LRRK2 mutations and neurotoxicant susceptibility

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Abstract

Interactions between genetic and environmental factors are thought to contribute to the pathogenesis of the majority of Parkinson's disease (PD) cases. However, our understanding of these interactions is at an early stage. Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common cause of hereditary PD. Penetrance of LRRK2 mutations is incomplete and variable, suggesting that other environmental or genetic factors may contribute to the development of the disorder. Recently, using animal models, several attempts have been made to understand if LRRK2 may mediate sensitivity to environmental neurotoxicants. Here, we critically review the most current data on how LRRK2 mutations influence neurotoxicity in PD models.

Keywords: Parkinson's disease, leucine-rich repeat kinase 2, gene–environment interaction

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Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder.[1] PD affects more than 1% of population over age 60. Primary motor symptoms typically include a combination bradykinesia, postural instability, and resting tremor. Non-motor symptoms are often present, including autonomic dysfunction, cognitive dysfunction, and mental health disorders.[1–3] PD is a progressive, age-related neurodegenerative disease with pathology characterized by the loss of dopamine (DA) neurons in the substantia nigra (SN), resulting in striatal DA depletion, and the presence of aggregated α-synuclein in cytoplasmic inclusions, known as Lewy bodies.[2,4] To date, no proven treatment has been discovered that is able to halt or slow disease progression.

The etiology of most PD cases is largely unknown, termed ''sporadic.'' Both epidemiological investigations and animal studies have repeatedly linked several classes of compounds to the disease, including pesticides, heavy metals, and other compounds.[5–7] Recent advanced epidemiology studies have begun to pinpoint specific interactions between environmental and genetic factors.[8–11] In the last decade, numerous genetic factors, including mutations, deletions, and expansions have also been identified that contribute to the etiology of PD.[12–14]

Currently, up to ~10% of PD cases can be directly attributed to genetic factors. Prominent risk genes include α-synuclein, leucine-rich repeat kinase 2 (LRRK2), glucocerebrosidase, DJ-1, PINK-1, and Parkin. In 1997, it was discovered that a mutation in a single gene, α-synuclein could cause a form of PD. Here, an Italian family with an autosomal-dominant form of parkinsonism resembling sporadic PD with early disease onset was discovered.[13] Additionally, other autosomal dominant mutations and duplication and triplications of the α-synuclein gene have also been found, but, all are rare.[15–17]

The discovery of LRRK2 mutations as a causative PD factor resulted in heightened awareness of the importance of genetic factors in PD. Currently, mutations in LRRK2 are the most common genetic cause of PD. For example, a monogenic mutation (G2019S) in certain ethnic groups (Tunisian Arab Berbers) can explain almost 40% of cases.[18,19] Nearly 50 variants of LRRK2 gene are associated with PD and a subset including, R1441C/G/H, I2020T, Y1699C, and G2019S LRRK2 mutant are known to be pathogenic.[3] However, the pathophysiological function as well as normal physiological function of the LRRK2 is not clearly understood. The underlying logic for studying genetic mutations of PD is based on the belief that inherited and sporadic forms of the disease share common mechanistic features that may lead to identification of molecular and biochemical pathways that influence disease pathogenesis. It is worth noting that PD directly attributable to LRRK2 mutations is typically indistinguishable from sporadic PD cases,[19,20] potentially indicating common pathogenic pathways. As such, progression in understanding of pathophysiological function of LRRK2 using animal models can contribute to the identification of underlying molecular or biochemical pathways of PD development. Evidence from epidemiological studies on incomplete penetrance of LRRK2 carriers suggests other genetic or environmental factors may be needed for pathogenic mutant LRRK2 to trigger

PD pathogenesis. In this mini review, our discussion focuses on a critical evaluation of data on how LRRK2 may mediate sensitivity to neurotoxicant exposures, representing gene–environment interactions with the potential to influence a significant number of PD cases.

LRRK2 in human PD

LRRK2 is a complex protein (~280 kDa, 2527 amino acids), containing multiple functional domains including, a Ras-like small GTPase domain, a MAPKKK-like kinase domain, and three potential protein interaction domains (an ankyrin domain, leucine-rich repeats, and WD40 repeats).[21,22] LRRK2 is expressed in diverse mammalian cell types. In the brain, LRRK2 is expressed in subcellular components, including cytoskeletal structures of diverse neuronal cell types.[23] In neurons, the physiological function of LRRK2 has been reported to mediate autophagy, neuroinflammation, regulation of cytoskeleton, synaptic vesicle storage, and mobilization, as well as MAPK kinase signaling.[24–28] Yet, the physiological function of LRRK2 is currently not well defined with an unknown substrate.

