Chapter 9
Models of LRRK2-Associated Parkinson's Disease

Yulan Xiong, Ted M. Dawson, and Valina L. Dawson

Y. Xiong (✉)
Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Department of Anatomy and Physiology, Kansas State University College of Veterinary Medicine, Manhattan, KS 66506, USA
e-mail: yulanxiong@ksu.edu

T.M. Dawson (✉)
Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Adrienne Helis Malvin Medical Research Foundation, New Orleans, LA 70130-2685, USA
e-mail: vdawson@jhmi.edu

V.L. Dawson (✉)
Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Adrienne Helis Malvin Medical Research Foundation, New Orleans, LA 70130-2685, USA

Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
e-mail: tdawson@jhmi.edu

Abstract Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most common genetic causes of Parkinson's disease (PD) and also one of the strongest genetic risk factors in sporadic PD. The LRRK2 protein contains a GTPase and a kinase domain and several protein-protein interaction domains. Both in vitro and in vivo assays in different model systems have provided tremendous insights into the molecular mechanisms underlying LRRK2-induced dopaminergic neurodegeneration. Among all the model systems, animal models are crucial tools to study the pathogenesis of human disease. How do the animal models recapitulate LRRK2-induced dopaminergic neuronal loss in human PD? To answer this question, this review focuses on the discussion of the animal models of LRRK2-associated PD including genetic- and viral-based models.

Keywords LRRK2 • Animal models • Parkinson's disease

Introduction

Parkinson's disease (PD) is recognized as the most common movement disorder, affecting up to 1% of the population above the age of 60 and 4–5% above the age of 85 [1]. Clinical symptoms in PD patients include akinesia, resting tremor, muscle rigidity, and postural imbalance [1]. The cardinal symptoms are caused by the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) [2]. Although the majority of PD cases appear to be sporadic, in the past couple of decades, several genes have been identified to be responsible for this progressive neurodegenerative disease [3]. To date, genes encoding LRRK2 (leucine-rich repeat kinase 2), α-synuclein, parkin, DJ-1, PINK1 (phosphatase and tensin homolog deleted on chromosome 10-induced putative kinase 1), VPS35 (vacuolar protein sorting 35), DNAJC13, GBA (glucocerebrosidase), and EIF4G1 (eukaryotic initiation factor 4G1), among others, are associated with genetic forms of PD [3–8]. Mutations in the *LRRK2* gene (PARK8, dardarin, OMIM 609007) cause late-onset, autosomal dominant PD and is the most frequent genetic cause of PD, accounting for 4% of familial PD and 1% of sporadic PD across all populations. Importantly, LRRK2-mediated PD is clinically and pathologically indistinguishable from sporadic PD [9, 10], suggesting that understanding LRRK2-associated PD may lead to an understanding of sporadic PD.

The LRRK2 protein contains two enzymatic domains, a GTPase and a kinase domain and multiple protein-protein interaction domains including a leucine-rich repeat (LRR), a WD40 repeat, and a LRRK2-specific repeat domain (Fig. 9.1) [11, 12]. LRRK2 interaction domains are thought to serve as protein-binding modules where LRRK2 acts as a signaling scaffold. LRRK2 GTPase and kinase enzyme activity are important in regulating LRRK2-dependent cellular signaling pathways and may reciprocally regulate each other to direct LRRK2's ultimate function [13]. Pathogenic mutations of LRRK2 are centered on LRRK2 enzymatic domains
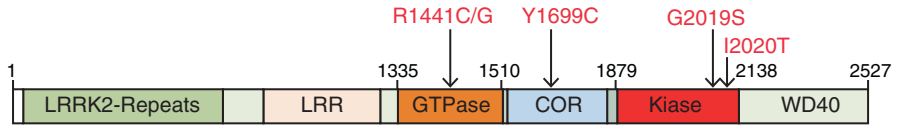
Fig. 9.1 Schematic showing the domain structure of LRRK2 protein and the position of pathogenic mutations. Residues 1–660 encode LRRK2-specific repeat sequences, 984–1278 encode the leucine-rich repeat (LRR), 1335–1510 encode the Roc GTPase domain, 1519–1795 encode the C-terminal of Ras (COR) domain, and 1879–2138 encode the kinase domain. Five confirmed LRRK2 pathogenic mutations: R1441C/G, Y1699C, G2019S, I2020T

(Fig. 9.1). Thus, LRRK2 enzymatic activity is important in PD. The most prevalent LRRK2 mutation, G2019S, is within the kinase domain. It accounts for 5–6% of autosomal dominant PD patients and ∼1% of sporadic late-onset PD. Patients with the G2019S mutation exhibit Lewy bodies in most cases [1]. However, mutations in the GTPase domain and COR domain, such as R1441 C/R1441 G and Y1669C often vary on Lewy body pathology [10, 14]. This raises the possibility that these mutations cause disease via distinct pathogenic mechanisms.

