Heterogeneity of Leucine-Rich Repeat Kinase 2 Mutations: Genetics, Mechanisms and Therapeutic Implications

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Abstract Variation within and around the leucine-rich repeat kinase 2 (*LRRK2*) gene is associated with familial and sporadic Parkinson's disease (PD). Here, we discuss the prevalence of *LRRK2* substitutions in different populations and their association with PD, as well as molecular and cellular mechanisms of pathologically relevant *LRRK2* mutations. Kinase activation was proposed as a universal molecular mechanism for all pathogenic *LRRK2* mutations, but later reports revealed heterogeneity in the effect of mutations on different activities of *LRRK2*. One mutation (G2019S) increases kinase activity, whereas mutations in the Ras of complex proteins (ROC)–C-terminus of ROC (COR) bidomain impair the GTPase function of *LRRK2*. Some risk factor variants, including G2385R in the WD40 domain, actually decrease the kinase activity of *LRRK2*. We suggest a model where *LRRK2* mutations exert different molecular mechanisms but interfere with normal cellular function of *LRRK2* at different levels of the same downstream pathway. Finally, we discuss the current state of therapeutic approaches for *LRRK2*-related PD.

Key Words: Parkinson's disease · kinase inhibitors · GTPase · vesicular trafficking · cytoskeleton · treatment.

Introduction

Parkinson's disease (PD) is a devastating neurodegenerative disorder affecting about 1.5 % of the population older than 65 years [1]. The typical clinical presentation of PD includes

resting tremor, rigidity, bradykinesia, and postural instability [2]. Initially, PD was considered as a purely idiopathic disorder, but a growing number of genetic factors are now recognized to contribute to lifetime risk of disease [3].

Among the known autosomal-dominant genes, mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are a relatively common cause of PD [4, 5]. Clinically, *LRRK2*-associated PD closely resembles idiopathic disease [6, 7], although pathological phenotypes in patients with the same mutation are variable [8]. The gene consists of 51 exons [9, 10] and is located on chromosome 12, in the locus originally designated as PARK8 [11]. The same locus has been identified by genome-wide association studies as conferring lifetime risk for sporadic PD.

LRRK2 encodes a large (286-kDa) complex protein composed of a central catalytic Ras of complex proteins (ROC)–C-terminus of ROC (COR)–kinase tridomain surrounded by several protein–protein interacting domains (Fig. 1). *LRRK2* is an active kinase [12–15], and, as a member of the ROCO family of proteins [16, 17], can both bind and hydrolyse GTP [18–20]. It was initially proposed that GTP binding could stimulate the kinase activity of *LRRK2*, although subsequent results suggested that kinase activity of *LRRK2* depends on the ability to bind guanosine nucleotides rather than the GTP-bound state [21–23], an important mechanistic distinction. Reciprocal regulation of GTP binding by the kinase domain may also occur, as *LRRK2* autophosphorylates several residues within the ROC domain. Phosphorylation of T1491 and T1503 residues apparently diminishes GTP binding [24–29], suggesting that *LRRK2* GTPase may be functionally downstream of the kinase activity [30], although more complex situations may occur. These suggestions show that there is intramolecular communication between different *LRRK2* domains, pointing out how complex the molecule is.

Furthermore, these activities are probably regulated by cellular signaling. Similar to many other GTPases, GTP

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Fig. 1 LRRK2 domain structure, localization of mutations, and proposed pathogenic mechanisms. ARM=armadillo repeats; ANK=ankyrin repeats; ROC=Ras of complex proteins; COR=C-terminus of ROC, kinase domain, and WD40 domain; GTP

hydrolysis of LRRK2 might be regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) [30, 31]. GEFs activate GTPases by promoting the exchange of GDP for GTP, while GAPs stimulate GTP hydrolysis and inactivation of enzymes. ARHGEF7 and ArfGAP1 were recently suggested to play these respective roles for LRRK2 [32–34]. The weak intrinsic GTPase activity of LRRK2 *in vitro* may be owing to the absence of the regulatory factors in assays of purified protein [35]. Dimerization is another potential mechanism of regulation of GTP binding and hydrolysis by LRRK2 [36]. In this model, GTP binding promotes dimerization and activation of the protein. Some studies have suggested that a dimer is the catalytically active form of LRRK2 [24, 37–39], although this has not yet been confirmed using structural analyses. The dimeric form of LRRK2 may also be essential for kinase activity of the protein [37, 38]. LRRK2 is also phosphorylated by other, as yet unknown, kinases.

