sensitivity to rotenone-induced neurodegeneration even at an earlier age.[71] BAC transgenic rats overexpressing wild-type human α-synuclein show significant loss of nigral TH neurons at age 18 months, reduced striatal dopamine at age 12 months, and locomotor deficits beginning at age 12 months, including decreased rearing behavior and increased time to traverse wide or narrow beams or grids.[72] These rats also develop aggregates of insoluble proteinase K-resistant α-synuclein and significant non-motor behavioral abnormalities including impaired odor discrimination, and changes in novelty-seeking behavior before onset of the progressive motor deficits.[72] Subsequent studies of the same line of BAC transgenic rats overexpressing wild-type human α-synuclein revealed significantly reduced serotonin levels in the hippocampus, with decreased hippocampal serotonergic fibers and unchanged raphe serotonergic neurons.[73] These abnormalities occurred together with α-synuclein aggregation and anxiety-like behavior, as measured by reduced exploration and feeding. This BAC transgenic rat model could be useful for research on PD serotonergic abnormalities and anxiety symptoms, which are frequently observed but understudied aspects of PD.

Although α-synuclein transgenic rats generally do not mimic the severe loss of nigral dopaminergic neurons compared to rats with viral overexpression of α-synuclein (discussed below), advantages of transgenic rat models of PD include less phenotypic variability compared to viral overexpression and avoidance of intracranial surgeries and associated inflammatory responses. Recently, a strain of Berlin-Druckrey rats bearing a synuclein gene promoter mutation has been identified that results in accumulation of synuclein in the mesencephalic area, striatum and frontal cortex, with altered dopaminergic neuron electrophysiology.[74] Together with transgenic rats, these provide valuable research tools that can be combined through breeding with other animal models relevant to PD, including transgenic rats that express green fluorescent protein (GFP) exclusively in dopaminergic neurons.[75]

LRRK2 Transgenic and KO Rats

Dominantly inherited point mutations in Leucine Rich Repeat Kinase 2 (LRRK2) are the most common cause of familial PD.[16, 17] LRRK2 is a large ~286 kDa protein, with multiple domains including enzymatically functional kinase and GTPase domains. It is highly unusual for a single polypeptide to contain both kinase and GTPase activities, which suggests these activities are crucially linked in the regulation or function of LRRK2.[76–78] *In vitro* studies show that PD-linked LRRK2 mutations increase LRRK2 kinase activity, which is required for LRRK2-mediated cell toxicity.[79–81] LRRK2 KO rats have no obvious phenotype but are surprisingly resistant to nigral dopaminergic neuron loss induced by adeno-associated viral (AAV) expression of wild-type human α-synuclein.[82] LRRK2 KO rats are also resistant to recruitment and activation of myeloid cells and nigral cell loss in response to intracranial injection of lipopolysaccharide (LPS) to elicit neuroinflammation.[82] These studies highlight the utility of LRRK2 KO rats for PD research, even though these rats do not have PD-related phenotypes.[83, 84] Several lines of transgenic rats expressing human wild-type LRRK2 or LRRK2 with the PD-linked point mutant G2019S, R1441C or R1441G

have been characterized.[85–89] Similar to mice, no LRRK2 transgenic rats show significant loss of nigral dopaminergic neurons. Nevertheless, Walker *et al.* reported that 6-month old rats overexpressing human G2019S LRRK2 had decreased ability to remain on top of a rotating rod (rotarod test of motor coordination) compared to non-transgenic rats.[88] No abnormalities were found in any of the other motor behavior tests including beam walking, rearing and forelimb use in a glass cylinder, and forepaw use while being dragged backwards at a constant speed. Sloan *et al.* observed age-dependent motor deficits on the rotarod test in BAC transgenic rats expressing human G2019S or R1441C mutant LRRK2, but not in rats expressing wild-type human LRRK2.[86] Intraperitoneal injection of L-DOPA/benserazide significantly increased the time before falling from the rotarod for the transgenic rats but had no effect on the non-transgenic controls. This suggests that these LRRK2 BAC transgenic rats have L-DOPA responsive nigrostriatal dysfunction without detectable cell death or protein aggregation. Consistent with this, fast-scan cyclic voltammetry showed impaired dopamine release evoked by a single pulse in the dorsal striatum in acute brain slices of BAC transgenic rats compared to non-transgenic controls. Furthermore, mutant but not wild-type LRRK2 BAC transgenic rats showed impaired cognitive function/spatial short-term memory, as measured by reduced performance on the alternating T-maze test. Gastrointestinal function was normal according to stool frequency and composition.[86] Inducible transgenic rats with expression of human G2019S LRRK2 starting at age 5 months (early adulthood) did not result in dopaminergic neuron loss but caused impaired dopamine reuptake, measured by microdialysis.[89] This model could potentially be useful for investigating the effects of elevated extracellular dopamine.