Tremendous work in both in vitro and in vivo systems suggests that LRRK2 is involved in diverse pathways and cellular signaling including regulation of protein translation, vesicle trafficking, neurite outgrowth, autophagy, and cytoskeletal dynamics [15–17]. Several model systems have been developed to study LRRK2 function from yeast to invertebrates such as *Drosophila* and *C. elegans*, rodents, and patient-derived induced pluripotent stem cells (iPScs) [18]. Yeast, a eukaryotic single-cell organism, has been widely used to uncover the fundamental pathobiology of proteins associated with neurodegenerative diseases including PD. The first LRRK2 yeast model that revealed LRRK2 GTPase function plays a key role in LRRK2 pathobiology [19, 20]. The toxicity is closely associated with GTPase activity and defects in endocytic vesicular trafficking and autophagy [19]. More importantly, using this yeast model, the first GTPase-activating protein (GAP) for LRRK2, ArfGAP1, was identified and characterized [20]. Patient-derived iPScs provide highly relevant models for PD studies as the well-developed capacity to generate iPSc-derived DA neurons. Several LRRK2 iPSc models have been developed. DA neurons derived from LRRK2 iPScs display reduced neurite length, accumulation of α-synuclein and tau, increased vulnerability to cellular stress, and impaired autophagy and mitochondrial function [18]. IPSc models allow us to study LRRK2 pathobiology directly in human context. However, both yeast and iPSc models cannot recapitulate the physiological cell diversity in the intact mammalian brain and the complexity of brain circuits.

While the eukaryotic yeast and iPS cell models provide an important yet complementary insights to animal models on understanding disease mechanisms, this review focuses on the animal models of LRRK2-associated PD and discusses the advantages and disadvantages of each model and how each of these different models have contributed to understanding the role of LRRK2 in PD pathogenesis.

Genetic Animal Models of LRRK2

LRRK2 Drosophila *Models*

Animal models are crucial tools for LRRK2 research. Invertebrate animals, especially *Drosophila*, have proven to play an important role in studying LRRK2 pathogenic mechanisms and developing therapeutics. *Drosophila* has well-defined nerve systems, which share similar neuronal transmitters with mammals. Importantly, *Drosophila* has DA neuronal clusters and can perform complicated behavioral tests, which mimic some DA-dependent behaviors in human. Several steps have been taken to establish and utilize LRRK2 *Drosophila* models:

1. Generation of *Drosophila* strains carrying mutations in genes linked to disease
2. Determination of the *Drosophila* models to see if they recapitulate the pathogenesis of the disease and in turn are good models of the disease
3. Investigation of the detailed molecular mechanisms underlying the phenotypes
4. Identification of genetic modifiers to dissect the signaling pathways involved in pathogenesis
5. Drug candidate screening

Several LRRK2 *Drosophila* models have been generated and are listed in Table 9.1.

LRRK2 Knockout *Drosophila* Models

Drosophila has one human LRRK2 homolog dLRRK, and residues affected by PD-causing mutations in LRRK2 are conserved in *Drosophila* LRRK2. To study the function of endogenous wild-type (WT) LRRK2, *Drosophila* LRRK2 knockouts have been generated [21–24]. Several groups reported that the homozygous mutant fly develops normally with a normal life span as well as unchanged number and pattern of DA neurons [21, 23, 24], although one group reported LRRK loss-of-function mutants exhibited a severely impaired locomotive activity and a severe reduction in tyrosine hydroxylase immunostaining and shrunken morphology of DA neurons in LRRK mutants [22]. In addition, Wang et al. showed that mutant flies containing C-terminal kinase domain truncated dLRRK are selectively sensitive to H_2O_2, but not to paraquat, rotenone, or β-mercaptoethanol [23]. By contrast, Imai et al. showed that *dLRRK* null flies are relatively resistant to general oxidative stress, such as paraquat and H_2O_2 treatment, compared to WT flies [21]. Thus, the exact role of dLRRK in oxidative stress remains unclear. The different phenotypes are possibly due to the different genomic loci of insertion for gene disruption and the different genetic backgrounds. As the majority of the reports support that *dLRRK* is dispensable for survival of DA neurons in flies and this is consistent with the phenotypes in LRRK2 knockout mice, the general consensus is that LRRK2 toxicity is from a gain-of-function and not a loss-of-function mechanism.

Table 9.1 *Drosophila* models for LRRK2-associated PD

LRRK2 variants	Genetic manipulations	Motor deficits/life span	DA neuronal loss	Nigrostriatal dysfunction	Sensitivity to oxidative stress	Other phenotypes	References
dLRRK	Loss of function	Locomotor activity ↓	No changes	TH staining ↓ DA neurons shrunken	ND	ND	[22]
dLRRK	Loss of function	Life span ↓	No changes	No changes	Sensitive to hydrogen peroxide, not to paraquat, rotenone, and β-mercaptoethanol	ND	[23]
dLRRK	Loss of function	Life span ↓ Fertility ↓ Malformed abdomen	No changes	DA content ↑	Hydrogen peroxide ↓ Paraquat ↓	ND	[21]
dLRRK	Loss of function	Locomotor activity ↓	ND	ND	ND	ND	[24]
dLRRK	O/E	No changes	No changes	No changes	ND	ND	[22]
hLRRK2	O/E	Locomotor activity ↓ Life span ↓ Response to L-Dopa	TH neurons ↓ No response to L-Dopa	ND	ND	Retinal degeneration	[25]
hG2019S		Locomotor activity ↓ Life span ↓ Response to L-Dopa	TH neurons ↓↓ No response to L-Dopa	ND	ND	Retinal degeneration	

Table 9.1 (continued)

