Published in final edited form as:

Biochem Soc Trans. 2017 February 08; 45(1): 163–172. doi:10.1042/BST20160264.

Mechanisms of LRRK2-dependent neurodegeneration: role of enzymatic activity and protein aggregation

Md. Shariful Islam and Darren J. Moore

Center for Neurodegenerative Science, Van Andel Research Institute, 333 Bostwick Ave NE, Grand Rapids, MI, USA

Abstract

Mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene are the most common cause of familial Parkinson's disease (PD) with autosomal dominant inheritance. Accordingly, LRRK2 has emerged as a promising therapeutic target for disease modification in PD. Since the first discovery of *LRRK2* mutations some 12 years ago, LRRK2 has been the subject of intense investigation. It has been established that LRRK2 can function as a protein kinase, with many putative substrates identified, and can also function as a GTPase that may serve in part to regulate kinase activity. Familial mutations influence both of these enzymatic activities, suggesting that they may be important for the development of PD. Many LRRK2 models have been established to understand the pathogenic effects and mechanisms of familial mutations. Here, we provide a focused discussion of the evidence supporting a role for kinase and GTPase activity in mediating the pathogenic effects of familial LRRK2 mutations in different model systems, with an emphasis on rodent models of PD. We also critically discuss the contribution and relevance of protein aggregation, namely of α -synuclein and tau-proteins, which are known to form aggregates in PD brains harboring *LRRK2* mutations, to neurodegeneration in LRRK2 rodent models. We aim to provide a clear and unbiased review of some of the key mechanisms that are important for LRRK2-dependent neurodegeneration in PD.

Leucine-rich repeat kinase 2 and Parkinson's disease

Parkinson's disease (PD) is a common, progressive, chronic neurodegenerative movement disorder which affects 1–2% of the US population above 60 years of age [1,2]. The disease is classically defined by the appearance of clinical motor symptoms, including resting tremor, muscular rigidity, bradykinesia, and postural instability, which collectively define parkinsonism, resulting from the relatively selective loss of nigrostriatal pathway dopaminergic neurons and their axonal projections [1,2]. While the majority of PD cases are idiopathic, 5–10% of PD cases are inherited in families with mutations identified in at least 12 genes so far [3]. The most frequently mutated gene in familial PD is *leucine-rich repeat kinase 2 (LRRK2)* [4]. Common variation at many genetic loci also confers risk for developing the common idiopathic form of PD, with variation at the *LRRK2* locus

Correspondence: Darren J. Moore (darren.moore@vai.org).

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

associated with increased disease risk [5]. It is anticipated that investigating these familial and risk genes will provide important insight into the molecular mechanisms and pathways that precipitate neurodegeneration in idiopathic PD.

Mutations in the *LRRK2* gene cause late-onset, autosomal dominant PD, and *LRRK2* mutations represent the most common cause of PD [4,6,7]. Among the identified *LRRK2* mutations, at least seven missense mutations (N1437H, R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) are considered to be truly pathogenic based upon segregation with disease in *LRRK2*-linked families (Figure 1) [4]. In addition, many *LRRK2*-coding variants are associated with PD risk, including G2385R and R1628P, which confer increased risk in Asian populations, whereas a N551K-R1398H haplotype may confer a protective effective [8,9]. Genome-wide association studies have also identified common variants (or single nucleotide polymorphisms) at the *LRRK2* locus that increase risk for idiopathic PD, although it is not yet clear how these SNPs that are enriched in gene regulatory regions influence *LRRK2* expression in different tissues [5,10]. G2019S represents the most frequent mutation being found in 5–6% of familial and 1–2% of idiopathic PD cases in the US population, and in up to 40% of cases in certain ethnicities [4]. Familial PD due to *LRRK2* mutations is clinically and neurochemically indistinguishable from idiopathic PD, including profound dopaminergic neuronal degeneration and gliosis in the substantia nigra pars compacta, reduced levels of dopamine in the caudate putamen, and the appearance of α -synuclein-positive Lewy body pathology in the brainstem (and in some cases diffuse Lewy pathology) [4,7,11]. However, in a small proportion of *LRRK2* mutant PD brains, irrespective of the specific mutation present, Lewy body pathology is absent and instead tau-positive neurofibrillary pathology, ubiquitin-positive inclusions, TDP-43-positive inclusions, or the absence of known protein aggregates have been reported [7,12–14]. Therefore, PD due to *LRRK2* mutations shares similar features to idiopathic disease although the neuropathology is pleomorphic. This raises important questions concerning the pathologic contribution of protein pathology to *LRRK2*-dependent neurodegenerative processes, especially for α -synuclein and tau-proteins, which are known to form pathological aggregates (and are often mutated) in neurodegenerative α -synucleinopathies and tauopathies, respectively.

In this review, we briefly discuss the evidence for mechanisms that could potentially mediate neuronal dysfunction and damage due to familial *LRRK2* mutations in PD animal models, including the contribution of *LRRK2* enzymatic activity and substrate phosphorylation, and protein aggregation especially of α -synuclein and tau-proteins.

LRRK2 structure and enzymatic activity

LRRK2 is a large multi-domain protein of 2527 amino acids belonging to the ROCO protein family [15]. *LRRK2* contains two catalytic domains, a Ras-of-Complex (Roc) GTPase domain and a serine/threonine-directed protein kinase domain separated by a C-terminal-of-Roc (COR) domain (Figure 1). *LRRK2* also contains many protein–protein interaction domains flanking the Roc-COR-kinase catalytic core, including armadillo, ankyrin, leucine-rich repeat (LRR), and WD40 repeat domains, potentially suggesting that *LRRK2* may serve as a scaffold for the assembly of protein complexes. *LRRK2* is predominantly found in a

