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Leucine-Rich Repeat Kinase 2 (LRRK2): A Key Player in the Pathogenesis of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is the most common neurodegenerative movement disorder with a prevalence of more than 1% after the age of 65 years. Mutations in the gene encoding leucine-rich repeat kinase 2 (*LRRK2*) have recently been linked to autosomal dominant, late-onset PD that is clinically indistinguishable from typical, idiopathic disease. *LRRK2* is a multi-domain protein containing several protein interaction motifs as well as dual enzymatic domains of GTPase and protein kinase activities. Disease-associated mutations are found throughout the multi-domain structure of the protein. *LRRK2*, however, is unique among the PD-causing genes because a missense mutation, G2019S, is a frequent determinant of not only familial, but also of sporadic PD. Thus, *LRRK2* has emerged as a promising therapeutic target for combating PD. This article reviews the current state of knowledge regarding the domain structure, amino acid substitutions, and potential functional roles of *LRRK2*.

Keywords

Parkinson's disease; leucine-rich repeat kinase 2 (*LRRK2*); neurodegeneration; signal transduction

Introduction

Parkinson's disease (PD)¹ is a chronic and progressive neurodegenerative disorder of the central nervous system that affects control of voluntary movement. Pathologically, PD is characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) and the presence of intraneuronal proteinaceous cytoplasmic inclusions, termed

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¹Abbreviations

PD, Parkinson's disease; SNpc, substantia nigra pars compacta; MPP+, 1-methyl-4-phenylpyridinium; *LRRK2*, leucine-rich repeat kinase 2; PINK1, phosphatase and tensin (PTEN)-induced kinase 1; UCH-L1, ubiquitin C-terminal hydrolase L1; Roc, Ras of Complex Protein; COR, C-terminal of Roc; LRR, leucine-rich repeat; MAPKKK, mitogen activated protein kinase kinase kinase; GDI, GDP dissociation inhibitor; TKL, tyrosine kinase-like; RIPK, receptor-interacting protein kinases; MAPK, mitogen activated protein kinase; ERK, extracellular-signal regulated kinase; JNK, c-Jun amino-terminal kinase; PET, positron emission tomography; PSP, progressive supranuclear palsy; AD, Alzheimer's disease; FTD, frontotemporal dementia; MBP, myelin basic protein; TUNEL, Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling; ERM, ezrin/radixin/moesin; CRMP-2, collapsing response mediator protein-2.

Lewy bodies, in surviving neurons. Clinically, patients typically exhibit motor symptoms including tremor, rigidity, bradykinesia, and postural instability, and beneficially respond to dopamine replacement therapy. Common to multi-factorial diseases, the incidence of PD increases with age. The percentage of affected individuals within a population rises from ~1% at 65 years of age to ~5% by 85 years of age, with a mean age at onset of 55 years (Dauer and Przedborski 2003). Because of an aging population, improved diagnosis, and prolonged survival, especially in developing countries, the number of patients suffering from PD is predicted to double by the year 2030 (Dorsey et al. 2007).

While enormous steps have been taken over nearly the past 200 years in understanding the neurobiology of PD, the exact etiology of this devastating disease is still largely unknown. Currently, several mechanisms are implicated in the selective degeneration of dopaminergic neurons in PD (Cookson 2005). For example, failure or impairment in the ubiquitin-proteasome system, potentially leading to the aggregation of oligomeric proteins, may play a role in PD pathogenesis. Mitochondrial complex I toxins, such as 1-methyl-4-phenylpyridinium (MPP+) and paraquat, have shown that mitochondrial dysfunction and increasing oxidative stress also likely contribute to disease pathogenesis. Dopaminergic neurons may be selectively vulnerable to such cellular insults because of the endogenous elevated level of oxidative stress arising from the presence of dopamine in these cells (Schulz and Falkenburger 2004).

Although the majority of PD cases are sporadic, ~5–15% of cases are due to genetic alterations. A major breakthrough in recent years in understanding PD pathogenesis has been the mapping of ten chromosomal regions, named *PARK1-11*, and the subsequent cloning of seven genes involved in familial PD (Table 1). These include three autosomal dominant genes (*α -synuclein*, *leucine-rich repeat kinase 2 [LRRK2]*, and *Grb10-interacting GYF protein 2 [GIGYF2]*), and three autosomal recessive genes (*parkin*, *DJ-1*, and *phosphatase and tensin homolog (PTEN)-induced kinase 1 [PINK1]*) (Bonifati et al. 2003; Chartier-Harlin et al. 2004; Kitada et al. 1998; Paisan-Ruiz C 2004; Polymeropoulos et al. 1997; Singleton et al. 2003; Valente et al. 2004; Zimprich A 2004). A mutation in the seventh gene, *ubiquitin C-terminal hydrolase L1 (UCH-L1)*, has only been found in one family and the importance of this gene in familial PD is still uncertain (Leroy et al. 1998). Although mutations in these genes account for only a small percentage of all PD cases, studying the encoded proteins has enabled and supported many of the proposed mechanisms underlying the pathogenesis of PD. Identifying the causative genes associated with the other remaining *PARK* loci, for which linkage has been identified, will likely further contribute to elucidating the etiology of PD.