LRRK2 also contains several domains that might be important for protein–protein interactions outside the catalytic core such as armadillo, ankyrin, leucine-rich, and WD40 repeats (Fig. 1). LRRK2 physically interacts with many proteins, such as microtubules [40–43], 14-3-3 proteins [44, 45], mitogen-activated protein kinase 6 [46], and Wnt signaling pathway proteins [47]. Therefore, LRRK2 may also function as a scaffold for many signaling pathways and might therefore be implicated in the number of cellular functions [48].

The physiological function of LRRK2 is unknown, but LRRK2 is involved in autophagy [49, 50], vesicle sorting and trafficking [51, 52], dopamine homeostasis [53, 54], dopamine receptor activation, synaptogenesis [55], and cytoskeleton dynamics (Fig. 2) [56, 57]. Many of these functions depend on LRRK2 activity and could, therefore, be affected by different mutations. In this review, we discuss the diversity of mechanisms of different pathogenic mutations and risk factor variants of LRRK2. We also discuss whether we can predict useful therapeutic strategies based on what we know about LRRK2 biology and pathogenesis.

Diversity of LRRK2 Mutations

Many mutations have been reported in *LRRK2* in patients with PD, but only 6 of these (R1441C/G/H, Y1699C, G2019S, and I2020T) found in the central catalytic ROC–COR–kinase triple domain clearly segregate with the disease (Fig. 1; Table 1) [48, 58, 59]. Some additional LRRK2 variants represent risk factors for the disease (G2385R, R1628P), while the role of other reported substitutions remains unclear. We will discuss these different mutations below based on where they are in the protein as this leads most easily into a discussion about variable effects on function.

Fig. 2 Pathogenic mechanisms of LRRK2 mutations (modified from [151]). Many pathogenic mechanisms have been reported for LRRK2 mutations. Among those there is a growing number of evidence of involvement of LRRK2 in different vesicle trafficking events, including endocytosis [152, 171], Golgi clearance [43, 51, 126, 150, 151], cytoskeleton dynamics [40–43, 53, 115, 126, 138, 139, 150, 172–174], lysosomal dynamics [143], and autophagy [124, 140, 142, 146, 175]. Different pathogenic LRRK2 mutations can cause alterations of at least some of these events. Please note that lack of indication that a specific mutation has a given effect does not necessarily mean that a negative result has been reported; in most cases not all mutations have been tested

Mutations in the Kinase Domain

The most common *LRRK2* mutation is G2019S, which was first identified in PD patients of European ancestry [60–63]. The highest incidence of this variant is in people of Ashkenazi Jewish descent (almost 20 %) [64] and in North African patients (about 40 %) [65]. The G2019S substitution is located in the activation loop of the kinase domain of LRRK2, and causes stabilization of the enzyme in the active form, leading to enhanced phosphorylation activity (Fig. 1) [31, 66]. A 2–3-fold increase in kinase activity of G2019S LRRK2 compared with wild-type protein has been consistently seen by many groups [12, 13, 15, 20, 56, 67–73].

The I2020T substitution is also located within the activation loop of the kinase domain (Fig. 1). This mutation is causative in the first Japanese family reported for the PARK8 locus [11, 74]. In contrast to the adjacent G2019S mutation, how I2020T affects protein function is controversial. Some reports suggest an increased kinase activity for I2020T mutant [14, 75, 76] presumably via stabilization of the active state confirmation of the kinase [77], while others revealed unchanged [44] or even decreased kinase activity [67]. Although methodological differences between assays may contribute to the apparent differences between studies [78], a more likely interpretation of these data is that the effect of this mutation is not more than a 40 % increase over the wild type. In most assays, this is within measurement error. Overall, this suggests that the effects of G2019S and I2020T on kinase activity of

LRRK2 are quite different from each other. The differential effects of these two mutations is surprising given that these are apparently similar substitutions at adjacent residues and a convincing structural explanation has not yet been proposed.