Rare missense mutations and more common genetic polymorphic variability in LRRK2 are found to be important risk factors to late-onset PD.[29] Several mutations in LRRK2 have been found to be pathogenic.[14,19,20] PD caused by LRRK2 mutations is typically clinically and pathologically indistinguishable from sporadic PD.[19] Interestingly, PD patients with LRRK2 mutations (R144C, Y1699C, I2020T) other than G2019S frequently do not have Lewy body pathology, but sometimes exhibit neurofibrillary tau tangles.[30] PD-causing mutations are mainly concentrated in the kinase, Roc, and COR domains: G2019S and I2020T occur in the kinase domain, R1441C/G in Roc domain, and Y1699C and N1437H in COR domains.[23] Of those, the G2019S mutation at the conserved Mg^{++}-binding motif in the kinase domain is the most common genetic cause of PD. Accumulating evidence suggests that gain of function in kinase activity by mutation likely mediates the neurotoxic mechanisms leading to PD phenotypes.[31–34] LRRK2 G2019S-induced PD cases explain 1% of patients with sporadic PD and 4% of patients with hereditary PD.[19,20] However, the frequency of LRRK2-induced parkinsonism varies by specific mutation and ethnicity.

LRRK2 mutations are more frequently found in certain ethnic groups.[19] LRRK2 G2019S-induced PD in Tunisian Arab Berbers cases explain 39% of patients with sporadic PD and 36% of patients with hereditary PD. LRRK2 G2019S-induced PD in Ashkenazi Jewish was observed in 10% of patients with sporadic PD and 28% of familial-linked patients.[19] Penetrance of carriers of mutated LRRK2 depends on specific mutations. Mutations within the kinase domain (G2019S and I2020T) have similar penetrance, but tend to be significantly more penetrant than mutations in the Roc domain (R1441C and R1441G). Within the Roc domain, R1441C and R1441G LRRK2 have similar penetrance. N1437H and Y1699C LRRK2 mutations in the COR domain are the most highly penetrant compared with other LRRK2 mutations. It should be noted that data on N1437H are derived from a very small sample size ($n = 7$).

In comparing penetrance between mutations, age of onset and percent of diagnosed carriers are two of the most commonly reported metrics. N1437H LRRK2 had a lower quartile of 46 years or younger, a median of 49 years of age, and an upper quartile of 50 years or older. Y1699C LRRK2 had a lower quartile of 44 years or younger, a median of 50 years of age, and an upper quartile of 56 years or older. I2020T LRRK2 had a lower quartile of 51 years or younger, a median of 55 years of age, and an upper quartile of 60 years or older ($n = 29$). G2019S LRRK2 had a lower quartile of 49 years or younger, a median of 57 years of age, and an upper quartile of 67 years or older ($n = 330$). R1441C LRRK2 had a lower quartile of 65 years or younger, a median of 71 years of age, and an upper quartile of 77 years or older ($n = 27$). R1441G LRRK2 had a lower quartile of 60 years or younger, a median of 65 years of age, and an upper quartile of 72 years or older ($n = 104$).[35]

In particular, Israeli Ashkenazi Jewish LRRK2 G2019S carriers had a 57.9-year-old mean age at onset, Tunisian Arab Berbers had 57.1 years, whereas Norwegian carriers had a 63-year-old mean age at onset.[36] The risk for an inherited G2019S LRRK2 carrier to develop PD was also estimated as 28% at age 59, 51% at 69, and 74% at 79 years, which suggests incomplete of the penetrance up to nearly 20 years after the average age of onset in sporadic PD.[19]

Considering the incomplete penetrance of pathogenic LRRK2 mutations, especially G2019S, other genetic or environmental factors must likely be added to induce disease development. Thus, it is critical to examine mechanistically and pathologically how LRRK2 mutations might modulate sensitivity to environmental insults.