LRRK2 variants	Genetic manipulations	Motor deficits/life span	DA neuronal loss	Nigrostriatal dysfunction	Sensitivity to oxidative stress	Other phenotypes	References
dLRRK	O/E	ND	No changes	No changes	No changes	ND	[21]
dY1383C dI1915T		ND	No changes	TH staining ↓ DA content ↓	Hydrogen peroxide ↑ Paraquat ↑	ND	
hLRRK2	O/E at 29°C	Locomotor activity: 10 days ↓ 20 days ↑ Life span ↑ Fertility ↑	TH neurons ↓	ND	Rotenone ↑	Retinal degeneration	[27]
hI1122V hY1699C hI2020T		Locomotor activity: 10 days ↓ 20 days ↑ Life span ↑ in hY1699C, hI2020T Fertility ↑ in hI1122V, hI2020T	TH neurons ↓ the most with I 2020 T	ND	Rotenone ↑	Retinal degeneration	
hLRRK2	O/E	No changes	No changes	ND	No changes	No changes	[26]
hG2019S hY1699C hG2385R		Locomotor activity ↓ Life span ↓	TH neurons ↓	ND	hG2019S, ↑ hG2385R ↑ hY1699C no change	No changes	

LRRK2 variants	Genetic manipulations	Motor deficits/life span	DA neuronal loss	Nigrostriatal dysfunction	Sensitivity to oxidative stress	Other phenotypes	References
hLRRK2	O/E	Locomotor activity: no changes Life span ↓	ND	Dendritic ends ↓	ND	ND	[28]
hG2019S		Locomotor activity ↓↓ Life span ↓↓	TH neurons ↓	Dendritic ends ↓↓ Axon degeneration ↑	ND	ND	
hR1441C hG2385R		Locomotor activity ↓ Life span ↓	ND	Dendritic ends ↓	ND	ND	
hLRRK2	O/E	ND	ND	ND	ND	Visual function: no changes	[29]
hG2019S		ND	ND	ND	ND	Visual function ↓	
hI1122V hR1441C hY1383C hI1915T hI2020T hG2385R hG2019/K1906M		ND	ND	ND	ND	Visual function: no changes	

Table 9.1 (continued)

LRRK2 variants	Genetic manipulations	Motor deficits/ life span	DA neuronal loss	Nigrostriatal dysfunction	Sensitivity to oxidative stress	Other phenotypes	References
hLRRK2 hG2019S	O/E	Locomotor activity: no changes	ND	Axon transport: no changes	ND	ND	[30]
hR1441C hY1699C		Locomotor activity ↓	ND	Axon transport ↓		ND	
dR1069C dY1383C		Locomotor activity ↓	ND	Axon transport ↓		ND	

ND not determined, *O/E* overexpression, ↑ increased, ↓ decreased

LRRK2 Transgenic *Drosophila* Models

In contrast to *dLRRK* knockout *Drosophila*, overexpression of both human LRRK2 and dLRRK pathogenic mutations in *Drosophila* leads to age-dependent DA-responsive reductions in locomotor activity and loss of DA neurons (Table 9.1) [21, 25–28]. Interestingly, in addition to the DA neurodegeneration, different LRRK2 mutations cause different phenotypes related to the degeneration. One recent study showed that LRRK2 G2019S induced extensive neurodegeneration throughout the visual system [29]. This degeneration is LRRK2 G2019S mutation specific and occurs in a kinase-dependent manner. Dopaminergic expression of LRRK2 G2019S led to nonautonomous cell death reminiscent of that seen in PD [29]. Another report showed that LRRK2 R1441C or Y1699C mutations in the GTPase-COR domain preferentially associates with deacetylated microtubules and inhibits axonal transport in *Drosophila*, causing locomotor deficits in vivo. These features are not seen with the LRRK2 G2019S mutation, suggesting that these defects are GTPase activity dependent [30]. A previous study suggested that reduced axonal transport rates caused by α-synuclein mutants might contribute to accumulation of α-synuclein and hence Lewy body formation and neuritic abnormalities in PD brain [31]. Taken together, reduced axonal transport rates may contribute to the formation of Lewy bodies or Lewy neurites in some PD cases carrying R1441C or Y1669C mutations. These studies suggest that different LRRK2 pathogenic mutations act at distinct pathways and cause varied neuropathology in that accompanies DA neurodegeneration.

Using LRRK2 *Drosophila* Models to Study Molecular Mechanisms Underlying LRRK2-Associated PD

Do LRRK2 *Drosophila* models reveal the pathogenic mechanisms underlying LRRK2-induced DA neurodegeneration? To address this, *Drosophila* offers a wide variety of genetic tools including genetic screens, which allow genome-wide analyses of genetic interactions based on the modification of a given phenotype, and candidate gene approaches, in which only those suspected genes are analyzed for modifications of the phenotype. Both strategies allow identification of components of signaling pathways involved in PD pathogenesis. Using LRRK2 *Drosophila* models, several in vivo LRRK2 interactors have been identified and characterized in different signaling pathways.

LRRK2 Function in Protein Synthesis/Translation

Drosophila dLRRK was shown to regulate protein translational pathways. Imai et al. first provided evidence that both dLRRK and human LRRK2 can phosphorylate eukaryotic initiation factor 4E-binding protein (4E-BP), a negative regulator of eukaryotic initiation factor 4E-mediated protein translation and a key mediator of

various stress responses [21]. A link between dLRRK and protein synthesis was further strengthened by the observation from the same group that LRRK2 interacts with the microRNA pathway to regulate protein synthesis [32]. However, these *Drosophila* studies have yet to be extended to mammalian systems. A recent study, using a combination of an LRRK2 *Drosophila* model and human dopamine neurons, demonstrated that LRRK2 phosphorylates ribosome protein s15 to enhance protein translation and mediate LRRK2-induced neurodegeneration [33]. Taken together, there is strong convergent evidence that LRRK2 regulates protein translation machinery in diverse species and tissues.