ROC–COR Bidomain Mutations

The second most common LRRK2 mutation, R1441C, has been found in several different populations [79]. Interestingly, two other variations of the same codon (R1441G and R1441H) have been reported. The R1441G variant is frequent in some Basque families, but rare elsewhere [80]. The R1441H mutation was reported in several PD families [81–85], but is generally infrequent worldwide [86].

The R1441 residue is located at the interface of the ROC and COR domains, and likely stabilizes the interactions between these regions [87]. The R1441 mutations cause a decrease in GTPase activity of LRRK2, although this activity is low and has been difficult to measure reliably, leading to some uncertainty about relevance of this observation [18–20, 88]. The mechanism of diminished activity is likely via disruption of hydrogen bonding and stacking interactions normally provided by the arginine side chain [18–20, 87], which may cause an enhancement in the affinity of LRRK2 for GTP with a subsequent decrease in GTP hydrolysis [89]. Substitution of the positively charged arginine to neutral amino acids (cytosine or glycine) is expected to cause misfolding of ROC domain [90]. R1441 substitutions may also alter the interaction between the ROC and COR domains [91]. Data on kinase activity of R1441C mutant of LRRK2 have been inconsistent. Some groups have reported increased kinase activity of R1441C LRRK2 [15, 20], while others did not detect any difference with the wild-type protein [13, 44, 67, 68]. As discussed for I2020T, the apparent differences between studies likely result from the fact that the effects on kinase activity are modest at best and often within measurement error.

The Y1699C mutation was originally discovered in a large family from Germany, Denmark, and Canada, and another family from the UK [9, 10], although it is relatively rare in most populations [4, 3]. The Y1699 residue is located on the outer surface of the COR domain and substitution with cytosine at this likely alters interactions between the ROC and COR domains, leading to a reduction in GTPase activity [88, 91]. As for R1441 mutations, the kinase function of Y1699C LRRK2 is similar to wild-type protein [13, 44, 67, 68].

Overall, it therefore seems most likely that the major effect of both ROC and COR mutations is on GTPase rather than kinase activity. In general, the GTP-bound form of proteins is the active version and it logically follows that that ROC and COR mutations increase the activation state of the protein. Therefore, despite these mutations having a loss of GTPase function, they likely result in a persistent function compared with wild-type protein.

Table 1 Pathogenic mutations and risk factor variants of *LRRK2*

Pathogenic mutations	Substitution	LRRK2 domain	Molecular mechanism	Cellular effects	Invertebrate and rodent models	Therapeutic strategy
Pathogenic mutations	G2019S	Kinase	Kinase activation	Acute toxicity, neurite shortening, endocytosis impairment, lysosomal positioning alteration, autophagy impairment, Golgi clearance, cytoskeleton dynamics imbalance	DA neurodegeneration, reduction of dopamine levels <i>in vivo</i> , locomotor dysfunction in <i>Caenorhabditis elegans</i> ; modest DA neuron loss, diminished dopamine transmission and motor deficits in mouse	Inhibition of kinase activity
	I2020T	Kinase	Unclear. May be kinase activation	Acute toxicity, neurite shortening, Golgi clearance, cytoskeleton dynamics imbalance	DA neuron loss in <i>Drosophila</i>	Inhibition of kinase activity (?)
	R1441C/G/H	ROC	GTP hydrolysis disruption, destabilization of ROC domain	Acute toxicity, neurite shortening, endocytosis impairment, autophagy impairment, Golgi clearance, cytoskeleton dynamics imbalance	DA neurodegeneration, reduction of dopamine levels <i>in vivo</i> , locomotor dysfunction in <i>C. elegans</i> ; diminished dopamine release, bradykinesia and coordination deficiency in mouse	Modulation of GTPase activity
	Y1699C	COR	GTP hydrolysis disruption, strengthening of ROC-COR interactions	Acute toxicity, neurite shortening, autophagy impairment, Golgi clearance, cytoskeleton dynamics imbalance	DA neuron loss and locomotor deficits in <i>Drosophila</i>	Modulation of GTPase activity
Risk variants	R1628P	COR	Unknown. Presumably may affect GTPase activity (?)	-	-	Modulation of GTPase activity (?)
	G2385R	WD40	Partial loss of kinase activity	Reversal of some effects of G2019S mutant, Golgi clearance	DA neuron loss and locomotor deficits in <i>Drosophila</i>	Restoration of kinase activity