dimeric conformation in cells and tissues primarily mediated by intermolecular interactions between the Roc-COR tandem domains of two monomers [16–19]. The dimeric form of LRRK2 associates with cellular membranes and most probably represents the ‘active’ conformation [20,21]. LRRK2 possesses kinase activity *in vitro* and in cells and many potential substrates have been identified including LRRK2 itself via autophosphorylation largely within the Roc domain (Figure 1) [15,22,23]. LRRK2 substrates include β -tubulin, moesin, FoxO1, Futsch, tau, endophilin A1, RPS15, snapin, NSF, MARK1, RGS2, ArfGAP1, and multiple Rab GTPases [15,24–26]. While most of these substrates are known to be directly phosphorylated by LRRK2 *in vitro*, it is not yet clear whether they can also be phosphorylated in a cellular context with the notable exception of Rab proteins (i.e. Rab8a, Rab10, and Rab12) that were identified as substrates in mouse embryonic fibroblasts from G2019S knockin mice [25]. Furthermore, there is a paucity of evidence for substrate phosphorylation by LRRK2 occurring in the mammalian brain. At this juncture, it remains to be clarified whether the kinase activity of LRRK2 simply serves to autoregulate its GTPase activity and/or whether kinase activity primarily serves to mediate substrate phosphorylation that subsequently modulates substrate activity or function in downstream signaling pathways (Figure 1). It remains unclear how or whether substrate phosphorylation plays a role in mediating the pathogenic effects of familial LRRK2 mutations in different PD models *in vivo*. In this context, ArfGAP1 and RPS15 are perhaps the best-characterized LRRK2 substrates with expression and/or phosphorylation of these proteins shown to be required for LRRK2-induced neurotoxicity in neuronal culture and *Drosophila* models [24,27,28], whereas a similar role for Rab phosphorylation remains to be explored [25].

LRRK2 can also function as a GTPase where it can bind to and hydrolyze GTP *in vitro* via its Roc domain (residues 1335–1510; Figure 1) [29,30]. LRRK2 can bind to guanine nucleotides via its phosphate-binding ‘P-loop’ region (residues 1341–1348), and mutation of key residues (i.e. Lys1347 or Thr1348) can impair this binding. GTP hydrolysis occurs at a slow rate involving the Switch II catalytic region with a key role for the Arg1398 residue belonging to a highly conserved DFAGR motif present in all small GTPases [29,31]. The GTPase domain can serve to regulate kinase activity. GDP/GTP-binding-deficient mutants of LRRK2 are kinase-inactive, whereas the binding of GTP to LRRK2 in cells (but not *in vitro*) enhances its kinase activity [29–33]. Therefore, GTP-binding capacity is important for the kinase activity of LRRK2 (Figure 1). Reciprocally, the kinase activity of LRRK2 may regulate its GTPase activity via autophosphorylation [34–36], with the phosphorylation of certain Roc domain residues shown to enhance GTP hydrolysis at least within the context of an isolated Roc domain [37]. At this juncture, however, the overall effects of Roc domain autophosphorylation on GTPase activity within the full-length LRRK2 protein are not yet clear although kinase-inactive variants of LRRK2 that lack autophosphorylation possess normal GTPase activity [31]. The GTP hydrolysis event itself may also serve to regulate kinase activity since hypothesis-testing mutations that impair GTP hydrolysis (i.e. R1398L/T1343V) also impair GTP-dependent kinase activation, and GTP induces kinase activity to a greater extent than non-hydrolyzable GTP analogs [31]. The existence of guanine nucleotide exchange factors and GTPase-activating proteins (GAPs) for LRRK2 that regulate the GTPase cycle is not yet clear although several candidates have been identified (i.e. ArhGEF7, ArfGAP1, and RGS2; Figure 1) [27,28,38,39]. Comparison of LRRK2 to the

crystal structures of related prokaryotic ROCO proteins from *Chlorobium tepidum* and *Methanosarcina barkeri* suggest instead that the LRRK2 GTPase cycle may be regulated by dimerization via the C-terminal region of the COR domain [40,41]. Whether this mechanism also holds true for LRRK2 awaits further confirmation. The function of the GTPase domain and its contribution to the pathogenic actions of familial LRRK2 mutations in PD models remains incompletely characterized.

Familial PD mutations have variable effects on the enzymatic activity of LRRK2 (Figure 1). Until recently, only a single mutation (G2019S) has been shown to consistently enhance kinase activity *in vitro* using LRRK2 autophosphorylation or putative substrates as readouts [22,42]. However, the recent identification of certain Rab GTPases as the first *bona fide in vivo* substrates of LRRK2, at least in cultured mouse fibroblasts, suggests that all familial mutations may share the capacity to enhance kinase activity [25]. The reason for the differences in kinase activity of familial PD mutations *in vitro* and in cells is not yet clear but may suggest that important regulatory protein cofactors are lacking from *in vitro* kinase assays. Similar to Rab phosphorylation, LRRK2 autophosphorylation occurring at Ser1292 is also consistently enhanced by all familial mutations [43]. Therefore, enhanced LRRK2 kinase activity, depending on the particular substrate, is potentially a common property of familial mutations located within the Roc, COR, or kinase domains. Familial mutations located in the Roc (R1441C/G/H) or COR (Y1699C) domains also share the capacity to impair GTP hydrolysis activity and potentially enhance GTP binding, whereas the kinase domain G2019S or I2020T mutations do not exhibit these effects [15,30,31,44,45]. The protective R1398H variant located in the Roc domain oppositely reduces GTP binding and enhances GTP hydrolysis activity [46]. It is possible that familial mutations within the kinase domain could exert an effect on GTPase activity via autophosphorylation of Roc domain residues (Figure 1) [34,35,37]. Alternatively, familial mutations may commonly lead to enhanced kinase activity either by acting directly on the kinase domain (G2019S, I2020T) or indirectly by impairing GTPase activity (R1441C/G/H, Y1699C) (Figure 1). Collectively, the GTPase and kinase domains and their activities are clearly important for *LRRK2-linked* PD and may represent key targets for the therapeutic inhibition of LRRK2 activity.