Viral Vector Animal Models of PD

More robust PD animal models of nigral dopaminergic neuron loss have been generated by injecting rats intracranially with viral vectors to overexpress α-synuclein in the substantia nigra. Recombinant adeno-associated viral (AAV) vectors and HIV-1-derived lentiviral vectors have been engineered to express transgenes, such as α-synuclein, driven by strong transcriptional promoters like the phosphoglycerate kinase (PGK) promoter or the chicken β-actin (CBA) promoter with enhancer elements from the cytomegalovirus (CMV) promoter.[90–92]. Unlike most other viral vector systems, these can efficiently deliver genes to mature neurons and different viral coat protein serotypes can be used to selectively target different cell types, such as glia versus neurons.[93–95] Unlike transgenic mice, rats with viral overexpression of α-synuclein have significant nigral dopaminergic neuron loss, denervation of the striatum and abundant inclusions immunoreactive for α-synuclein.[90–92]

Numerous recent studies have used recombinant AAV with the genomic elements of AAV serotype 2 packaged with capsid proteins from AAV serotypes 1, 2, 5, 6, 7, 8 or 9 to enhance expression of α-synuclein in the substantia nigra of wild-type rats and many other rat models of PD.[58, 91, 96–119] AAV vectors are typically injected unilaterally into the substantia nigra, which allows the uninjected hemisphere to be used as an internal control for histological analyses and allows locomotor asymmetry behavior to be studied as a measure of nigrostriatal function or degeneration. High purity and high titer ($1 \times 10^{10} – 1 \times 10^{14}$ viral genome copies per ml) viral preparations are generally required to cause greater than 50% loss of nigral dopaminergic neurons, which is typically measured ~8 weeks after injection.

In some studies ~50% nigral cell loss occurs within 1 week, however, greater nigral cell loss is generally observed after 4 weeks post-injection consistent with progressive neurodegeneration.[105]

Methods for viral expression of LRRK2 are limited due to the large size of full-length LRRK2 cDNA (~7.6 kilobases) that is difficult to package within viral capsids, along with the other required sequences. Second-generation recombinant human serotype 5 adenoviruses have been successfully used to express in rats full-length human wild-type and G2019S mutant LRRK2 driven by the neuron-specific synapsin-1 promoter.[120] Recombinant adenoviral vectors were injected unilaterally at six locations in the striatum of adult female Wistar rats and brains were harvested and analyzed by immunohistochemistry 10, 21, and 42 days after injection. Viral titers were matched to 2.3×10^9 viral particles/microliter and 2 microliters were injected at each site. Compared to the uninjected side, injection of G2019S LRRK2 but not wild-type LRRK2 or GFP control viral vectors caused a nearly 10% reduction in nigral dopaminergic neurons by 10 days and a nearly 20% reduction at 21 and 42 days post-injection.[120] This suggests that the PD-linked G2019S mutation is significantly more toxic and causes progressive loss of nigral neurons, presumably due to increased kinase activity. To further test the dependence on kinase activity, the same adenoviral system was used to inject at the same stereotaxic coordinates at similar titers (2.1×10^9 viral particles/microliter) human wild-type, G2019S and G2019S/D1994N kinase inactive control LRRK2. Surprisingly, no significant loss of nigral dopaminergic neurons was observed at 42 days post-injection, even for rats injected with the G2019S LRRK2 expressing adenovirus.[121] Consistent with the absence of nigral cell loss, there was no change in motor behavior. The difference in outcome compared to the previous study was attributed to inefficient or inconsistent retrograde transport of adenoviral vectors from axon terminals in the striatum to neuronal cell bodies in the substantia nigra.[121] Nevertheless, rats injected with G2019S but not wild-type or kinase inactive LRRK2 developed ubiquitin-immunoreactive inclusions in the striatum consistent with kinase-dependent pathology induced by LRRK2, even when LRRK2 fails to be transported from the striatum to the substantia nigra.[121]