ROC Ras of complex proteins; *COR* C-terminus of ROC; *DA* dopamine

The ROC–COR bidomain mutations have another shared effect in that they all decrease S910, S935 phosphorylation-dependent 14-3-3 binding [44]. I2020T is also dephosphorylated but G2019S is not. Decreased phosphorylation of Ser935 is a result of an enhanced affinity of R1441C and Y1699C mutants for protein phosphatase 1 α [92]. Additionally, loss of 14-3-3 binding correlates with an increased number of inclusion bodies formed in the cytoplasm of cells [44]. Collectively, the effects of ROC–COR domain mutations, and perhaps I2020T, are likely driven by altered protein folding.

Genetic Risk Factors Associated with Parkinson's Disease

Two variants in LRRK2 that are predominantly found in Asian populations, G2385R [93–97] and R1628P [98–101], increase the lifetime risk of PD about 2-fold [102]. Molecular modeling predicts that the G2385 residue is located on the outer surface of the WD40 domain towards the C-terminus of LRRK2 [31]. The substitution of a neutral and flexible glycine for a positively charged arginine at this position could interfere with interdomain interactions within LRRK2. The WD40 and extreme C-terminus of LRRK2 are also required for kinase activity of LRRK2 as C-terminally truncated constructs have a loss of kinase activity [67, 73, 103]. In our hands, using a range of different constructs and assays, the G2385R substitution causes a 40–50 % loss of LRRK2 kinase function. Interestingly, a double G2019S/G2385R LRRK2 mutant had kinase activity indistinguishable from wild-type protein, suggesting an opposite effect of G2019S and G2385R substitutions on kinase function of LRRK2 within the same molecule [73]. These results reinforce that there are multiple intramolecular interactions within LRRK2 and that some disease-associated variants affect such communications.

LRRK2 Polymorphisms that may Affect Protein Function

A number of LRRK2 polymorphisms have been reported, but only few are likely to be pathogenically relevant. The N1437H substitution was found in 2 Norwegian families and 1 patient from Sweden [58, 104]. Owing to the limited genetic data, whether this mutation is pathogenic is uncertain. Direct evaluation of GTPase and kinase activity of N1437H LRRK2 has not yet been reported, although this variant does cause increased GTP binding [58]. When overexpressed in HEK293 cells, N1437H LRRK2 has an increased phosphorylation at Ser1292 [105] and diminished phosphorylation of Ser935 and, hence, 14-3-3 binding [92]. The measured effects are similar to other ROC–COR domain mutations, supporting pathogenicity.

An I2012T substitution was initially suggested to be pathogenic [10]. However, owing to the limited data about familial segregation it is difficult to be certain that this is a causative mutation. The I2012T variant lies within the predicted Mg²⁺

binding region of the kinase domain and thus could influence LRRK2 kinase activity [31]. Supporting this, Jaleel et al. [67] demonstrated the I2012T mutant has decreased kinase activity, but subsequent studies did not find any difference in phosphorylation activity compared with wild-type LRRK2 [44]. Therefore, further genetic and biochemical evaluation of the I2012T substitution is still needed.

The R1398H LRRK2 polymorphism is quite common (>5 %) in several populations and apparently plays a protective role against development of PD [102, 106–109]. The R1398 residue is located in the switch II motif of the ROC domain and could be important for the interaction with the γ -phosphate of GTP [87]. Intriguingly, the hypothesis-testing R1398L substitution has increased GTPase activity *in vitro* [33, 110, 111]. However, R1398Q does not change GTPase activity of LRRK2, in contrast to expectations [110]. It would be interesting to check if R1398H LRRK2 has enhanced GTPase activity, as having the opposite effect of R1441C or Y1699C mutations might explain why this is a protective variant.