LRRK2 Function in Vesicular Trafficking

Studies using LRRK2 *Drosophila* models have revealed potential roles for LRRK2 in multiple aspects of vesicle trafficking including endolysosomal pathways, synaptic vesicle (SV) endocytosis, ER-Golgi, and retromer trafficking. First, dLRRK was reported to localize to the membranes of late endosomes and lysosomes, physically interacts with the crucial mediator of late endosomal transport Rab7, and negatively regulates Rab7-dependent perinuclear localization of lysosomes [34]. LRRK2 has been further shown to localize at endosomes and interacts with clathrin light chains (CLCs) to limit Rac1 activation. These data identify a new pathway in which CLCs function with LRRK2 to control Rac1 activation on endosomes [35]. The function of LRRK2 in endolysosomal pathways is further strengthened by a study on novel ethyl methanesulfonate (EMS)-induced nonsense alleles in dLRRK, which cause striking defects in the endolysosomal and autophagy pathways [36]. Second, a study in *Drosophila* shows that LRRK2 functions on SV endocytosis at the neuromuscular junctions by phosphorylating endophilin A (EndoA) at S75 and mediating EndoA-dependent membrane tubulation and membrane association [37]. In addition, dLRRK has been demonstrated to regulate Golgi outpost (GOP) dynamics in dendrites through the golgin Lava lamp [38]. Moreover, genetic interactions between VPS35, Rab7L1, ArfGAP1, and LRRK2 in *Drosophila* highlight LRRK2's role in retromer and ER-Golgi trafficking [20, 39, 40]. All data taken together strongly support that LRRK2 plays a crucial role in vesicular trafficking pathway, which may provide potential mechanisms for accumulation of α-synuclein in LRRK2-associated PD.

LRRK2 Function in Dendritic Degeneration and Synaptic Morphology

Expression of LRRK2 G2019S in *Drosophila* dendritic arborization neurons induces mislocalization of the axonal protein tau in dendrites and causes dendrite degeneration. This may act through a mechanism in which LRRK2 G2019S promotes tau phosphorylation by the glycogen synthase kinase 3β (GSK3β) [28]. In addition, LRRK2 regulates synaptic morphology through interacting with 4E-BP at the postsynaptic site and phosphorylating Futsch at the presynaptic compartments

of the *Drosophila* neuromuscular junctions [41]. These studies point out a possible role for LRRK2 in dendrite degeneration and synaptic dysfunction.

LRRK2 Genetic Interaction with Other PD Genes

As the number of genetic alterations linked to PD pathogenesis increases, establishing functional pathways and whether these genes or risk factors interact with each other will be crucial. *Drosophila* as a classical genetic model provides powerful tools to study genetic interactions between different genes. Using LRRK2 *Drosophila* models, genetic dissection revealed that LRRK2 interacts with other PD genes or risk factors such as VPS35, RAB7L, parkin, DJ-1, and PINK1 [27, 39, 40] and implicates several potential LRRK2 functions. Genetic interaction between LRRK2, VPS35, and Rab7L implicates LRRK2 function in retromer and lysosomal pathways that contribute to PD [39, 40]. Coexpression of human parkin in LRRK2 G2019S-expressing flies provides significant protection against DA neurodegeneration that occurs with age or in response to rotenone, suggesting a potential link between LRRK2, parkin, and mitochondria in the pathogenesis of LRRK2-related parkinsonism [27]. Genetic interaction between LRRK2 and parkin, DJ-1 or PINK1 also suggests that dominant PD genes may act via common pathways with the recessive PD genes.

Using LRRK2 *Drosophila* Models to Identify Potentially Therapeutic Compounds

The genetic LRRK2 *Drosophila* model represents a promising platform for inhibitor identification and validation. Studies have shown that GW5074, curcumin, or sorafenib significantly suppressed LRRK2 PD-like phenotypes in *Drosophila* [42, 43]. Although candidate compounds have been used in these studies, they open the possibility of performing compound screens, which may be useful for finding new drugs for treatment of LRRK2-associated PD.

LRRK2 C. elegans *Models*

The nematode *Caenorhabditis elegans* has a well-defined and genetically tractable nervous system that offers an effective model to explore basic mechanistic pathways that might underpin complex human neurological diseases. *C. elegans* contains only one *lrk-1* gene encoding a LRRK-like protein. Lrk-1 is localized in the Golgi apparatus and is required for polarized localization of SV proteins. The loss of *lrk-1* causes SV protein mislocalization to both presynaptic and dendritic endings in neurons, which are dependent on the AP-1 clathrin adaptor UNC-101 [44]. The results raise the possibility that the LRK-1 functions on the *trans*-Golgi network

(TGN) to exclude SV proteins from the dendrite-specific transport mechanisms mediated by the AP-1 clathrin adaptor complex. This study suggests that LRRK2 might function in the Golgi network. Recent identification of ArfGAP1, a Golgi protein that reciprocally regulates LRRK2-induced toxicity both in vitro and in vivo, might provide a new insight into LRRK2 function in ER to Golgi trafficking [20]. Other loss-of-function studies in *C. elegans* revealed that LRRK2 acts to protect *C. elegans* DA neurons from the toxicity of 6-hydroxydopamine and/or human α-synuclein, possibly through the p38 pathway, by supporting upregulation of GRP78 [45]. The loss of lrk-1 renders animals hypersensitive to the endoplasmic reticulum stressor tunicamycin, which is rescued by *PINK1* [46]. These studies suggest a functional link between LRRK2 and ER stress [45, 46].