Mechanisms of LRRK2-mediated neuronal toxicity

Contribution of kinase and GTPase activity

Many model systems have been used as tools to understand the molecular and cellular mechanisms of familial LRRK2 mutations [47]. Primary neuronal culture models in particular have been used extensively to understand the pathogenic effects of LRRK2 mutations. The transient overexpression of familial LRRK2 mutants (i.e. R1441C, Y1699C, and G2019S) consistently induces neuronal cell death, impairs neurite outgrowth, and disrupts Golgi complex morphology relative to wild-type LRRK2 [27,48–50]. Familial mutations also increase the formation of LRRK2-positive inclusions in many cell types including neurons [48]. A critical role for kinase activity in mutant LRRK2-induced neuronal toxicity and inclusion formation has been demonstrated by introducing kinase-inactive mutations at key residues within the proton acceptor site (Asp1994) or ATP-binding site (Lys1906) of the kinase domain. Much of the evidence so far supports the kinase

dependency of the G2019S mutation although a limited number of studies suggest that the R1441C mutation may also exert its actions via a kinase-dependent mechanism [32,48,50–52]. Kinase-inactive mutations at Asp1994 have been shown to selectively destabilize LRRK2 proteins in cultured neurons and rodent brain, an undesirable effect that would inadvertently serve to phenocopy a neuroprotective effect by reducing LRRK2 levels [31,53–55]. Caution is, therefore, warranted in ascribing the neuroprotective effects of kinase inhibition when using kinase-inactive Asp1994 mutants, without carefully controlling for total LRRK2 levels in each assay. Inactivating mutations at Lys1906 do not similarly influence LRRK2 stability, making null mutations at this residue the preferred choice for *in vivo* studies [31]. Pharmacological inhibition of LRRK2 kinase activity has also been shown to provide protection against familial G2019S or R1441C mutants in neuronal cultures, using compounds such as GW5074, indirubin-3'-monooxime, and CZC-25146 [51,52]. However, these original inhibitors exhibit relatively low potency and selectivity for LRRK2 compared with other kinases and have now been superseded by highly selective and potent third-generation kinase inhibitors that are largely yet to be characterized in neuronal toxicity models for LRRK2. One exception is the kinase inhibitor G1023, which was reported to rescue G2019S LRRK2-induced neuronal toxicity in cultures [43]. It is not yet clear whether familial LRRK2 mutations outside of the kinase domain also induce neuronal toxicity through a kinase-dependent mechanism, although both genetic and pharmacological kinase inhibition of the R1441C mutant was shown to be neuroprotective in culture models in one study [52].

In addition to neuronal culture models, many model organisms have been shown to exhibit dopaminergic neurodegeneration and neuropathology following the transgenic overexpression of familial LRRK2 mutants, including in nematode worm, fruit fly, mouse, and rat models [47]. Importantly, the genetic deletion of LRRK2 or the knockin of familial LRRK2 mutations fails to induce neuronal loss in model organisms, thereby supporting a toxic gain-of-function mechanism [47,55]. In *Caenorhabditis elegans*, the expression of G2019S LRRK2 induces progressive dopaminergic neuronal loss that can be partly rescued by pharmacological kinase inhibition with GW5074 or LRRK2-IN-1 as well as by genetic kinase inactivation [56,57]. In *Drosophila*, dopaminergic neuronal loss, climbing deficits, and reduced survival induced by G2019S LRRK2 expression can be rescued in part by kinase inhibition with GW5074 [56]. In rodent models, only two studies have so far demonstrated that genetic (i.e. D1994A/N) and/or pharmacological (GW5074 or indirubin-3'-monooxime) inhibition of kinase activity provides neuroprotection against dopaminergic neurodegeneration and neuropathology induced by G2019S LRRK2 [47]. These models comprise the stereotactic delivery of large-capacity recombinant herpes simplex virus amplicon vectors to the mouse striatum or human adenovirus serotype 5 vectors to the rat striatum that express human LRRK2 variants with neurodegenerative phenotypes developing over a period of 3–6 weeks [51,54]. Similar studies have not yet been conducted using more stable kinase-inactive mutants of LRRK2 (i.e. K1096M) or the latest generation of LRRK2 kinase inhibitors with markedly improved potency, selectivity, and brain penetration (i.e. MLi-2, PF-06447475, or GNE-7915 [25,43,58]). Furthermore, it is not yet clear whether LRRK2-mediated substrate phosphorylation plays a pathogenic role in these rodent models and future studies of this nature may depend on the availability of

substrate knockout mice/rats or on the viral-based co-expression of LRRK2 with phospho-null and phospho-mimic mutants of each substrate in the rodent brain. Transgenic co-expression studies have been conducted for the substrates ArfGAP1 and RPS15 in *Drosophila* G2019S LRRK2 models of PD supporting their functional interaction *in vivo* in dopaminergic neurons but these await further confirmation in rodent LRRK2 models [24,28]. At present, a range of rodent transgenic models that express additional familial LRRK2 mutations such as R1441C or R1441G have been developed but do not exhibit sufficiently pronounced or robust neuropathology compared with G2019S models, which would allow a rigorous assessment of the role of LRRK2 kinase activity [47]. Collectively, numerous studies involving genetic and pharmacological kinase inhibition support a role for kinase activity in the neurotoxic actions of the G2019S mutation. However, additional validation is now required to carefully control for the protein destabilizing effects of some kinase-inactive mutations *in vivo* and for the potential offtarget effects of first generation LRRK2 kinase inhibitors.

The contribution of GTPase activity to LRRK2-induced neurotoxicity has been investigated less intensively than kinase activity [15]. Familial LRRK2 mutations within the Roc and COR domains, such as R1441C, R1441G, and Y1699C that decrease GTPase activity *in vitro*, are able to induce neuronal toxicity in primary culture models [48–50]. Introduction of the guanine nucleotide binding-deficient mutation, K1347A, has been reported to provide protection against neuronal toxicity induced by G2019S LRRK2 [32]. However, K1347A and the adjacent T1348N mutations, which are commonly used to abolish GDP/GTP binding, are known to markedly reduce the protein stability of LRRK2 in mammalian cells particularly in cultured neurons, warranting caution with the interpretation of their neuroprotective effects [31]. The contribution of GTP hydrolysis has also been explored using hypothesis-testing mutations that either increase (R1398L) or decrease (R1398L/T1343V) the GTPase activity of LRRK2 but do not adversely influence LRRK2 protein stability in neurons [31,44]. While neither mutation is able to alter the neurotoxic effects of G2019S LRRK2 in cortical cultures, decreasing GTPase activity alone is considerably more toxic than increasing activity [31], potentially akin to the functional effects of familial mutations in the Roc-COR tandem domain. Whether the neurotoxic actions of Roc-COR domain familial mutations (i.e. R1441C, R1441G, or Y1699C) are exerted solely through their effects on impairing GTPase activity, such as by modulating GTPase activity-dependent signaling pathways or interactions with effector proteins, is not yet known. Alternatively, Roc-COR familial mutations could potentially act by prolonging the GTP-bound state of LRRK2 (via impaired GTP hydrolysis activity), which would indirectly enhance kinase activity and the phosphorylation of certain substrates (i.e. Rab proteins) that modulate downstream signaling events leading to neuronal toxicity [25,45]. Either of these potential neurotoxic mechanisms for Roc-COR mutations has not yet been confirmed. Although GTP-binding-deficient mutations reduce LRRK2 stability [31], similar studies have used compounds to address the effects of inhibiting GTP binding. Two structurally related compounds, 68 and FX2149, have been identified in unbiased screens that are reported to inhibit LRRK2 GTP binding and kinase activity in cells and brain, and afford some protection against G2019S LRRK2-induced neuronal toxicity in culture [59,60]. While these compounds potentially represent the first pharmacological tools to manipulate the

GTPase activity of LRRK2, their mechanism of action on GTP binding is not known and their selectivity toward other small GTPases remains to be determined.