Finally, it should be noted that there are many more variants in LRRK2, many of which are very rare and/or only found in controls and not PD cases. It is likely that the LRRK2 protein tolerates a fairly extensive series of amino acid substitutions and many of these are not associated with altered risk of PD and are instead benign polymorphisms.

Biological Effects of LRRK2 Mutations

Strikingly, despite how the different mutations in LRRK2 have varied effects on activities of the molecule, some aspects of their effects in cells and animals are similar. Revealing the specific and detailed cellular pathogenic mechanisms for each LRRK2 mutation that result from the primary effects of mutations may help not only to understand physiological functions of the protein better, but also allow the development of pharmacological interventions to halt the progression of PD.

Direct Toxic Effects of LRRK2 Expression in Neurons

An obvious question about LRRK2 mutations is whether they directly cause neuronal cell death. Overexpression of G2019S LRRK2 in cultured primary neurons and neuroblastoma cell lines mediates cellular toxicity and triggers apoptotic cell death [13, 15, 70, 103]. Similarly, overexpression of R1441C or Y1699C also triggers cell death [13]. In some cases, the acute toxic effects could be attenuated by mutation in kinase domain to render it inactive [13, 15, 112] or by pharmacological inhibition of LRRK2 kinase [113]. However, whether this is strictly owing to loss of kinase activity of LRRK2 itself is not clear as the hybrid mutants with artificial kinase dead mutations are less

stable than kinase active LRRK2 [112]. Similarly, kinase inhibition can destabilize LRRK2 [50].

Transgenic expression of human G2019S LRRK2 in *Drosophila* causes dendrite degeneration and dopaminergic (DA) neurons loss followed by locomotor dysfunction [114, 115]. Importantly, this effect was diminished by pharmacological inhibition of kinase activity of G2019S mutant or by overexpression of parkin, a protein implicated in autosomal-recessive Parkinsonism [116, 117]. It was also suggested that DA neuron loss in *Drosophila* triggered by overexpression of G2019S hLRRK2 is caused by an increase in bulk protein synthesis and be prevented by overexpression of the phosphodeficient T136A ribosomal protein s15 [118]. Transgenic overexpression of human I2020T LRRK2 in *Drosophila* also induces DA neuron degeneration by a mechanism that is proposed to involve phosphorylation of 4E-BP [75], although this mechanism is controversial [72, 119]. Overexpression of human LRRK2 in *Caenorhabditis elegans* also mediate age-dependent DA neurodegeneration, reduction of dopamine levels *in vivo*, behavioral deficits, and locomotor dysfunction that is more profound for G2019S than wild-type protein [120, 121]. Transgenic expression of human R1441C mutant LRRK2 in *C. elegans* induces age-dependent DA neurodegeneration and locomotor deficit accompanied by a reduction in dopamine levels [120]. In *Drosophila*, a dLRRK Y1383C mutant (analogous to human Y1699C) caused prominent vesicular aggregation of the protein and DA neuron loss [75]. Transgenic overexpression of human Y1699C or G2385R LRRK2 in *Drosophila* is also associated with DA neuron loss accompanied by locomotor deficits [117, 122].

Gene transfer models have been used to further support the idea that mutant LRRK2 can be directly toxic to neurons *in vivo*. Injection of adenoviruses expressing human G2019S but not wild-type LRRK2 to the striatum of adult rats induces hyperphosphorylation of tau and progressive DA neuron loss [123]. In mice, herpes simplex virus amplicon vectors expressing human G2019S LRRK2 caused degeneration of DA neurons, which was attenuated by pharmacological inhibition of LRRK2 kinase activity [113].