While loss of the LRRK2 homolog in *C. elegans* provided information of the biological function of LRRK2, overexpression of human LRRK2 in *C. elegans* established a model that recapitulates key features of PD. Overexpression of human LRRK2 WT, R1441C, or G2019S in DA neurons in *C. elegans* causes age-dependent DA neurodegeneration, behavioral deficits, and locomotor dysfunction that is accompanied by a reduction of dopamine levels in vivo [47, 48]. Several studies suggested that these phenotypes could be caused by mitochondrial dysfunction, autophagy inhibition, and ER stress. Expressing human LRRK2 WT increased nematode survival by protecting against mitochondrial stress, but mutant forms of LRRK2 (G2019S or R1441C) enhanced vulnerability to mitochondrial dysfunction and inhibition of autophagy [47, 49]. Although LRRK2 G2019S consistently inhibits autophagy in multiple studies, the effects of LRRK2 WT appear to vary between studies even from the same group [47, 49]. The explanation for this variation appears to depend on whether or not α-synuclein is present [50]. Coexpressing LRRK2 WT with α-synuclein produces a modest age-dependent inhibition of autophagy [50]. Since *C. elegans*, like *Drosophila*, does not express endogenous α-synuclein, caution needs to be taken in interpreting studies using *C. elegans* models.

The observations from LRRK2 *C. elegans* models support a role for LRRK2 kinase and GTPase activity as critical mediators of neurotoxicity induced by mutant LRRK2. Overexpression of the GTP-binding defective mutant, K1347A, prevents the LRRK2-induced neurodegeneration and behavioral abnormalities [48]. LRRK2 kinase inhibitors TTT-3002 and LRRK2-IN1 protect against LRRK2 R1441C- or LRRK2 G2019S-induced neurodegeneration [42, 48]. These studies suggested that both LRRK2 GTPase and kinase activity play crucial roles in LRRK2-induced neurodegeneration in *C. elegans*.

LRRK2 Zebrafish Models

Although zebrafish has been established as an excellent vertebrate model for the study of human disease, zebrafish LRRK2 (zLRRK2) models are not well developed. There is one human LRRK2 homolog in zebrafish, zLRRK2, which has a high degree conservation of amino acid sequences with human LRRK2 (hLRRK2)

proteins and the highest conservation within the kinase domain. Two groups reported the generation of loss-of-function zLRRK2 models, but with conflicting results. Sheng et al. first reported that the deletion of the WD40 domain of zLRRK2 by morpholino-targeted splicing caused parkinsonism-like phenotypes, including loss of DA neurons in the diencephalon and locomotion defects [51]. These neurodegenerative and locomotion defects could be rescued by overexpressing zLRRK2 or hLRRK2 mRNA. The administration of L-Dopa could also rescue the locomotion defects, but not the neurodegeneration [51]. However, a later study reported by Ren et al. demonstrated that the deletion of the WD40 domain of zLRRK2 using the same methods does not cause the loss of DA neurons [52]. Given the opposite results from two similar studies, the loss-of-function zLRRK2 models need further evaluation. Transient co-overexpression of human WT or GS LRRK2 with GFP-tagged ubiquitin in WT zebrafish embryos causes impaired clearance of transiently expressed ubiquitin, suggesting of ubiquitin proteasome system disruption [53]. The characterization on DA system was not performed [53]. Taken together, LRRK2 zebrafish models are underdeveloped and need more evaluation and characterization.

LRRK2 Mouse Models

Whereas all the models are important and can be used in a variety of research directions, generally more effort is placed on developing mouse models to study human genetic disorders because mice possess similar neuronal networks and basal ganglia circuitry with high conservation of homologs with the human disease-causing genes. Then, what are the criteria for the effective modeling of human diseases in mice? A good model should recapitulate the genetic and pathological features of the disease in human patients while avoiding spurious phenotypes that are not involved in human diseases [54–56]. For PD, mouse models that faithfully recapitulate the characteristic neurodegeneration and motor deficits as well as other hallmarks of PD such as α-synuclein aggregation are necessary. They would provide in vivo platforms to validate pathogenic molecular pathways and therapeutic strategies in more controlled physiological systems [55].

LRRK2 Knockout Mouse Models

A question frequently raised is whether LRRK2 pathology could be the result of a loss of function. To address this question, several groups generated and analyzed LRRK2 knockout mice. Consistent among the knockouts is that observation that there is no DA neurodegeneration although some abnormalities are observed outside the nervous system (Table 9.2) [57–63]. Andres-Mateo et al. reported the first LRRK2 knockout mouse model showing an intact nigrostriatal DA pathway up to 2 years of age and no altered sensitivity to MPTP-induced neurotoxicity [57]. Tong

Table 9.2 Mouse models for LRRK2-associated PD

LRRK2 variants	Genetic manipulations	Motor deficits	DA neuronal loss	Nigrostriatal dysfunction	Other phenotypes	References
LRRK2 −/−	Knockout	ND	No changes	No changes	Lack of hypersensitivity to MPTP	[57]
LRRK2 −/−	Knockout	No changes	No changes	ND	ND	[60]
LRRK2 −/−	Knockout	ND	No changes	No changes	Accumulation of alpha-synuclein and ubiquitinated proteins; impaired autophagy-lysosomal pathway; increased apoptotic cell death, inflammatory responses, and oxidative damage	[63]
LRRK2 −/−	Knockout	No changes	No changes	No changes	An early-onset increase in number and size of secondary lysosomes in the kidney; hypertension blood pressure	[58]
LRRK2 −/−	Knockout	Abnormal exploratory behavior at 7 and 16 months	No changes	No changes	Degeneration in the kidney, increased autophagic activity	[59]