One of the advantages of viral vector rat models of PD is the relative ease of overexpressing multiple genes or different variants of the same gene, compared to generating different lines of transgenic rats. Unlike genetic rat models of PD, viral vectors can be injected unilaterally so that the contralateral hemisphere can be used as an uninjected or vehicle injected control within the same animal for neuropathological or biochemical analyses. Moreover, different species and different brain regions can be targeted with the same recombinant viral vector, making comparisons facile relative to transgenic animal models. Another major advantage of this model is that robust behavioral, biochemical, neurochemical and neuropathological outcomes can be observed in as little as little as 3–4 weeks after viral injection. This is advantageous to minimize the time and expense of screening and testing new PD therapeutic strategies. However, genetic PD animal models generally have more consistent spatial and temporal transgene expression. The purity, titer and delivery of viral vectors can drastically affect the percentage of cells that are transduced.[97] The level of viral transgene expression can diminish over time, depending on the promoter or other factors, which could confound longitudinal study designs or studies comparing interventions at different time points. Generating viral vector animal models is usually more labor intensive compared to breeding

genetic PD animal models because each animal must undergo stereotaxic surgery to inject the virus into one or more brain regions.

α-Synuclein Pre-formed Fibril Rat PD Models

Direct injection of pre-formed fibrils (not monomer) of purified recombinant α-synuclein into the striatum or the substantia nigra of rats and mice has recently been shown to generate excellent PD animal models of synuclein pathology and, in some cases, loss of nigral dopaminergic neurons, depending on the length of time between injection and analysis.[122–125] Paumier *et al.* found that unilateral injection of α-synuclein pre-formed fibrils into rat striatum causes bilateral α-synuclein-immunoreactive pathology in many brain regions that innervate the striatum (including cortex, substantia nigra and amygdala) 30 and 60 days post-injection and bilateral loss of ~20–30% of nigral dopamine neurons 180 days post-injection.[124] The bilateral nigral cell loss observed in unilaterally injected rats differs significantly from mice injected the same way, which only showed ipsilateral nigral degeneration.[122] Although nigral cell loss was bilateral, the fibril-injected rats had reduced striatal dopamine only in the ipsilateral hemisphere. This was insufficient to cause apparent motor deficits but caused significant impairment of ultrasonic vocalizations.[124] Injection of α-synuclein pre-formed fibrils into rat substantia nigra causes remarkably similar α-synuclein-immunoreactive pathology and similar ~50% nigral cell loss compared to injection of fibrils into rat striatum, as measured 6 months post-injection.[125]

Unique aspects of this model include the conversion of endogenous α-synuclein into visible brain inclusions or aggregates and the time-dependent spreading of the α-synuclein pathology throughout the brain. Importantly, the brain inclusions are immunoreactive for α-synuclein phosphorylated at serine 129, which is a relatively selective marker of synuclein pathology in human postmortem brains and animal models.[126] The pre-formed fibrils themselves do not appear to form visible inclusions but they presumably catalyze the conversion of endogenous monomeric α-synuclein into visible inclusions as previously shown *in vitro*.[127] This animal model may be particularly useful for determining the relationship between α-synuclein fibrilization and PD-related neurodegeneration, which has long been the subject of intense research.[128] Both this model, and the model generated by intracranial injection of AAV-α-synuclein could be used to test therapies targeting α-synuclein, such as testing anti-α-synuclein antibodies to deplete normal or pathogenic forms of α-synuclein,[129] testing compounds that can mitigate the effects of neuroinflammation caused by α-synuclein aggregates,[130] or testing compounds that can prevent α-synuclein aggregation.[131]

Conclusions

A major impediment to discovering and developing more effective treatments for PD has been the lack of preclinical animal models that reproduce the age-dependent onset and progression of PD symptoms and neuropathology. Recent advances in genetics, transgenic technology and basic neuroscience research have yielded substantial improvements in PD animal models. New rat models of PD, such as those described here, provide unprecedented opportunities for advancement of preclinical research on specific aspects of PD including α-

synuclein neuropathology, progressive nigral cell loss, motor deficits, and non-motor symptoms. Many of the recently developed rat PD models appear to have greater face validity by reproducing more robust and progressive nigral cell loss and α-synuclein pathology compared to mice with the same PD-linked mutations or injections.[105] There is hope that this signifies greater predictive validity for determining the extent to which treatments that prevent or ameliorate neurodegeneration in these animal models will be clinically effective.