Besides genetic or pharmacological modulation of GTPase activity, many cofactors have been identified that may regulate the GTPase cycle of LRRK2, including ArfGAP1, RGS2, and ArhGEF7. ArfGAP1 was first identified as a genetic modifier of human LRRK2-induced toxicity in a yeast model and was subsequently shown to interact with LRRK2 in mammalian cells and brain and promote the GTPase activity of LRRK2 *in vitro* [27,28,44]. ArfGAP1 also serves as a robust substrate of LRRK2-mediated phosphorylation [27,28]. Importantly, reducing the expression of ArfGAP1 protects against neuronal toxicity induced by G2019S LRRK2 in primary cultures, suggesting that it is critically required for mediating the downstream pathogenic actions of LRRK2 mutations [27,28]. It is not yet clear whether this mechanism involves the actions of ArfGAP1 on LRRK2 GTP hydrolysis or via ArfGAP1 phosphorylation. RGS2, another GAP protein, was identified in *C. elegans* as a genetic modifier of LRRK2 that also promotes its GTPase activity but has the opposite effect to ArfGAP1, whereby RGS2 overexpression is neuroprotective against LRRK2 [38]. The mechanisms of action of ArfGAP1 and RGS2 require further clarification and their effects on modulating neurodegeneration in rodent LRRK2 models of PD still remain to be evaluated. Both GAPs, however, represent potential therapeutic targets for modulating LRRK2 activity and neurotoxicity *in vivo*. Collectively, some studies have provided clues for the role of GTPase activity in neurotoxicity induced by G2019S LRRK2; however, many technical caveats and mechanistic questions have been identified with the genetic and pharmacological modulation of LRRK2 GTPase activity that will need to be resolved in future studies.

Protein aggregation pathways: role of α -synuclein and tau

PD brains harboring *LRRK2* mutations predominantly exhibit α -synuclein-positive Lewy body pathology, yet in some cases tau-positive neurofibrillary tangles, reminiscent of the tauopathy progressive supranuclear palsy, are the only detectable neuropathology [3,4,7,11]. Common variation in the *SNCA* and *MAPT* genes, which encode α -synuclein and tau, respectively, have been identified as the major risk factors for idiopathic PD [5]. Whether this protein aggregation pathology contributes to neurodegenerative processes in these *LRRK2*-linked PD cases or whether it represents benign pathology that occurs secondary to neurodegeneration is not known. Many studies using rodent models have attempted to address these questions albeit indirectly for the most part. LRRK2 transgenic or viral-based mouse or rat models consistently do not exhibit signs of α -synuclein pathology as measured by aggregation, insoluble species, or Ser129 phosphorylation [47]. However, some mutant LRRK2 transgenic mouse and viral-based rat models reveal modest alterations in tau accumulation or hyperphosphorylation but no evidence of tau aggregation *per se* [61–63]. Therefore, LRRK2 transgenic rodent models do not generally recapitulate the protein aggregation pathology observed in *LRRK2*-linked PD brains.

To further explore the pathological interaction between LRRK2 and α -synuclein *in vivo*, models have been developed that co-express both proteins. In mouse forebrain neurons, the inducible transgenic expression of human G2019S LRRK2 markedly enhances the

progression and extent of human A53T α -synuclein-induced neurodegeneration although G2019S LRRK2 expression itself does not induce neuronal damage [64]. The deletion of LRRK2 in these mice oppositely protects against α -synuclein-induced neuronal damage [64]. Such studies may appear to place LRRK2 downstream from α -synuclein-induced neurodegenerative pathways, whereas human genetic data indicate that *LRRK2* mutations alone are sufficient to induce α -synuclein aggregation [11]. Alternatively, it is possible that LRRK2-mediated phosphorylation or signaling is required to initiate the full neurotoxic effects of mutant α -synuclein. Two related studies using human α -synuclein transgenic mouse models that develop prominent hindbrain neuropathology, within the brainstem and spinal cord, have failed to reveal a pathological interaction with LRRK2 transgenic or knockout mice, potentially revealing regional limitations to this interaction or reflecting technical caveats related to the crossbreeding of mouse models versus the conditional co-expression of two transgenes in the same neuronal populations [65,66]. Recent studies using rat models have further extended this interaction to midbrain dopaminergic neurons and demonstrate that dopaminergic neurodegeneration and microgliosis induced by the AAV2-mediated expression of human α -synuclein can be abrogated either by LRRK2 deletion or by LRRK2 kinase inhibition with PF-06447475 [58,67]. BAC transgenic rats expressing human G2019S LRRK2 reveal enhanced dopaminergic neurodegeneration induced by AAV2- α -synuclein delivery that can similarly be rescued by kinase inhibition with PF-06447475 [58]. LRRK2 knockout rats are also protected against dopaminergic neurodegeneration and gliosis induced by lipopolysaccharide (LPS), a potent inducer of neuroinflammation, and, therefore, it is not yet clear whether LRRK2 deletion or inhibition is generally neuroprotective toward pro-inflammatory stimuli including LPS- or AAV2- α -synuclein-induced neurotoxicity [67]. A recent study involving the direct delivery of preformed fibrils of α -synuclein, instead of AAV2-based expression, to the substantia nigra suggests that the formation of α -synuclein aggregates may be enhanced by human G2019S LRRK2 expression in rats [68]. Sufficient evidence now supports an intriguing role for LRRK2, and in particular its kinase activity, in mediating α -synuclein-induced aggregation and neurodegeneration in rodent models. These studies may lay the foundation for the use of LRRK2 kinase inhibitors to prevent the neurotoxic actions of α -synuclein in PD. However, so far, only a single study has attempted to address whether α -synuclein is required downstream from LRRK2-induced neurotoxicity [53], as implied by human genetic data [4,11]. Gene silencing of α -synuclein in neuronal culture models protects against mutant LRRK2-induced cell death by unexpectedly reducing LRRK2 levels [53]. While these data support a role for α -synuclein in mediating the neurotoxic effects of familial LRRK2 mutations, the mechanism of LRRK2 destabilization by α -synuclein remains unclear and additional confirmation is now required in rodent α -synuclein knockout or knockdown models.