LRRK2 variants	Genetic manipulations	Motor deficits	DA neuronal loss	Nigrostriatal dysfunction	Other phenotypes	References
WT	BAC transgenic	No changes	No changes	No changes	ND	[67]
R1441G		Decline in rearing starting from 3 to 6 months; Response to L-Dopa	No changes	Axonal and tau pathology; Impaired dopamine release	ND	
WT	BAC transgenic	Hyperactive at 6 months	No changes	No changes	No changes	[66]
G2019S		No changes at 12 months	No changes	Decreased DA content and DA release and uptake	Enhanced kinase activity and phospho-tau	
WT	BAC transgenic	No changes	No changes	Reduction of extracellular dopamine levels	No changes	[68]
G2019S		No changes	No changes	Reduction of extracellular dopamine levels	Increased phospho-tau	
WT	Tet-off/CamKII-tTA inducible transgenic	No changes	ND	ND	ND	[60]
G2019S		Increased ambulatory activities at 12 months	ND	ND	ND	

(continued)

Table 9.2 (continued)

LRRK2 variants	Genetic manipulations	Motor deficits	DA neuronal loss	Nigrostriatal dysfunction	Other phenotypes	References
WT	Tet-off/Pitx3-tTA inducible	No changes	No changes	No changes	Decreased expression of DA genes	[71]
G2019S	transgenic	No changes	No changes	Impaired dopamine homeostasis and release	Decreased expression of DA genes	
G2019S	CMVE-PDGFβ drived transgenic	No changes	TH neurodegeneration at 20 months	No changes	Reduced neurite complexity and autophagic abnormalities	[69]
R1441C		Decreased at 15 months	No changes	No changes	ND	
WT	CMVE-PDGFβ drived transgenic	No changes	No changes	No changes	No changes	[65]
G2019S		Impaired motor activity, response to L-Dopa	TH neurodegeneration starting from 12 months	Decreased dopamine transporters or TH staining	Activated MKK4-JNK pathway	
R1441C	ROSA26 drived transgenic	No changes	No changes	No changes	Nuclear abnormalities	[70]
WT	Thy1.2 drived transgenic	ND	No changes	No changes	No changes on neurite outgrowth	[73]
G2019S		ND	No changes	No changes	No changes on neurite outgrowth	
R1441C	Knock-in	Reduced response to AMPH in locomotor activity	No changes	Reduced catecholamine release in cultured mutant chromaffin cells	ND	[74]

LRRK2 variants	Genetic manipulations	Motor deficits	DA neuronal loss	Nigrostriatal dysfunction	Other phenotypes	References
G2019S	Knock-in	No changes	No changes	No changes	No changes in autophagy	[58]
G2019S	Knock-in	Homo-G2019Smice travel longer distances at 12 months	No changes	Impaired dopamine release, altered DA metabolism	Mitochondrial abnormalities; elevated glutamate release; increased phosphor-tau	[72, 75]

et al. demonstrated that LRRK2 knockouts develop striking kidney pathology and impaired autophagy-lysosomal function [62, 63]. The kidney phenotype was observed in two other LRRK2 knockouts, although the defects in autophagy changes were not observed [58, 59]. A recent study using LRRK2 knockouts suggests that LRRK2 influences neurogenesis and particularly neuronal morphogenesis [61].

Since the majority of LRRK2 PD patients exhibit α-synuclein deposition, the role of LRRK2 in α-synuclein pathology has been explored. Lin et al. showed that knockout of LRRK2-rescued A53T α-synuclein overexpression induced Golgi fragmentation, α-synuclein accumulation and aggregation, microglial activation, and forebrain neuronal degeneration [60]. On the other hand, Tong et al. demonstrated that LRRK2 knockout mice develop striking accumulation and aggregation of α-synuclein and Daher et al. showed that deletion of LRRK2 had no influence on the lethal neurodegenerative phenotype of the A53T α-synuclein transgenic mice [63, 64]. The different findings between these studies could be due to the different levels of α-synuclein expression and or technical concerns. Whether inhibition of LRRK2 could be employed as a therapeutic strategy to attenuate α-synuclein-mediated neuronal damage relevant to PD needs further investigation.

All the observations from the LRRK2 knockout mice suggest that LRRK2 plays little if any role in the development and survival of DA neurons is under physiologic conditions. Thus, PD caused by LRRK2 mutations are likely not due to a loss of LRRK2 function.