Each PD animal model has advantages and disadvantages. Table 1 lists the major neuropathological and behavioral features of published rat PD models and their potential pre-clinical utility. Table 2 lists the specific mutations in genetic rat models of PD related to human PD-linked mutations. The primary advantage of genetic animal models of PD is the knowledge that PD-linked mutations are the root cause of all the neuropathological or behavioral phenotypes that emerge, even if they do not perfectly match humans in every way. This is a significant advantage because the pathogenic mechanisms of the more prevalent idiopathic PD remain uncertain but could overlap with familial PD. Mechanistic insight gained from genetic PD animal models could expedite the development of more effective treatments for idiopathic PD. The limited predictive validity of current PD animal models for screening and development of therapeutically effective drugs can be ascribed to insufficient understanding of the cellular and molecular abnormalities that must be targeted and specifically manipulated in order to prevent or slow PD progression. A shift in reliance away from models that phenocopy PD, even if they may not be etiologically related, toward models that share common pathogenic (e.g. genetic) mechanisms, even if they may not be perfect phenocopies, could have a profound impact on the progress of both basic and translational PD research.

Currently, α-synuclein has potentially the greatest mechanistic overlap between familial and idiopathic PD. There have been substantial recent advances in PD animal models generated by intracranial injection of pre-formed fibrils of α-synuclein.[22] Because these models have exceptional spread of neuropathology immunoreactive to the pathological marker serine-129-phosphorylated α-synuclein, they could be particularly useful for studying the pathogenic role of abnormal forms of α-synuclein, for studying disease spread or progression, and for studying non-motor symptoms of disease that may be related to peripheral α-synuclein pathology. Both the model generated by injection of AAV α-synuclein and the model generated by the injection of α-synuclein pre-formed fibrils could be used to test anti-α-synuclein therapies, such as clearing, depleting, or preventing α-synuclein aggregation. The pre-formed fibril model may also be useful for developing PD biomarkers and imaging methods capable of specifically detecting abnormal α-synuclein. Disadvantages of this model include the requirement for surgically injecting the material into the brain, which is labor intensive, adds technical variability, and perturbs the system by eliciting some neuroinflammation and repair, all of which can increase variability of outcomes.

Future steps to optimize genetic rat models of PD include reducing variability caused by different “strains” of α-synuclein having different propensities to induce the aggregation of α-synuclein and other proteins, such as Tau.[123, 132–134] There is also a need to reduce

variability caused by the effect of endogenous rodent α-synuclein on the aggregation of human wild-type or mutant α-synuclein.[135] It may be necessary to "humanize" genetic animal models of PD by replacing the endogenous α-synuclein with wild-type human α-synuclein in order to obtain consistent, reproducible and robust PD models. It will also be important to determine the extent to which genetic animal models depend on background strain, because this is known to be a major source of variability in neurotoxin and other PD animal models, including intracranial injection of α-synuclein pre-formed fibrils and viral expression vectors.[22] Viral gene expression can be targeted to specific subsets of cells using cell-type specific promoters and viral capsid proteins with different tropism and spreading properties.[94, 136] The increasing number cell-type specific Cre transgenic rat lines, including lines for expression in selective subsets of dopaminergic neurons, will enable increased spatiotemporal gene manipulations in genetic rat models of PD.[137] Much as optogenetics has revolutionized the ability to study the selective effect of specific circuits, pathways, and nuclei on neurological functions, more spatially and temporally focused manipulations in genetic rat models of PD will enhance the ability to study the role of specific proteins, pathways, and cell types in PD initiation, progression and manifestation in motor as well as non-motor symptoms.