In contrast to cell culture or acute experiments, dopamine cell loss is not generally seen using transgenic rodent models. Two transgenic mouse models overexpressing human G2019S LRRK2 exhibit modest age-dependent DA neuron loss associated with accumulation of autophagosomes and mitochondrial alterations [124, 125], but other G2019S LRRK2 transgenic mouse models had diminished dopamine transmission and motor deficits without detectable neuron loss [53, 54]. The only I2020T LRRK2 transgenic mouse model reported to date exhibits no obvious loss of DA neurons [126]. R1441G bacterial artificial chromosome transgenic mice do not demonstrate detectable nigral neuron loss but develop age-dependent bradykinesia and coordination deficiency presumably due to diminished dopamine release, which could be

partially reversed upon levodopa administration [127, 128]. Similar results were received for R1441C knock-in mouse model, where the point mutation of LRRK2 caused deterioration in stimulated DA neurotransmission and D2 receptor function but, again, no cell loss [129].

Collectively, these results show that while LRRK2 may be directly toxic to DA neurons, whether this holds true in systems with more chronic lower level expression is unclear. As this situation may more closely mirror the situation in patients with physiological levels of expression over lifetime, it might be argued that the transgenic results are more relevant and predict that LRRK2 is not directly toxic to neurons *in vivo*. Further complicating the picture, endogenous LRRK2 is not actually very highly expressed in presynaptic dopamine neurons but is expressed in postsynaptic striatal neurons and in microglia [130–135]. Therefore, an open question is whether the effects of LRRK2 mutations are really neuronal cell-autonomous. Because of these uncertainties, it is valid to consider other effects of LRRK2 mutations that may be relevant to the development of pathophysiological states.

Effects of LRRK2 on Cellular Phenotypes and Physiology

In neurons, G2019S LRRK2 induces shortening of neurite length and, perhaps, alteration of their branching [53, 56, 57]. I2020T also induces the shortening and debranching of neurites [56, 126]. Acute overexpression of R1441C LRRK2 induces neurite shortening [13, 56, 70, 103]. Primary neurons derived from bacterial artificial chromosome transgenic (Y1699C) mice have reduced neurite outgrowth and branching, which was partially rescued with staurosporine, a nonspecific kinase inhibitor [136]. It should be noted that wild-type LRRK2 may share the essential ability of mutants to shorten neurites as neurons from LRRK2 knockout mice have longer neurites [57]. This might support the idea that LRRK2 mutations can induce a persistent functional state.

Neurite shortening might therefore be a way to assay pathogenic mutations in LRRK2. However, the G2385R LRRK2 variant does not induce neurite shortening (at least in M17 neuroblastoma cells), and even alleviated the effect of G2019S mutant when present on the same construct [73]. Furthermore, the effects of mutant LRRK2 on neurite length have a limited developmental window [55]. It might be that altered development of neurons changes their later sensitivity to the PD process, but that hypothesis is difficult to assess without an *in vivo* model that shows both neurite length changes and neurodegeneration, both of which are lacking in current models. Therefore, neurite shortening is a helpful assay for LRRK2 but the relevance either to the effects of all mutations or disease pathogenesis is unclear.

What the neurite shortening effects have done is to provide a platform to explore mechanisms related to mutant LRRK2. The effects of the G2019S can be overcome by

overexpression of Rac1 [137], a Rho GTPase involved in actin cytoskeleton remodeling. I2020T-mediated neurite shortening may involve changes in tubulin-associated tau [138]. Abnormally phosphorylated tau was found in the brainstem of LRRK2 I2020T mutation carriers [139]. Alterations in autophagy may also contribute to neurite shortening caused by G2019S LRRK2 [140]. These results highlight 2 functional themes for LRRK2 that may be important in understanding pathogenesis; altered cytoskeletal dynamics and changes in vesicular trafficking, particularly related to the autophagy–lysosome system. This latter idea is particularly intriguing as endogenous LRRK2 is associated with vesicular structures in cells and brain [141], and is particularly associated with vesicles related to late endosomes and autophagosomes [142]. Several groups have shown that LRRK2 mutations induce the formation of autophagy-related intracellular inclusions [35].