LRRK2 modulation of DA neurotoxicity

Many *in vitro* studies suggest that enhanced LRRK2 kinase activity in a GTPase-dependent manner is a major pathogenic mechanism of LRRK2-induced PD. In an *in vitro* model, R1441C-mutated LRRK2 was found to increase kinase activity in a GTPase-dependent manner. It has also been shown that G2019S or R144C mutant LRRK2 has a gain of function in kinase activity.[37] LRRK2 kinase activity is required for induction of inflammation and activities of microglia, which plays a critical role in the pathogenesis of PD.[27] Furthermore, mutant (G2019S, R1441C, Y1699C) LRRK2 expression was also found to cause significant cell death in a neuronal cell line (SH-SY5Y) and in rodent primary neuronal culture compared to WT LRRK2.[38] Pathogenic G2019S LRRK2 has also been reported to induce cytotoxicity through increased GTPase-dependent kinase activity compared to WT LRRK2.[33] Overexpression of mutated (R1441C, Y1699C) LRRK2 caused significant cytotoxicity in a kinase-dependent manner compared to WT LRRK2.[31] Therefore, overexpression of G2019S, R1441C, Y1699C LRRK2 in cell lines and primary neuronal culture has been repeatedly found to elicit marked neuronal toxicity compared to WT LRRK2.

Drosophila models

Drosophila is suitable for studying late stage PD because of a short lifespan and possession of a DA system. A highly conserved single ortholog of LRRK2 was identified in Drosophila genome and it encodes a 2445 amino-acid protein containing GTPase and kinase domains homologous to human LRRK2.[39] Critical sequences mutated in familial LRRK2 PD are conserved between human LRRK2 (hLRRK2) and Drosophila LRRK (dLRRK).[40]

Previous studies in *drosophila* have reported conflicting evidence for on whether LRKK2 knockdown is protective in PD models. This controversy illustrates a need for increased understanding for the role of LRRK2 in normal and pathological neurobiology.

Flies expressing dLRRK (*Drosophila* ortholog of LRRK2) with a deleted kinase domain live normally without any overt developmental or DA neuron abnormalities. However, mutant dLRRK flies exhibit increased mortality resulting from hydrogen peroxide treatment, but not in response to rotenone, paraquat, or β-mercaptoethanol treatments.[41] Another set of experiments using dLRRK knockout flies did not show DA neurodegeneration compared to non-mutant controls. However, significantly elevated DA levels compared to non-mutant control were observed. In this experiment, when exposed to oxidative stress inducers (H_2O_2 or paraquat), knock-out flies were more resistant to mortality in comparison to non-mutant WT flies.[40] However, the authors did not report any changes in DA neuronal cell number when the knock-out mutant flies were exposed to the toxicants (H_2O_2 or paraquat). These results may suggest that dLRRK provides general, non-DA-mediated protection from toxic insults. In contrast, it was reported that (1) ectopic overexpression of WT or R1069C (*Drosophila* ortholog of human R1441C mutant) dLRRK did not induce any DA neurodegeneration, compared to non-transgenic endogenous dLRRK; (2) loss-of-function (through imprecise excision) mutant dLRRK flies showed DA neuron degeneration and locomotor deficit compared to their non-mutant counterpart.[42] However, the results provided by Lee et al.[42] are difficult to interpret due to lack of statistical analysis.

Other studies have utilized expression of human LRRK2. In *Drosophila*, overexpression of WT human LRRK2 did not affect DA neurons, but expression of disease-causing mutations such as G2019S, Y1699C, or G2385R induced late-onset loss of DA neurons with locomotor deficits.[43] In this study, LRRK2 mutant (G2019S and G2385R, but not the Y1699C) flies showed increased DA neurodegeneration in response to the DA neurotoxicant rotenone. Interestingly, co-expression of human parkin, a protein linked to PD and important in protein degradation, provided significant protection in G2019S LRRK2 flies from DA neurodegeneration induced by aging or exposure to rotenone.[43] Previous work also demonstrated that flies overexpressing human WT parkin were significantly resistant to rotenone-induced DA neurodegeneration.[44] In addition, parkin was found to physically interact with LRRK2 protein *in vitro*.[38] It remains unclear whether this protective effect by parkin against rotenone is through direct interaction with mutant LRRK2 or indirectly with oxidative stress and/or mitochondrial homeostasis.[43] Similarly, overexpression of Y1383C or I1915TdLRRK (Drosophila ortholog of human Y1699C or I2020T LRRK2) induced DA neurodegeneration in flies. In that study, WT dLRRK expression did not cause DA neurodegeneration compared to normal flies. Paraquat or H_2O_2 exposure in Y1383C or I1915T dLRRK expressing flies resulted in significantly heightened mortality compared to toxicant-treated flies expressing WT dLRRK or non-transgenic flies.[40] Unfortunately in this study there was no systematic comparison in mortality between Y1383C or I1915T dLRRK flies and WT dLRRK flies. Also, only survival was reported. More specific endpoints, such as DA cell loss were not examined.