LRRK2 Transgenic Mouse Models

Many groups have generated LRRK2-related PD mouse models expressing LRRK2 WT or PD-associated mutant LRRK2 G2019S or R1441C/G (Table 9.2) [58, 60, 65–75]. Several transgenic techniques for LRRK2-related PD modeling in mice have been utilized, including conventional [65, 69, 70, 73], BAC transgenic [66–68], tet-inducible transgenic [60, 71], and mutant LRRK2 knock-in techniques [58, 72, 74, 75]. However, to date only two of the LRRK2 models exhibit age-dependent SNpc DA neurodegeneration [65, 69]. Most LRRK2 transgenic animals manifest deficits in DA transmission and DA-responsive behavior. Between the two studies with SNpc DA neurodegeneration, both used conventional transgenic techniques utilizing the PDGF-β promoter to generate LRRK2 mutant G2019S mouse lines. Ramonet et al. show that LRRK2 G2019S mice developed about 20% SNpc DA neurodegeneration at 20 months of age [69], while Chen et al. demonstrated more robust degeneration in the SNpc starting from 12 months of age with about 50% degeneration at 16 months of age without a phenotype in LRRK2 WT transgenic mice [65]. The different degrees of the degeneration may be due to the different overexpression levels of the transgenes.

Why don't most LRRK2 transgenic models exhibit SNpc DA degeneration? One potential explanation could be a lack of robust transgene overexpression in SNpc DA neurons. The BAC and knock-in models express mutant LRRK2 during development, and thus there may be compensatory mechanisms in the mouse that prevent

loss of DA neurons. Thus, conditional and selective expression of LRRK2 in SNpc DA neurons may overcome this problem. A recent study reported an LRRK2 G2019S conditional transgenic mouse model using the tet-off system and a PitX3-tTA driver line to drive transgene expression in DA neurons. However, no SNpc DA degeneration was observed in this model [71]. The reason is unclear but may be related to not aging the mice to 24 months of age, or perhaps expression of LRRK2 only in DA neurons is not sufficient for DA degeneration to occur given that the endogenous LRRK2 expression levels are comparatively low in SNpc DA neurons and LRRK2 is also expressed in other neurons. Thus, overexpression of LRRK2 in other neurons at the same time as in DA neurons or other genetic and/or environmental factors may be required for degeneration of DA neurons.

LRRK2 Rat Models

For the last several decades, investigators have chosen to use mouse models because of the technologies that were available. Now the same technologies are available in the rat. As a model of human disease, the rat offers many advantages over the mouse and other organisms. Physiology is easier to monitor in the rat. Moreover, in many cases, the physiology is more like the corresponding human condition. The rat is more intelligent than the mouse and is capable of learning a wider variety of tasks that are important in mimicking human behavioral symptoms. Recently, both LRRK2 knockout and transgenic rat models have been generated and characterized.

LRRK2 Knockout Rat Models

Like other LRRK2 animal models, LRRK2 knockout rats have no significant loss of SNpc neurons. Similar to LRRK2 knockout mice, the loss of LRRK2 in rats leads to abnormal phenotypes in peripheral organs. Two studies have observed abnormal kidneys [76, 77]. Besides the kidney phenotype, Ness et al. observed significant weight gain in the LRRK2 knockout rats accompanied by significant increases in insulin and insulin-like growth factors [77]. They also found significant alterations in the cellular composition of the spleen in LRRK2 knockout animals, which Baptista et al. did not observe [76, 77]. Instead, they found LRRK2 knockout rats displayed an abnormal lung and liver phenotype. Using LRRK2 knockout rats, the West group demonstrated resistance to DA neurodegeneration elicited by intracranial administration of LPS and protection from α-synuclein-induced DA neurodegeneration and rhabdomyolysis-induced kidney injury [78, 79]. The abnormal peripheral phenotype of the LRRK2 knockout rat is suggestive of a complex LRRK2 biology influencing metabolism, immune function, and kidney homeostasis. The phenotype of LRRK2 knockout rat is consistent with LRRK2 knockout in other organisms such as *Drosophila*, *C. elegans*, and mouse, supporting the concept that

LRRK2 plays little role in the development and survival of DA neurons under physiologic conditions.

LRRK2 Transgenic Rat Models

The first LRRK2 transgenic rat model was developed by Zhou et al. using an inducible system [80]. Temporal expression of human LRRK2 G2019S in rats did not lead to DA neurodegeneration, but enhanced locomotor activity with age accompanied with impaired dopamine reuptake by the dopamine transporter (DAT) was observed. As a result of compromised DAT activity, amphetamine-evoked dopamine release and amphetamine-elicited locomotor activity were reduced in LRRK2 G2019S transgenic rats [80]. Since only two copies of LRRK2 transgene were expressed in this model, there may have been insufficient protein to produce DA neurodegeneration. Human BAC-LRRK2 G2019S or R1441G rats were developed, and mutant LRRK2 expression was approximately 5 ~ 8 times higher than endogenous rat LRRK2. However, both BAC-LRRK2 R1441G and G2019S transgenic rats do not show signs of neurodegeneration and do not develop significant motor or cognitive deficits with age [81–83]. Instead, LRRK2 G2019S induced oxidative stress in the striatum and substantia nigra and increased inducible nitric oxide synthase expression in SNpc DA neurons and abnormal morphology of SNpc DA neurons [81, 83]. Although this model does not reproduce the key features of end-stage PD, it may be useful in studying gene-environment interactions. However, a recent study indicates that BAC-LRRK2 R1441G transgenic rats did not show increased vulnerability to sub-toxic doses of paraquat [82]. Since these studies lacked a wild-type human LRRK2 transgenic rat as a control, it is not possible to conclude that the phenotype induced by mutant LRRK2 is due to the LRRK2 PD mutation or overexpression of the LRRK2 protein. All results from different LRRK2 transgenic rats suggest that rats compensate and accommodate LRRK2's toxic effects.