The *Drosophila* LRRK2 homolog (*lrrk*) interacts with Rab7, a small GTPase involved in late endosome processing, and introduction of point mutation analogous to human G2019S substitution promotes formation of Rab7-positive inclusions in *Drosophila* follicle cells [143]. Overexpression of another GTPase implicated in vesicle sorting and trafficking, the *Drosophila* Rab7L1 ortholog, rescued DA neuron loss mediated by G2019S mutant [51]. This effect may be related to retromer function [51]; mutations in a retromer protein, VPS35, are a genetic cause of PD in humans [144, 145]. The control of vesicular sorting and recycling is critical to most cells as a major way of regulating organelle distribution and turnover, again often by the autophagy–lysosome system. Interestingly, all mutations tested in LRRK2 cause alterations in markers of autophagy function [146], suggesting that this is a common effect of mutations.

In *C. elegans*, LRRK-1 plays a pivotal role in polarized sorting of synaptic vesicle proteins to the Golgi [147]. A LRRK2 interactor, ArfGAP1, associates with the Golgi apparatus as a part of the coat protein I complex [148, 149]. Golgi apparatus fragmentation is a prominent pathological feature of DA neurons in G2019S LRRK2 transgenic mice [150]. The fragmentation could result in or be a consequence of altered vesicle sorting and trafficking, both of which are essential steps in the autophagy pathway. We have recently suggested that the effects of LRRK2 on autophagy and on Golgi turnover are, in fact, related. By looking for direct protein interactors of LRRK2, we recovered both Rab7L1 (also identified by Macleod et al. [51]) and cyclin-G associated kinase (GAK) [151]. Both Rab7L1 and GAK are associated with the Golgi apparatus and, specifically, overlap in their distribution at the trans-Golgi network. We were able to show both in cells and in an acute expression model *in vivo* that LRRK2 promotes turnover of trans-Golgi network-derived vesicles. Previous results in mice showed that trans- rather than cis- Golgi markers are better measures of LRRK2-induced Golgi

fragmentation [150]. Importantly, all mutations in LRRK2 exaggerated this phenomenon, including the risk factor G2385R, which had an effect in between wild-type LRRK2 and the Mendelian mutants. Although these results need to be confirmed and extended, they suggest that the pathogenic effects of LRRK2 are shared by all mutations, despite their different biochemical effects. Our interpretation is that all mutations cause, to some extent, persistent or poorly regulated function and could cause alterations at different levels of the same pathophysiological pathway.

An open question is whether this general cell biological event has particular relevance to neurons. LRRK2 plays a role in the regulation of synaptic vesicle endocytosis and recycling, which involves vesicle sorting and trafficking [52, 152]. Surprisingly, both knockdown and overexpression of wild-type or mutant LRRK2 impairs synaptic vesicle endocytosis, meaning that the relationship between mutation and normal function is more complex than for Golgi turnover. The precise mechanism of these phenomena is unresolved but it may be mediated via interaction of LRRK2 with the number of proteins involved in orchestration of synaptic vesicle endocytosis and trafficking including synaptobrevin 1A and Rab5 GTPase [52, 152]. Considering that the WD40 domain of LRRK2 interacts with many proteins involved in orchestration of synaptic vesicle dynamics [52], it is possible that G2385R mutant LRRK2 may have similar effects. However, because of the limited expression of LRRK2 in presynaptic dopamine neurons it remains to be determined how changes in presynaptic sorting cause disease.

If vesicular sorting in general, or synaptic recycling specifically, is a direct effect of LRRK2 mutations, then this may have secondary effects that are measurable in cells and could contribute to pathogenesis. Dopamine neurons derived from pluripotent stem cells from R1441C mutation carriers revealed mitochondrial pathology presumably due to mitochondrial DNA damage, which could be rescued pharmacologically or by gene correction [153, 154]. Similar effects on mitochondrial function have been seen in G2019S fibroblasts [155]. Because the Golgi is a major source of lipids for many organelles, it would be of interest to see if the effects of LRRK2 on mitochondria are a consequence of Golgi dysfunction or represent an independent pathway for pathogenesis.

Overall, both vesicle sorting impairment and cytoskeleton dynamic dysregulation could potentially lead to disruption of vesicle trafficking and turnover in DA neurons followed by neurodegeneration and development of frank PD symptoms.