In contrast to earlier studies, others have found that both overexpression of WT LRRK2 and mutated LRRK2 caused selective loss of DA neurons and age-dependent behavior deficits.[45–47] In a fly model using *GAL4/UAS* (*GAL4* promoter), flies expressing WT or G2019S LRRK2 showed loss of DA neurons and photoreceptor cells, locomotor deficits, and early mortality. Here, expression of G2019S LRRK2 induced a more severe phenotype than WT LRRK2.[45] Similarly, Yang et al.[34] reported that (1) H_2O_2 did not affect DA neurons and survival in non-transgenic flies; (2) H_2O_2 induced significant DA neuron loss and mortality in WT LRRK2 and G2019S LRRK2. Again, H_2O_2 induced more severe DA neuron loss in G2019S than WT LRRK2 flies. Importantly, treatment with a kinase inhibitor or an anti-oxidant significantly suppressed the PD-like phenotype. In this study, WT and G2019S LRRK2 expression alone decreased the number of DA neurons and survival in the flies compared to non-transgenic flies, with G2019S LRRK2 resulting in heightened sensitivity in both endpoints.

Using targeted expression, Venderova et al.[47] observed that human LRRK2 expression (WT, Y1699C, I1122V, I2020T; under tyrosine hydroxylase gene promoter) in *Drosophila* induced loss of DA neurons, without significant differences between mutant LRRK2 and WT LRRK2. The addition of chronic rotenone treatment (100 μM) further enhanced DA neurodegeneration of I1122V and I2020T LRRK2 flies compared to WT LRRK2 flies. However, ubiquitous expression (under *Da-GAL4* driver) of either WT or mutant human LRRK2 in flies extended the basal lifespan of the flies compared to control at room temperature, although these animals were more sensitive to rotenone. These discrepancies are difficult to interpret, as mammalian studies have shown that LRRK2 expression occurs in specific neuronal and glial populations and also systemically in immune cells.[48–50]

In *Drosophila*, the majority of studies show that expression of human LRRK2 may be sufficient to induce DA neurodegeneration. Expression of disease-causing mutations typically results in heightened DA cell loss. Additionally, when flies were exposed to H_2O_2 or rotenone, flies expressing human LRRK2 exhibited heightened DA neurodegeneration compared to non-transgenic flies. Interestingly, only LRRK2 mutants (G2019S, G2385R, I2020T, I1122C) in the kinase domain showed significantly

heightened DA neurotoxicant sensitivity compared to WT LRRK2. Mechanistic experiments on LRRK2 activity suggest that increased kinase activity may mediate toxicant sensitivity. G2019S LRRK2-induced neurodegeneration in *Drosophila* models has been shown to be rescued by inhibition of kinase activity using GW5074 or sorafenib.[46] Taken together, these studies strongly suggest that increased neurotoxicant sensitivity is dependent on mutations that increase kinase activity.

C. elegans models

In *C. elegans*, either WT or G2019S human LRRK2 expression in DA neurons (under DA transporter promoter) decreased the fluorescent intensity of the DA transporter (DA neuronal cell marker), with G2019S LRRK2 expression resulting in heightened loss relative to WT.[51] Yao et al.[52] also found that overexpression (under the DA transporter promoter) of WT, R1441C, G2019S LRRK2 in DA neurons of *C. elegans* all induced DA neurodegeneration, locomotor, and other behavioral dysfunction. Furthermore, in that study, R1441C or G2019S LRRK2 caused more severe phenotypes than WT LRRK2. G2019S LRRK2 expression only in DA neurons (under DA transporter promoter) of *C. elegans* induced significant DA neurodegeneration compared to non-transgenic *C. elegans*.[53] Similarly, another study reported that G2019S LRRK2 overexpression (under DA transporter promoter) in *C. elegans* induced significant DA neurodegeneration in a kinase activity-dependent manner compared to non-transgenic controls.[46]

Overall, it seems that overexpression of WT or mutated (G2019S, R1441C) in DA neurons induces DA neurodegeneration in *C. elegans*. However, mutated LRRK2 (at least G2019S and R144C) overexpression seems to cause more severe DA neurodegeneration compared to WT LRRK2, even though the two studies by Ray et al.[53] and Liu et al.[46] did not include *C. elegans* overexpressing WT LRRK2 as a controls, rendering it difficult to determine if mutation expression was directly responsible for neurodegeneration.