Similar mouse crossbreeding studies have also been reported for LRRK2 and tau models. The expression of human wild-type LRRK2 in a conditional mouse model expressing human P301L tau, a mutant form linked to the tauopathy FTDP-17, selectively in forebrain neurons was suggested to increase the aggregation and phosphorylation of tau in the cerebral cortex at a single time point (~5.5 months) [69]. Whether this increased tau pathology progressed further with age, or was associated with neuronal damage or behavioral deficits, was not

assessed. An independent study that crossed BAC transgenic mice expressing human R1441G LRRK2 with human P301S tau transgenic mice (prion protein promoter) revealed no impact of R1441G LRRK2 on tau hyperphosphorylation and aggregation, and no worsening of the reduced survival, gliosis, and selective neurodegeneration that normally develop in these tau mice [70]. The effects of LRRK2 deletion or kinase inhibition on these tau-related neurodegenerative pathologies are not known. As with α -synuclein, it is not yet clear whether tau protein is required for mediating mutant LRRK2-induced neurodegeneration in rodent models (i.e. using tau knockout mice), but this approach is warranted based on the development of tau neuropathology in *LRRK2-linked* PD brains [7]. Whether or not LRRK2 can modulate neurodegenerative tauopathy *in vivo* has not been clearly established. There could be pronounced differences between BAC transgenic mice expressing WT or R1441G LRRK2, or potential differences between the location of tau pathology that develops in each tau transgenic model and its interaction with LRRK2 [69,70]. The significance of tau pathology for neurodegenerative processes induced by familial *LRRK2* mutations in PD awaits further investigation.

Conclusions and future perspectives

Here, we have focused on the potential contribution of LRRK2 enzymatic activity and protein aggregation pathways to neurodegeneration induced by familial LRRK2 mutations in models of PD. However, there are many cellular pathways and processes not discussed here that may be regulated by mutant LRRK2, which may contribute to neurodegeneration, including effects on protein translation, autophagy, the endolysosomal pathway, Golgi complex integrity, microtubule and actin networks, and synaptic vesicle recycling, to name a few (Figure 1) [22]. One could consider the GTPase and kinase activities of LRRK2 the most straightforward and selective to target from a therapeutic standpoint, whereas protein aggregation, namely of α -synuclein and tau, is intimately linked to the effects of *LRRK2* mutations in PD brains (Figure 1). The contribution of kinase activity to mutant LRRK2-induced neurotoxicity has so far gained the majority of experimental support but now requires additional validation using disease-relevant rodent LRRK2 models and the latest kinase inhibitors that will form the foundation for human clinical trials. Important mechanistic questions also need to be addressed regarding how substrate phosphorylation by LRRK2 contributes to neuronal damage *in vivo*. The contribution of GTPase activity to regulating LRRK2 kinase activity, the physiological function of LRRK2 in general, and to neuronal toxicity induced by familial LRRK2 mutations needs to be better understood and awaits the development of additional genetic and pharmacological reagents for selectively manipulating LRRK2 GTPase activity *in vivo*. The contributions of α -synuclein and tau aggregation to mutant LRRK2-dependent neurodegenerative processes have not been convincingly answered, and suitable rodent models are now in place to do so, but what has emerged instead is a hitherto unanticipated role for LRRK2 activity in initiating and/or mediating α -synuclein-mediated neurodegeneration in rodent models. There are many hints that LRRK2, α -synuclein, and tau may coalesce in common pathological pathways but establishing the hierarchy and significance of these pathological interactions remains an important goal (Figure 1). LRRK2 rodent models continue to provide critical tools for

discovering and validating new disease mechanisms and are poised to play an important role in the evaluation of disease-modifying therapeutic agents for PD.

Acknowledgments

Funding

The authors are grateful for funding support from the National Institutes of Health [R01 NS091719], Swiss National Science Foundation [grant no. 31003A_144063], and the Van Andel Research Institute.

Abbreviations

COR	<u>C</u> -terminal-of- <u>Roc</u>
GAP	GTPase-activating protein
LPS	lipopolysaccharide
LRR	leucine-rich repeat
LRRK2	leucine-rich repeat kinase 2
PD	Parkinson's disease
Roc	<u>Ras</u> -of- <u>C</u> omplex

References

1. Lang AE, Lozano AM. Parkinson's disease. *N Engl J Med*. 1998; 339:1130–1143. DOI: 10.1056/NEJM199810153391607 [PubMed: 9770561]
2. Lang AE, Lozano AM. Parkinson's disease. *N Engl J Med*. 1998; 339:1044–1053. DOI: 10.1056/NEJM199810083391506 [PubMed: 9761807]
3. Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. *J Neurochem*. 2016; 139(Suppl 1):59–74. [PubMed: 27090875]
4. Paisan-Ruiz C, Lewis PA, Singleton AB. LRRK2: cause, risk, and mechanism. *J Parkinsons Dis*. 2013; 3:85–103. [PubMed: 23938341]
5. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. 2014; 46:989–993. DOI: 10.1038/ng.3043 [PubMed: 25064009]
6. Paisán-Ruiz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*. 2004; 44:595–600. DOI: 10.1016/j.neuron.2004.10.023 [PubMed: 15541308]
7. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*. 2004; 44:601–607. DOI: 10.1016/j.neuron.2004.11.005 [PubMed: 15541309]
8. Ross OA, Soto-Ortolaza AI, Heckman MG, Aasly JO, Abahuni N, Annesi G, et al. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. *Lancet Neurol*. 2011; 10:898–908. DOI: 10.1016/S1474-4422(11)70175-2 [PubMed: 21885347]
9. Heckman MG, Soto-Ortolaza AI, Aasly JO, Abahuni N, Annesi G, Bacon JA, et al. Population-specific frequencies for *LRRK2* susceptibility variants in the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium. *Mov Disord*. 2013; 28:1740–1744. DOI: 10.1002/mds.25600 [PubMed: 23913756]