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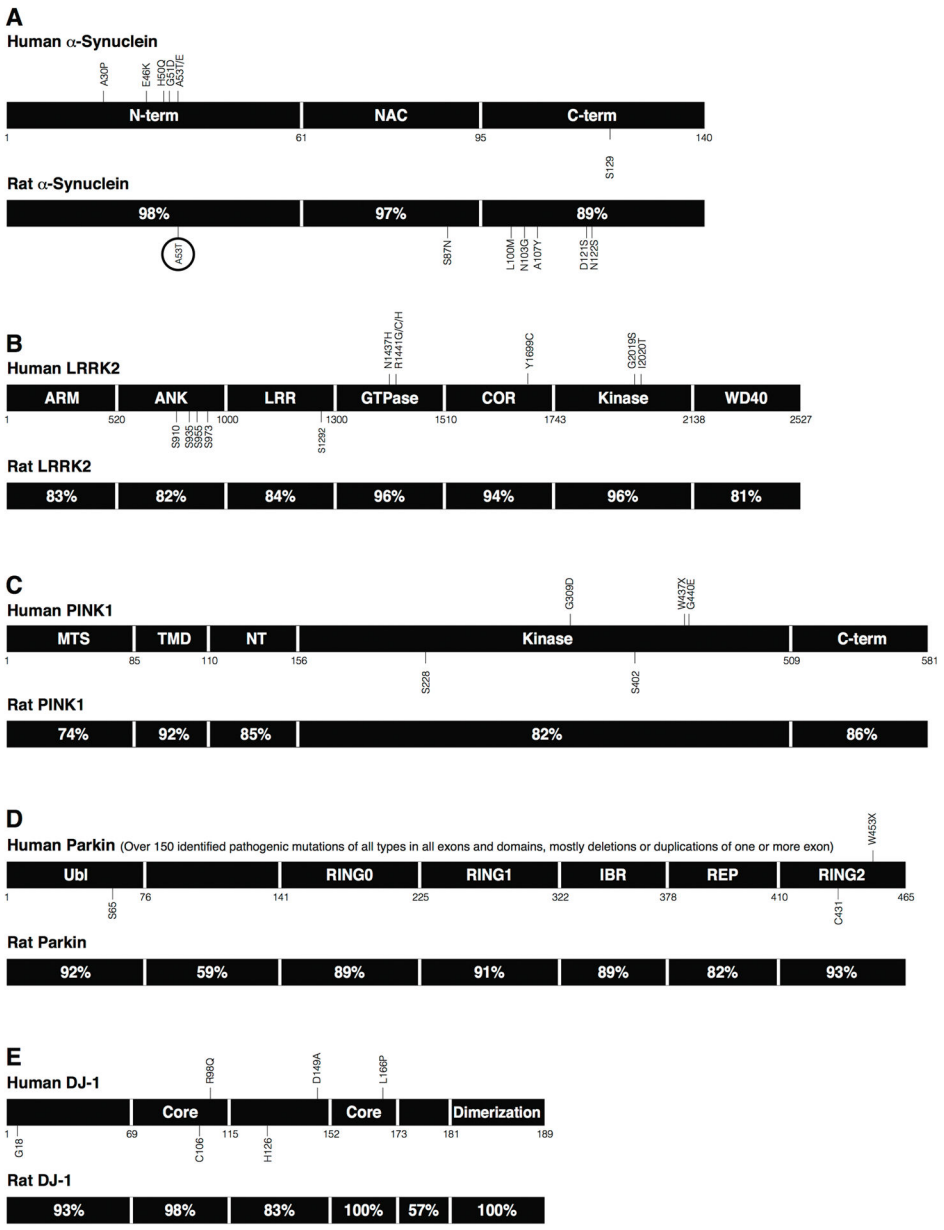

Figure 1.

Human and rat protein primary sequence comparisons for PD-linked genes. For each gene, the amino acid residues at the beginning and end of each domain are shown below the human sequence. PD-linked missense and nonsense mutations are shown on top of the human protein sequence. Functionally important amino acids are indicated at the bottom of the human protein sequence. The percent sequence identity between rat and human for each domain is indicated in the rat primary sequence. **(A)** α-Synuclein domains are annotated as follows: N-terminal domain (N-term); Non-Amyloid component domain (NAC); C-terminal domain (C-term). Phosphorylation of serine 129, shown below, is a marker of pathological forms of α-synuclein. **(B)** LRRK2 domains are as follows: Armadillo-like repeat (ARM); Ankyrin repeat (ANK); Leucine rich repeat (LRR); Roc (ras of complex) GTPase domain