Data on toxicant sensitivity modulation by LRRK2 are more limited in C. *elegans* than *Drosophila*. For example, Ray et al.[53] reported that when exposed to mitochondrial complex I inhibiting bacterial metabolites produced by *Streptomyces venezuelae*, G2019S LRRK2 mutant *C. elegans* showed significantly enhanced loss of DA neurons compared to non-transgenic counterparts. Saha et al.[51] also found that rotenone treatment (250 nM) induced increased loss of DA neurons in G2019S LRRK2 *C. elegans* compared to WT LRRK2 *C. elegans*.

One study demonstrated an antagonistic role of pink-1 and lrk-1 (C. *elegans* homolog of human LRRK2) in stress response and neuronal activity.[54] A loss-of-function mutation in pink-1 induced mitochondrial cristae length reduction, axonal outgrowth defect, and increased paraquat sensitivity, which was suppressed in lrk-1 mutants lacking expression of the kinase domain. Conversely, lrk-1 mutant *C. elegans* showed hypersensitivity to the endoplasmic reticulum stressor tunicamycin, which was rescued by in pink-1 mutants.

It is worth noting that both *Drosophila* and *C. elegans* do not express α-synuclein. While many attempts have been made to express WT and disease-causing mutations in α-synuclein in these animals, the interactions with LRRK2 and neurotoxicants have yet to be examined. It may be that LRRK2 mutations may modulate neurotoxicity differently in organisms lacking α-synuclein, which has been repeatedly shown to be a key mediator of pathogenesis in mammalian models.

Rodent models

To date, numerous LRRK2 rodent models have been created. In mice, knock-out of LRRK2 did not affect survival of the animal or nigral DA neuron numbers.[55,56] Interestingly, knock-out mice showed α-synuclein accumulation, impaired protein degradation pathways, and apoptotic cell death in the kidney of aged mice.[57] Overall, LRRK2 knock-out mice did not show any overt abnormalities in growth, viability, or nigrostriatal DA neuronal system.

R1441C knock-in and G2019S knock-in mice did not produce detectable neuronal cell loss.[58,59] Bacterial artificial chromosome (BAC) transgenic mice overexpressing WT, R1441G, G2019S LRRK2 also did not show overt DA degeneration, but these animals exhibited alternations in DA transmission and motor function.[60–62] A previous study, using WT or G2019S LRRK2 BAC transgenic mice reported that WT LRRK2 overexpression induced hyperactivity and increased performance in motor function tests, but G2019S LRRK2 overexpression did not induce changes.[60] Overexpression of G2019S LRRK2 under control of calcium/calmodulin-dependent protein kinase II (CaMKII) promoter also did not induce obvious neuropathological abnormalities in mice.[41,56] This may have occurred because LRRK2 was expressed at low levels due to the CaMKII promoter. Overexpression of G2019S LRRK2 under control of Thy1 regulatory sequences also did not cause DA neuropathology. Here, transient increased motor performance by G2019S LRRK2 mice was observed. It is worth noting that Thy1 based lines do not express LRRK2 in the DA neurons of SN.[59] In contrast, under the control of CMV-enhanced human platelet-derived growth factor β-chain (CMVE-PDGFβ) promoter, human G2019S LRRK2 overexpression did induce nigrostriatal DA neurodegeneration (stereological counts in SN pars compacta and stereological measurements of neurite density in SN pars reticulata) in an age-dependent manner in mice without obvious behavioral abnormalities.[63] This hybrid promoter was demonstrated to drive long-term neuronal specific transgene expression in rat brain, including DA neurons in SN.[64] However, this CMVE-PDGFβ-lined G2019S LRRK2 model as of yet has failed to reproduce a motor phenotype. Also, due to the low levels of WT LRRK2 expression in mice, with unknown reason, it is not possible to discern whether the DA neurodegeneration observed in G2019S LRRK2 mice was caused purely by the mutation or simply LRRK2 overexpression.