10. Coetzee SG, Pierce S, Brundin P, Brundin L, Hazelett DJ, Coetzee GA. Enrichment of risk SNPs in regulatory regions implicate diverse tissues in Parkinson's disease etiology. *Sci Rep*. 2016; 6:30509.doi: 10.1038/srep30509 [PubMed: 27461410]
11. Ross OA, Toft M, Whittle AJ, Johnson JL, Papapetropoulos S, Mash DC, et al. Lrrk2 and Lewy body disease. *Ann Neurol*. 2006; 59:388–393. DOI: 10.1002/ana.20731 [PubMed: 16437559]
12. Biskup S, West AB. Zeroing in on LRRK2-linked pathogenic mechanisms in Parkinson's disease. *Biochim Biophys Acta*. 2009; 1792:625–633. DOI: 10.1016/j.bbadis.2008.09.015 [PubMed: 18973807]
13. Wider C, Dickson DW, Wszolek ZK. Leucine-rich repeat kinase 2 gene-associated disease: redefining genotype-phenotype correlation. *Neurodegener Dis*. 2010; 7:175–179. DOI: 10.1159/000289232 [PubMed: 20197701]
14. Ling H, Kara E, Bandopadhyay R, Hardy J, Holton J, Xiomerisiou G, et al. TDP-43 pathology in a patient carrying G2019S LRRK2 mutation and a novel p.Q124E MAPT. *Neurobiol Aging*. 2013; 34:2889.e5–2889.e9. DOI: 10.1016/j.neurobiolaging.2013.04.011
15. Tsika E, Moore DJ. Contribution of GTPase activity to LRRK2-associated Parkinson disease. *Small GTPases*. 2013; 4:164–170. DOI: 10.4161/sgtp.25130 [PubMed: 24025585]
16. Greggio E, Zambrano I, Kaganovich A, Beilina A, Taymans JM, Daniels V, et al. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J Biol Chem*. 2008; 283:16906–16914. DOI: 10.1074/jbc.M708718200 [PubMed: 18397888]
17. Daniels V, Vancraenenbroeck R, Law BMH, Greggio E, Lobbetael E, Gao F, et al. Insight into the mode of action of the LRRK2 Y1699C pathogenic mutant. *J Neurochem*. 2011; 116:304–315. DOI: 10.1111/j.1471-4159.2010.07105.x [PubMed: 21073465]
18. Civiero L, Vancraenenbroeck R, Belluzzi E, Beilina A, Lobbetael E, Reyniers L, et al. Biochemical characterization of highly purified leucine-rich repeat kinases 1 and 2 demonstrates formation of homodimers. *PLoS One*. 2012; 7:e43472.doi: 10.1371/journal.pone.0043472 [PubMed: 22952686]
19. Guaitoli G, Raimondi F, Gilsbach BK, Gomez-Llorente Y, Deyaert E, Renzi F, et al. Structural model of the dimeric Parkinson's protein LRRK2 reveals a compact architecture involving distant interdomain contacts. *Proc Natl Acad Sci USA*. 2016; 113:E4357–E4366. DOI: 10.1073/pnas.1523708113 [PubMed: 27357661]
20. Berger Z, Smith KA, LaVoie MJ. Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry*. 2010; 49:5511–5523. DOI: 10.1021/bi100157u [PubMed: 20515039]
21. Sen S, Webber PJ, West AB. Dependence of leucine-rich repeat kinase 2 (LRRK2) kinase activity on dimerization. *J Biol Chem*. 2009; 284:36346–36356. DOI: 10.1074/jbc.M109.025437 [PubMed: 19826009]
22. Tsika E, Moore DJ. Mechanisms of LRRK2-mediated neurodegeneration. *Curr Neurol Neurosci Rep*. 2012; 12:251–260. DOI: 10.1007/s11910-012-0265-8 [PubMed: 22441981]
23. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA*. 2005; 102:16842–16847. DOI: 10.1073/pnas.0507360102 [PubMed: 16269541]
24. Martin I, Kim JW, Lee BD, Kang HC, Xu JC, Jia H, et al. Ribosomal protein s15 phosphorylation mediates LRRK2 neurodegeneration in Parkinson's disease. *Cell*. 2014; 157:472–485. DOI: 10.1016/j.cell.2014.01.064 [PubMed: 24725412]
25. Steger M, Tonelli F, Ito G, Davies P, Trost M, Vetter M, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *Elife*. 2016; 5:e12813.doi: 10.7554/eLife.12813 [PubMed: 26824392]
26. Belluzzi E, Gonnelli A, Cinaru MD, Marte A, Plotegher N, Russo I, et al. LRRK2 phosphorylates pre-synaptic N-ethylmaleimide sensitive fusion (NSF) protein enhancing its ATPase activity and SNARE complex disassembling rate. *Mol Neurodegener*. 2016; 11:1.doi: 10.1186/s13024-015-0066-z [PubMed: 26758690]