Therapeutic Strategies for LRRK2-associated PD

All current therapeutic strategies of PD are symptomatic and do not halt or slow down progression of the disease. The identification of LRRK2 mutations has led to several

proposals for mechanism-based therapies [3]. As the most common *LRRK2* mutation (G2019S) augments kinase activity, development of *LRRK2* kinase inhibitors has been proposed as a promising approach [156]. Many kinase inhibitors will block *LRRK2* activity, with CZC-25146 and *LRRK2*-IN1 having high specificity for *LRRK2* [157–159]. Neither of these kinase inhibitors would be taken into clinical trials owing to their poor blood–brain barrier permeability [159], although more recent inhibitors may have better distribution in the brain [160–162].

However, even if selective potent and bioavailable *LRRK2* kinase inhibitors were developed, there are some concerns about likely side effects. Because *LRRK2* is heavily expressed in kidneys and lungs [163], and deletion of *LRRK2* in mice causes primarily accumulation of cytoplasmic inclusions in those tissues [49, 50], *LRRK2* inhibitors could cause significant impairment of kidney and lung functions. Furthermore, apart from G2019S none of other *LRRK2* mutations consistently augment kinase activity of the protein [78]. Therefore, application of kinase inhibitors in case of these substitutions may or may not be sensible, and alternative therapeutic approaches might be considered in these cases [164, 165]. Moreover, some variants, like G2385R, have diminished kinase activity and are still associated with disease [73]. Given these data, it is unclear if kinase inhibitors are a viable option for *LRRK2*-based therapeutics.

However, there are still other possible regions within *LRRK2* that might be targeted therapeutically. Because pathogenic mutations in ROC–COR have impaired GTP hydrolysis, it might be that the GTP-bound form of *LRRK2* is pathogenic [18–20]. It has been suggested that GTP binding is essential for *LRRK2*-mediated toxicity [15, 111]. The proposed facilitation of GTP hydrolysis by *LRRK2* via activation of its GAP (ArfGAP1) might also be a target [165], although this would require targeting protein–protein interactions. Along the same lines, our recent results showing enhancement of *LRRK2* activity in cells by interactions with Rab7L1 and GAK may suggest additional targets. Dimerization of *LRRK2* itself is also thought to contribute to its activity and might be targetable if the structural basis of this phenomenon were elucidated [164].

Kinases usually participate in intracellular signaling cascades. Therefore, modulation of up- and downstream effectors of *LRRK2* could compensate for the pathogenic effects of mutations [164]. For example, activation of 4E-BP by rapamycin or transgenic overexpression of 4E-BP prevents dopamine neuron loss in *Drosophila* models [75, 166]. 4E-BP binds and negatively regulates eukaryotic initiation factor 4E (eIF4E). Strikingly, mutations in EIF4G1, another interacting partner of eIF4E, are also implicated in autosomal-dominant PD [167]. Therefore, it would be of interest to study functional interactions of *LRRK2*, 4E-BP, and EIF4G1 in mammalian models.

Because the death of DA neurons starts several years before the onset of PD symptoms [168], the most effective time to intervene in disease would be before motor symptoms occur. However, whether this is ethically appropriate is uncertain, as *LRRK2* mutations are not fully penetrant, even by the age of 80 [7, 169, 170]. Therefore, identification of the very earliest symptoms of *LRRK2* patients is likely to be an important step in deciding when to apply therapeutics. However, the limiting factor right now is having therapeutics on hand, including potentially different approaches for different mutations.

Conclusions

Substantial progress has been made in understanding the molecular mechanisms of pathogenic *LRRK2* mutations. However, the intracellular pathophysiologic effects of mutations remains unclear. Therefore, a major challenge is to identify the normal cellular function of *LRRK2* and to understand how *LRRK2* mutations consistently affect this function. To do so would, in our opinion, help facilitate development of new *LRRK2*-targeted etiological medications for the treatment of PD. There is, as there has been for many years now, still a need to develop better *in vivo* models that have robust degeneration such that any proposed events can be tied into one of the major events in the disease process. Eventually, such efforts may help to prevent or halt neuronal damage in PD and establish etiological treatment of the disorder.

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