As previously discussed, mutated (human R1441G and G2019S) LRRK2 expression in mice did not reproduce gross nigrostriatal DA neuron degeneration, but rather, subtle changes such as axonal pathology in DA neurons,

decreased DA release, and hyperphosphorylated tau.[61,62] In contrast, using an adenoviral vector to achieve ectopic human LRRK2 overexpression in rats, G2019S LRRK2 caused a progressive degeneration of nigral DA neurons, where expression of WT human LRRK2 did not induce any significant neurodegeneration.[65] It should be noted that viral vector studies utilize stereotaxic infusion, resulting in targeted cell transduction, a route and temporal expression profile that differs from transgenic lines.

Previously developed transgenic LRRK2 mouse models did not confirm overexpression of LRRK2 in SN pars compacta, nor produce DA neuron loss. Although two G2019S LRRK2 transgenic mouse models constructed using intrastriatal viral vector injection reproduced neurodegeneration of striatal DA neurons, they may have limited relevance in LRRK2 expression because of restricted expression only in the nigrostriatal DA system. This expression pattern is in contrast to LRRK2 expression observed in the postmortem brain of LRRK2-linked PD patients.[66] Further, in these animal models, it was found that LRRK2 expression diminished over time,[32,65] which is unlikely to occur in humans. However, a key advantage of such studies is the ability to test and compare multiple mutations and WT controls, where it is much simpler to create an additional test vector, rather than an additional transgenic rodent line. Thus, these studies are often better controlled, including a WT human expression group.

In a viral vector injection study, the authors could not detect any protein at the size expected for monomeric LRRK2 in SN, nor in striatal extracts.[65] Further, LRRK2 expression was shown to diminish over time. In contrast, BAC transgenic mice studies have reported 5–10 times increased LRRK2 expression in comparison to endogenous levels.[60,61] These ranges of LRRK2 expression levels are very similar to those in BAC G2019S transgenic rat in previous studies.[22,49,50] Our own study on the characterization of BAC transgenic G2019S LRRK2 rat also confirmed a high level of localization of LRRK2 in DA neurons in SN as well as overexpression within cells of other brain regions such as striatum, frontal cortex, and hippocampus.[49] The overexpression of G2019S LRRK2 in the rat brain did not induce gross nigral DA neurodegeneration, alterations in striatal DA levels, or PD-related behavioral changes up to 12 months of age. Nevertheless, G2019S LRRK2 expression induced more subtle changes, such as significant elongation of nigral DA neurons, increases of oxidative stress (GSSG/GSH ratio and nitrotyrosine levels), and inflammation markers (increased neuronal iNOS in the absence of microglia or astrocyte activation). Interestingly, while the study in rats did not produce evidence of end-stage PD pathology, there was evidence of oxidative damage and neuroinflammation in the nigrostriatal DA system that may be representative of preclinical PD. Such animals are well suited for testing whether mutated LRRK2 will modulate sensitivity to environmental neurotoxicants.[49]

Current LRRK2 rodent models do not typically exhibit nigrostriatal DA degeneration, nor behavior deficits characteristic of late-stage PD. It is worth noting that the vast majority of transgenic PD models have typically produced limited evidence of DA neurodegeneration characteristic of end-stage PD. With respect to LRRK2 rodent models, a lack of PD relevant pathology might result from differences between rodents and humans in terms of lifespan. Additionally, penetrance of PD-inducing mutated LRRK2 may be incomplete in rodents, as in humans, requiring other genetic and/or environmental insults to achieve detectable DA neuron loss. Also, as observed in transgenic mutant LRRK2 knock-in mice under different regulatory promoters, LRRK2 expression levels and patterns in different brain regions, especially SN and duration of the LRRK2 expression varies under different promoters which might contribute to the absence of DA neuron degeneration. Furthermore, different genetic backgrounds, or ages of animals might render rodents more resistant to LRRK2-induced neurodegeneration, considering LRRK2 mutations cause late onset PD and ages of rodents used in the aforementioned studies need to be noted. While late-stage pathology is not reproduced in rodent LRRK2 models, there are several major advantages and reasons for optimism: (1) Expression of known causative factors that influence a significant number of cases; (2) the replication of specific preclinical disease factors. Thus, it is expected that the models discussed earlier will be highly useful in exploring mechanisms of the earliest disease stages and also testing interactions with other pathogenetic factors.