27. Stafa K, Trancikova A, Webber PJ, Glauser L, West AB, Moore DJ. GTPase activity and neuronal toxicity of Parkinson's disease — associated LRRK2 is regulated by ArfGAP1. *PLoS Genet.* 2012; 8:e1002526.doi: 10.1371/journal.pgen.1002526 [PubMed: 22363216]
28. Xiong Y, Yuan C, Chen R, Dawson TM, Dawson VL. ArfGAP1 is a GTPase activating protein for LRRK2: reciprocal regulation of ArfGAP1 by LRRK2. *J Neurosci.* 2012; 32:3877–3886. DOI: 10.1523/JNEUROSCI.4566-11.2012 [PubMed: 22423108]
29. Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, et al. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. *Biochemistry.* 2007; 46:1380–1388. DOI: 10.1021/bi061960m [PubMed: 17260967]
30. West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, et al. Parkinson's disease-associated mutations in *LRRK2* link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum Mol Genet.* 2006; 16:223–232. DOI: 10.1093/hmg/ddl471
31. Biosia A, Trancikova A, Civiero L, Glauser L, Bubacco L, Greggio E, et al. GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. *Hum Mol Genet.* 2013; 22:1140–1156. DOI: 10.1093/hmg/ddt522 [PubMed: 23241358]
32. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci.* 2006; 9:1231–1233. DOI: 10.1038/nn1776 [PubMed: 16980962]
33. Taymans JM, Vancraenenbroeck R, Ollikainen P, Beilina A, Lobbstaël E, De Maeyer M, et al. LRRK2 kinase activity is dependent on LRRK2 GTP binding capacity but independent of LRRK2 GTP binding. *PLoS One.* 2011; 6:e23207.doi: 10.1371/journal.pone.0023207 [PubMed: 21858031]
34. Greggio E, Taymans JM, Zhen EY, Ryder J, Vancraenenbroeck R, Beilina A, et al. The Parkinson's disease kinase LRRK2 autophosphorylates its GTPase domain at multiple sites. *Biochem Biophys Res Commun.* 2009; 389:449–454. DOI: 10.1016/j.bbrc.2009.08.163 [PubMed: 19733152]
35. Webber PJ, Smith AD, Sen S, Renfrow MB, Mobley JA, West AB. Autophosphorylation in the leucine-rich repeat kinase 2 (LRRK2) GTPase domain modifies kinase and GTP-binding activities. *J Mol Biol.* 2011; 412:94–110. DOI: 10.1016/j.jmb.2011.07.033 [PubMed: 21806997]
36. Gloeckner CJ, Boldt K, von Zweydt F, Helm S, Wiesent L, et al. Phosphopeptide analysis reveals two discrete clusters of phosphorylation in the N-terminus and the Roc domain of the Parkinson-disease associated protein kinase LRRK2. *J Proteome Res.* 2010; 9:1738–1745. DOI: 10.1021/pr9008578 [PubMed: 20108944]
37. Liu Z, Mobley JA, DeLucas LJ, Kahn RA, West AB. LRRK2 autophosphorylation enhances its GTPase activity. *FASEB J.* 2016; 30:336–347. DOI: 10.1096/fj.15-277095 [PubMed: 26396237]
38. Dusanochet J, Li H, Guillily M, Liu M, Stafa K, Derada Troletti C, et al. A Parkinson's disease gene regulatory network identifies the signaling protein RGS2 as a modulator of LRRK2 activity and neuronal toxicity. *Hum Mol Genet.* 2014; 23:4887–4905. DOI: 10.1093/hmg/ddu202 [PubMed: 24794857]
39. Haebig K, Gloeckner CJ, Miralles MG, Gillardon F, Schulte C, Riess O, et al. ARHGEF7 (Beta-PIX) acts as guanine nucleotide exchange factor for leucine-rich repeat kinase 2. *PloS One.* 2010; 5:e13762.doi: 10.1371/journal.pone.0013762 [PubMed: 21048939]
40. Gotthardt K, Weyand M, Kortholt A, Van Haastert PJM, Wittinghofer A. Structure of the Roc-COR domain tandem of *tepidum*, *C. tepidum*, a prokaryotic homologue of the human LRRK2 Parkinson kinase. *EMBO J.* 2008; 27:2239–2249. DOI: 10.1038/emboj.2008.150 [PubMed: 18650931]
41. Terheyden S, Ho FY, Gilsbach BK, Wittinghofer A, Kortholt A. Revisiting the Roco G-protein cycle. *Biochem J.* 2015; 465:139–147. DOI: 10.1042/BJ20141095 [PubMed: 25317655]
42. Greggio E, Cookson MR. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro.* 2009; 1:e00002.doi: 10.1042/AN20090007 [PubMed: 19570025]
43. Sheng Z, Zhang S, Bustos D, Kleinheinz T, Le Pichon CE, et al. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Sci Transl Med.* 2012; 4:164ra161.doi: 10.1126/scitranslmed.3004485
44. Xiong Y, Coombes CE, Kilaru A, Li X, Gitler AD, Bowers WJ, et al. GTPase activity plays a key role in the pathobiology of LRRK2. *PLoS Genet.* 2010; 6:e1000902.doi: 10.1371/journal.pgen.1000902 [PubMed: 20386743]

45. Liao J, Wu CX, Burlak C, Zhang S, Sahm H, Wang M, et al. Parkinson disease-associated mutation R1441H in LRRK2 prolongs the 'active state' of its GTPase domain. *Proc Natl Acad Sci USA*. 2014; 111:4055–4060. DOI: 10.1073/pnas.1323285111 [PubMed: 24591621]
46. Nixon-Abell J, Berwick DC, Granno S, Spain VA, Blackstone C, Harvey K. Protective LRRK2 R1398H variant enhances GTPase and Wnt signaling activity. *Front Mol Neurosci*. 2016; 9:18.doi: 10.3389/fnmol.2016.00018 [PubMed: 27013965]
47. Daniel G, Moore DJ. Modeling LRRK2 pathobiology in Parkinson's disease: from yeast to rodents. *Curr Top Behav Neurosci*. 2015; 22:331–368. DOI: 10.1007/7854_2014_311 [PubMed: 24850078]
48. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis*. 2006; 23:329–341. DOI: 10.1016/j.nbd.2006.04.001 [PubMed: 16750377]
49. Smith WW, Pei Z, Jiang H, Moore DJ, Liang Y, West AB, et al. Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci USA*. 2005; 102:18676–18681. DOI: 10.1073/pnas.0508052102 [PubMed: 16352719]
50. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron*. 2006; 52:587–593. DOI: 10.1016/j.neuron.2006.10.008 [PubMed: 17114044]
51. Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, et al. Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. *Nat Med*. 2010; 16:998–1000. DOI: 10.1038/nm.2199 [PubMed: 20729864]
52. Ramsden N, Perrin J, Ren Z, Lee BD, Zinn N, Dawson VL, et al. Chemoproteomics-based design of potent LRRK2-selective lead compounds that attenuate Parkinson's disease-related toxicity in human neurons. *ACS Chem Biol*. 2011; 6:1021–1028. DOI: 10.1021/cb2002413 [PubMed: 21812418]
53. Skibinski G, Nakamura K, Cookson MR, Finkbeiner S. Mutant LRRK2 toxicity in neurons depends on LRRK2 levels and synuclein but not kinase activity or inclusion bodies. *J Neurosci*. 2014; 34:418–433. DOI: 10.1523/JNEUROSCI.2712-13.2014 [PubMed: 24403142]
54. Tsika E, Nguyen APT, Dushonchet J, Colin P, Schneider BL, Moore DJ. Adenoviral-mediated expression of G2019S LRRK2 induces striatal pathology in a kinase-dependent manner in a rat model of Parkinson's disease. *Neurobiol Dis*. 2015; 77:49–61. DOI: 10.1016/j.nbd.2015.02.019 [PubMed: 25731749]
55. Herzig MC, Kolly C, Persohn E, Theil D, Schweizer T, Hafner T, et al. LRRK2 protein levels are determined by kinase function and are crucial for kidney and lung homeostasis in mice. *Hum Mol Genet*. 2011; 20:4209–4223. DOI: 10.1093/hmg/ddr348 [PubMed: 21828077]
56. Liu Z, Hamamichi S, Lee DB, Yang D, Ray A, Caldwell GA, et al. Inhibitors of LRRK2 kinase attenuate neurodegeneration and Parkinson-like phenotypes in *Caenorhabditis elegans* and *Drosophila* Parkinson's disease models. *Hum Mol Genet*. 2011; 20:3933–3942. DOI: 10.1093/hmg/ddr312 [PubMed: 21768216]
57. Yao C, Johnson WM, Gao Y, Wang W, Zhang J, Deak M, et al. Kinase inhibitors arrest neurodegeneration in cell and *C. elegans* models of LRRK2 toxicity. *Hum Mol Genet*. 2013; 22:328–344. DOI: 10.1093/hmg/ddr431 [PubMed: 23065705]
58. Daher JPL, Abdelmotilib HA, Hu X, Volpicelli-Daley LA, Moehle MS, Fraser KB, et al. Leucine-rich repeat kinase 2 (LRRK2) pharmacological inhibition abates α -Synuclein gene-induced neurodegeneration. *J Biol Chem*. 2015; 290:19433–19444. DOI: 10.1074/jbc.M115.660001 [PubMed: 26078453]
59. Li T, He X, Thomas JM, Yang D, Zhong S, Xue F, et al. A novel GTP-binding inhibitor, FX2149, attenuates LRRK2 toxicity in Parkinson's disease models. *PLoS One*. 2015; 10:e0122461.doi: 10.1371/journal.pone.0122461 [PubMed: 25816252]
60. Li T, Yang D, Zhong S, Thomas JM, Xue F, Liu J, et al. Novel LRRK2 GTP-binding inhibitors reduced degeneration in Parkinson's disease cell and mouse models. *Hum Mol Genet*. 2014; 23:6212–6222. DOI: 10.1093/hmg/ddu341 [PubMed: 24993787]
61. Dushonchet J, Kochubey O, Stafa K, Young SM, Zufferey R, Moore DJ, et al. A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S