Viral-Mediated Animal Models of LRRK2

While the genetic LRRK2 models shed light on LRRK2 cellular functions and pathogenic pathways, development of recombinant viral vectors for in vivo delivery of transgenes has opened up a new possibility to model diseases in the CNS. The viral-mediated gene transfer approach in adulthood bypasses the development of compensatory effects. This approach also allows researchers to target specific neuronal populations, such as SNpc DA neurons. Another advantage of the viral-mediated gene transfer approach is that it allows researchers to control transgene dosage by modulating copy number of the transgene. While viral models allow us to recapitulate some of the neurodegeneration processes observed in PD patients that have so far been difficult to show in other models, there are caveats of non-physiological doses of transgenes and potential alterations in RNA translation.

Due to large size of LRRK2 gene and the limited packaging capacity of different viral vectors, so far only two LRRK2 viral models have been developed and characterized.

Herpes Simplex Virus (HSV)-LRRK2 Viral Model

The first LRRK2 viral model was developed by Lee et al. by carrying LRRK2 into HSV amplicons expressing a CMV-driven GFP reporter [84]. One advantage of HSV is that it is injected in the striatum and retrogradely transported into SNpc DA neurons, which avoids nonspecific inflammatory damage to the substantia nigra. In this model, after 3 weeks injection, the HSV-WT-LRRK2 induced modest SNpc DA neurodegeneration of about 10–20%, whereas the HSV-LRRK2 G2019S induced up to 50% neuronal loss in SNpc DA neurons. Interestingly, the kinase-dead LRRK2 does not induce neuronal loss, which strongly suggested that kinase activity of LRRK2 mediates LRRK2-induced DA neurodegeneration. This notion is further supported by the protective effects of pharmacological inhibition of LRRK2 kinase activity in this HSV model [84].

Adenoviruses (rAd)-LRRK2 Viral Model

Second-generation E1, E3, and E2a-deleted recombinant human serotype 5 adenoviruses (rAd) carrying LRRK2 WT and G2019S were generated by Dusonchet et al. [85]. Similar to HSV, adenoviral particles can be efficiently retrogradely transported to DA neurons within the SNpc following intrastriatal injections. Injection of rAd-LRRK2 G2019S into rat striatum causes a progressive loss of TH-positive DA neurons in the SNpc, reaching about 21% at 42 days postinjection, but no cell loss is detected in the rAd-GFP- or rAd-LRRK2 WT injected groups. Abnormal transient hyperphosphorylation of tau in dystrophic SNpc neuritic processes was observed upon LRRK2 overexpression at 10 days [85]. Tsika et al. further characterized the striatal pathology in this model [86]. Expression of LRRK2 G2019S selectively induces the accumulation of neuronal ubiquitin-positive inclusions accompanied by neurite degeneration and the altered distribution of axonal phosphorylated neurofilaments in the striatum. The pathological phenotype is dependent on LRRK2 kinase activity as a kinase-inactive mutation (LRRK2 G2019S/D1994N) completely ameliorates the pathological effects of LRRK2 G2019S [86].

Another LRRK2 viral model has been briefly mentioned in another study. The authors delivered lentiviral vectors carrying enhanced green fluorescent protein (eGFP)-tagged LRRK2 G2019S in adult mouse striatum and observed LRRK2 function in TGN turnover [87]. However, there was no characterization of this model in terms of pathology in the nigrostriatal pathways.

Concluding Remarks

Modeling of LRRK2-associated PD in various animal models has provided unprecedented insights into the potential mechanisms of LRRK2-mediated neurodegeneration such as regulation of protein translation, vesicle trafficking, neurite outgrowth, autophagy, and cytoskeletal dynamics. However, none of the current LRRK2 animal models fulfills all the key features of PD. Different LRRK2 animal models recapitulate different clinical and neuropathological features of LRRK2-associated PD, including the degeneration of nigrostriatal DA neurons, neuropathology, α-synuclein accumulation, abnormal striatal DA neurotransmission, and behavioral deficits.

Why are the animal models "imperfect" for modeling LRRK2-associated PD? First, for the simple animal models such as *Drosophila* and *C. elegans*, they do not have α-synuclein homolog and a true human LRRK2 homolog. PD patients harboring LRRK2 mutations frequently exhibit α-synuclein neuropathology in the form of Lewy bodies. A question about whether α-synuclein is required for LRRK2 pathology or vice visa has been raised. The challenge remains to validate the mechanisms identified in these model systems in human PD. Second, for LRRK2 rodent models, perhaps rodent DA neurons are particularly resistant to LRRK2 toxicity. In addition, there may be compensatory mechanisms in the rodents that prevent loss of DA neuron. Third, the fact is that LRRK2 mutations in humans are partially penetrant, implicating that there may be additional factors such as genetic and/or environmental stressors that are required for degeneration of DA neurons. Indeed, in both LRRK2 *Drosophila* and *C. elegans* models, treatments with mitochondrial function inhibitors exacerbate neurodegeneration. Fourth, the HSV- or adenovirus-mediated LRRK2 rodent models induce robust DA neurodegeneration, supporting the notion that both non-cell-autonomous and cell-autonomous processes contribute to the degeneration of DA neurons. The transgene can be virally expressed in both neurons and glia to activate the inflammatory pathway in glial cells and elicit neurodegeneration in DA neurons, which is largely absent in the genetic LRRK2 models. Therefore, non-cell-autonomous effects may provide a promising mechanism for LRRK2-induced PD in humans. All these possibilities need to be taken into consideration in developing future LRRK2 animal models.

Conflict of Interest The author declares no conflicts of interest.

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