Limited studies have examined how LRRK2 may modulate neurotoxicity in rodents. In a knock-out mouse model, sensitivity to MPTP was not altered.[55] In contrast, LRRK2 knock-out in rats abrogated DA degeneration induced by intracranial LPS administration or adenovirus-mediated human α-synuclein overexpression.[48] Similarly, inhibition of LRRK2 kinase activity (inhibitors) or LRRK2 knockdown (RNAi) attenuated microglial inflammatory responses that were induced by intracranial LPS infusion.[27]

In experiments examining gene–gene interactions, WT LRRK2 or G2019S LRRK2 equally exacerbated accumulation of α-synuclein, neuronal loss, and microglial activation that were induced in transgenic mice overexpressing A53T α-synuclein. Here, LRRK2 knock-out ameliorated α-synuclein mediated neuropathology.[56] In contrast, another study reported that WT LRRK2 or G2019S LRRK2 overexpression under Thy1 promoter did not exacerbate α-synucleinopathy induced in transgenic mice overexpressing A53T α-synuclein.[59] A few key points can be gleaned from these studies: (1) LRRK2 partially modulates neurotoxicity stemming from mutant α-synuclein; (2) such modulation may be dependent on an LRRK2 expression—the models discussed earlier are quite different in terms of how they were generated. Going forward, to compare models, it will be crucial to understand how LRRK2 expression in neuronal and non-neuronal cells differ. Histological approaches to characterizing LRRK2 expression have improved in the last few years, making such an endeavor much more feasible.

Intracranial LPS injection or adenovirus-mediated overexpression of human α-synuclein in the SN causes DA neurodegeneration in WT rats. However, LRRK2 knock-out rats have been found to be resistant to LPS- or α-synuclein-induced DA neurodegeneration with significantly reduced proinflammatory immune cells recruited in the brain

compared to the treated WT rats.[48] The authors also found high LRRK2 expression in proinflammatory immune cells in response to α-synuclein overexpression or LPS exposures, compared to the undetectable LRRK2 levels in untreated rats.[48] This result suggests a role of LRRK2 in mediating proinflammatory response in immune cells.

More recently, sensitivity to environmentally relevant toxicants has been examined in rodent LRRK2 models. Desplats et al.[67] reported that G2019S LRRK2 expression under Thy1 promoter in mice co-exposed to paraquat and maneb induced significant increases in hippocampal neurogenesis-related gene expression compared to exposed controls, even though the single genetic factor, G2019S LRRK2, had a minor impact on the gene expression.

In summary, a lack of LRRK2 protein or kinase function of LRRK2 does not seem to adversely affect nigrostriatal DA function. However, LRRK2 and in particular, PD-causing mutations seem to positively mediate inflammatory responses in the brain, which is also supported by *in vitro* and *in vivo* studies.[33,68]

Future needs and direction

To date, LRRK2 genetic animal models primarily utilizing either overexpression or knock-out technology have mainly failed to reproduce late-stage PD phenotype. From human studies, it is clear that other factors, possibly additional genetic risk factors or environmental exposures may be required to elicit the key behavioral and pathological PD features. Thus, the inability to reproduce late-stage PD in relatively short-lived animals is perhaps unsurprising. These animals express common mutations known to produce PD in humans. Given that preclinical changes discussed earlier have been detected in some models, there is optimism that the such models can be utilized to study mechanisms of the earliest disease stages and also identify interactions with other pathogenic factors required to precipitate a neurological phenotype consistent with clinical PD.

Rodent LRRK2 genetic models with common mutations such as G2019S exposed to environmentally relevant chemicals such as rotenone, paraquat, and maneb can provide more useful information on interactions between genetic and environmental factors in the pathogenesis of PD. Further, LRRK2 animal models may potentially be utilized to test interactions with factors not yet strongly linked to PD.

It is worth noting that several of the pathogenic LRRK2 mutations have been linked to increased kinase activity. The role that LRRK2 kinase activity may play in mediating neurodegeneration resulting from environmental exposures has yet to be investigated.

In summary, the clearest advantages to LRRK2 animal models are expression of known causative factors. Thus, such models are expected to be critical in our understanding of PD pathogenesis. Inability to reproduce late-stage disease phenotypes suggests that the inclusion of preclinical endpoints is absolutely essential to studies in these models. Examples of important preclinical endpoints that might be considered are: sensitive behavioral studies (motor and autonomic); oxidative stress/damage; olfactory and brainstem pathology, neuronal morphology changes.[49,69] The inclusion of these endpoints may be critical to identify pathological changes resulting from multiple insults.

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