- mutation in LRRK2. *J Neurosci Nurs.* 2011; 31:907–912. DOI: 10.1523/JNEUROSCI.5092-10.2011
62. Li X, Patel JC, Wang J, Avshalumov MV, Nicholson C, Buxbaum JD, et al. Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. *J Neurosci Nurs.* 2010; 30:1788–1797. DOI: 10.1523/JNEUROSCI.5604-09.2010
63. Melrose HL, Dächsel JC, Behrouz B, Lincoln SJ, Yue M, Hinkle KM, et al. Impaired dopaminergic neurotransmission and microtubule-associated protein tau alterations in human LRRK2 transgenic mice. *Neurobiol Dis.* 2010; 40:503–517. DOI: 10.1016/j.nbd.2010.07.010 [PubMed: 20659558]
64. Lin X, Parisiadou L, Gu XL, Wang L, Shim H, Sun L, et al. Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant α -synuclein. *Neuron.* 2009; 64:807–827. DOI: 10.1016/j.neuron.2009.11.006 [PubMed: 20064389]
65. Daher JPL, Pletnikova O, Biskup S, Musso A, Gellhaar S, Galter D, et al. Neurodegenerative phenotypes in an A53T α -synuclein transgenic mouse model are independent of LRRK2. *Hum Mol Genet.* 2012; 21:2420–2431. DOI: 10.1093/hmg/dds057 [PubMed: 22357653]
66. Herzig MC, Bidinosti M, Schweizer T, Hafner T, Stemmelen C, Weiss A, et al. High LRRK2 levels fail to induce or exacerbate neuronal alpha-synucleinopathy in mouse brain. *PLoS One.* 2012; 7:e36581.doi: 10.1371/journal.pone.0036581 [PubMed: 22615783]
67. Daher JPL, Volpicelli-Daley LA, Blackburn JP, Moehle MS, West AB. Abrogation of alpha-synuclein-mediated dopaminergic neurodegeneration in LRRK2-deficient rats. *Proc Natl Acad Sci USA.* 2014; 111:9289–9294. DOI: 10.1073/pnas.1403215111 [PubMed: 24927544]
68. Volpicelli-Daley LA, Abdelmotilib H, Liu Z, Stoyka L, Daher JPL, Milnerwood AJ, et al. G2019S-LRRK2 expression augments α -Synuclein sequestration into inclusions in neurons. *J Neurosci.* 2016; 36:7415–7427. DOI: 10.1523/JNEUROSCI.3642-15.2016 [PubMed: 27413152]
69. Bailey RM, Covy JP, Melrose HL, Rousseau L, Watkinson R, Knight J, et al. LRRK2 phosphorylates novel tau epitopes and promotes tauopathy. *Acta Neuropathol.* 2013; 126:809–827. DOI: 10.1007/s00401-013-1188-4 [PubMed: 24113872]
70. Mikhail F, Calingasan N, Parolari L, Subramanian A, Yang L, Flint Beal M. Lack of exacerbation of neurodegeneration in a double transgenic mouse model of mutant LRRK2 and tau. *Hum Mol Genet.* 2015; 24:3545–3556. DOI: 10.1093/hmg/ddv105 [PubMed: 25804954]

Figure 1. Summary of putative mechanisms of LRRK2-dependent neurotoxicity in PD
 LRRK2 protein domain architecture and the location and functional effects of familial PD mutations are indicated. The intramolecular regulation of the GTPase and kinase domains is also shown, including autophosphorylation within the Roc domain, the critical requirement of GTP binding and hydrolysis activities for kinase activity, and putative kinase substrates. Cellular pathways or processes known to be influenced by familial LRRK2 mutations as well as protein aggregation pathways involving α -synuclein and tau proteins are shown that potentially converge to induce neuronal damage and toxicity in PD through unknown mechanisms. Potential therapeutic strategies are also indicated including options for kinase inhibition and GTPase modulation that may mitigate the neurotoxic actions of LRRK2 mutations.