(GTPase); C-terminal of Roc (COR); Kinase domain (Kinase); WD40 domain (WD40). Known sites of LRRK2 phosphorylation are shown below. **(C)** PINK1 domains are annotated as follows: Mitochondrial Targeting Sequence (MTS); transmembrane domain (TMD); N-terminal regulatory region (NT); Kinase domain (Kinase); C-terminal regulatory sequence (C-term). Known sites of PINK1 phosphorylation are shown below. **(D)** Parkin domains are as follows: Ubiquitin Like domain (Ubl); really interesting new gene (RING0, RING1, RING2, respectively); In between RING domain (IBR); repressor domain (REP). Parkin is activated by PINK1 phosphorylation of serine 65, shown below the ubiquitin-like domain, and the identical serine 65 in ubiquitin. Cysteine 431 is required for Parkin mitochondrial translocation and Parkin E3 ligase activity. **(E)** DJ-1 domains are as follows: Core domains (Core); dimerization domain (Dimerization). Cysteine 106, shown below, is highly sensitive to oxidation and required for mitochondrial translocation.

Table 1

Table of Rat Models of PD.

Rat model	Nigral neuron loss	Alpha synuclein pathology*	Motor features	Non-motor features	Pre- clinical relevance	References
Neurotoxin models						
6-OHDA	Yes (Acute)	None reported	Yes	Cognitive impairments; DI disorder	Neurodegeneration	Reviewed in 24
Rotenone	Yes	Yes	Yes (age and dose dependent)	GI disorder	Neurodegeneration; alpha synuclein clearance	Reviewed in 24
Genetic Models						
PINK1 KO rats	Yes (progressive ~50% neuron loss)	Yes (endogenous)	Present- 4 mo.	Impairments in vocalization and approach behavior	Targeting mitochondrial dysfunction; Therapies that clear or reduce alpha synuclein inclusions	31, 39, 40, 49, 50
DJ-1 KO rats	Yes (progressive ~50% neuron loss)	None reported	Present- 4 mo.	None reported	Targeting mitochondrial dysfunction	31, 50, 51
Parkin KO rats	None observed	None reported	None observed	None reported	Targeting mitochondrial dysfunction	31, 50
LRKK2-G2019S	None observed	None reported	Rotarod defect- 3 mo.	Impaired spatial learning and memory	LRRK2 Biomarkers; LRRK2 kinase inhibitors	86
LRKK2-R1441C	None observed	None reported	Rotarod defect- 18–21 mo.	Impaired spatial learning and memory	LRRK2 Biomarkers; LRRK2 kinase inhibitors	86
LRKK2 KO rat	None observed	None reported	None observed	None reported	LRRK2 and alpha synuclein pathological relationship	83, 84
Synuclein E46K	None observed	Yes (12 months)	None observed	None reported	Therapies that clear or reduce alpha synuclein inclusions	71
Other models						
AAV-α-synuclein	Yes	Yes (All AAV-overexpressing models)	cylinder test deficits and forelimb use deficits	None reported	Therapies that clear or reduce alpha synuclein inclusions	Reviewed in 22
Lentiviral-α– synuclein	Yes	Yes	None reported	None reported	Therapies that clear or reduce alpha synuclein inclusions	90
α–Synuclein pre-formed fibrils	Yes (progressive 2–6 months post injection)	Yes (4 weeks post injection)	None observed	Impaired ultrasonic vocalizations	Therapies that clear or reduce alpha synuclein inclusions	22, 124, 125

*Synuclein-immunoreactive aggregates but no Lewy bodies have been reported in rat models of PD.

Table 2

Human gene mutations linked to PD and modeled in rats

Gene	Human Mutations	Mode of Inheritance	Rat Model Mutations	References
Alpha synuclein (PRK1)	A53T, A53E, A30P, H50Q, E46K, G51D	Dominant	Human E46K expressed via BAC transgenic	14, 15, 71
LRRK2 (PRK8)	N1437H, R1441C/G/H, Y1699C, G2019S, I2020T	Dominant	Human G2019S expressed via BAC transgenic; Human R1441C expressed via BAC transgenic	16, 17
Parkin (PRK2)	Deletions, Insertions, Frameshifts, Missense, and Nonsense mutations in every exon; Deletions and duplications of one or more exons are most common mutations.	Recessive	5 bp deletion in exon 4	12, 31
PINK1 (PRK6)	Missense and Nonsense mutations Q21X, G440E, Q456X, Q129fsX157, W437X, A271D, G309D, Exon deletions or duplications	Recessive	26 bp deletion in exon 4	11, 31
DJ-1 (PRK7)	L166P, D149A, R98Q, Exon deletions or splice-site alterations.	Recessive	9 bp deletion and 1 bp insertion in exon 5	13, 31