Although these key genetic discoveries have played an important role in understanding PD, mutations in most PD-associated genes have been linked to early-onset or pathologically atypical forms of the disease in only a few families. Distinctively, mutations in the most recently identified PD-associated gene, *LRRK2*, which is linked to the *PARK8* locus, have been associated with late-onset, autosomal-dominantly inherited PD (Paisan-Ruiz C 2004; Zimprich A 2004). *LRRK2* is also known as dardarin, after dardara, the Basque word for tremor. The *LRRK2* gene spans 51 exons and encodes a rather large (2,527 amino acids) protein of 286KDa that contains multiple, independent domains. Because mutations in

LRRK2 cause a familial form of PD that pathologically resembles idiopathic disease and are also found in up to 3% of sporadic PD cases, this protein has emerged as the most significant player in PD pathogenesis identified to date (Aasly et al. 2005; Berg D 2005; Taylor JP 2006).

Though much has been learned about *LRRK2* in the last few years, many important questions remain unanswered. For example, what is the normal function of *LRRK2*? What functional effect do *LRRK2* PD-associated mutations have on neurons? Why are dopaminergic neurons particularly sensitive to *LRRK2* mutations when the protein has a widespread expression pattern? This article reviews the current state of knowledge regarding the domain structure, amino acid substitutions, and potential functional roles of *LRRK2*.

LRRK2 Multi-Domain Structure

LRRK2 is a member of the ROCO protein family. This class of multi-domain proteins is characterized by the presence of a 200–250 amino acid Roc (Ras of Complex protein) domain, immediately followed by a 300–400 amino acid domain termed COR (C-terminal of Roc). The Roc and COR domains are usually followed by a kinase domain, and various protein-protein interaction motifs may also exist within ROCO proteins. There are at least 40 members in the ROCO superfamily and these proteins are found in a variety of species including mammals, metazoa, dictyostelium, plants, and prokaryotes (Bosgraaf L 2003).

Sequence analysis predicts that *LRRK2* comprises several independent domains including a leucine-rich repeat (LRR) domain, a Roc domain followed by its associated COR domain, a mitogen-activated protein kinase kinase kinase (MAPKKK) domain, and a C-terminal WD40 domain (Fig. 1) (Bosgraaf L 2003; Guo L 2006). Genetic variations are found throughout the functional domains of *LRRK2* (Fig. 1), suggesting that this protein may serve as an upstream central integrator of multiple signaling pathways that are crucial for proper neuronal functioning. Also, the presence of both protein interaction domains (LRR and WD40) as well as enzymatic domains (Roc and MAPKKK) within *LRRK2* suggests that this protein may serve as a scaffold for assembly of a multi-protein signaling complex. The predicted functions of these multiple domains are outlined in Figure 1.

Leucine-rich repeat (LRR) domain

This motif is present in over 60 proteins and the primary function appears to be to provide a versatile structural framework for the formation of protein-protein interactions. LRR containing proteins participate in many biologically important processes, such as hormone-receptor interactions, enzyme inhibition, cell adhesion and cellular trafficking, early mammalian development, neural development, cell polarization, regulation of gene expression, apoptosis, and regulation of cytoskeletal dynamics (Kobe and Deisenhofer 1995). Based on homology modeling, many of the *LRRK2* amino acid substitutions in the LRR domain are predicted to lie on the surface of this structural motif and, therefore, may be involved in forming protein-protein interactions (Mata et al. 2006b).

Ras of complex protein (Roc) and C-terminal of Roc (COR) domains

The Roc domain of ROCO family members stands out as a separate monophyletic group of the Ras-related superfamily of small GTPases (Bosgraaf L 2003). Three LRRK2 pathogenic amino acid substitutions in the Roc domain occur at the R1441 position (R1441C, R1441G, and R1441H). The COR domain is common to all ROCO proteins and is always found immediately C-terminal to the Roc domain. This Roc-COR module is conserved throughout evolution, suggesting a functional interdependence of these two motifs. Recent crystallographic structural determinations of Roc and Roc-COR suggest two different mechanisms of dimerization within these regions of LRRK2. Cookson and colleagues have reported that the Roc domain alone exists as a dimer with extensive domain swapping while Wittinghofer and colleagues have identified the COR domain as the dimerization motif (Deng et al. 2008; Gotthardt et al. 2008). Two PD-associated mutations, Y1699C and R1628P, are located in the COR domain.

Mitogen-activated protein kinase kinase kinase (MAPKKK) domain

Based on sequence similarity, the MAPKKK domain of LRRK2 belongs to the tyrosine kinase-like (TKL) subfamily of human protein kinases, whose members show sequence similarity to both serine/threonine and tyrosine kinases (Manning G 2002). The MAPKKK domain of LRRK2 most resembles receptor-interacting protein kinases (RIPKs), which are crucial sensors of cellular stress and can activate mitogen-activated protein kinase (MAPK) pathways (Meylan 2005). The PD-linked mutations G2019S and I2020T occur at the catalytic sites of the MAPKKK domain and are predicted to alter kinase activity (Guo L 2006; Marin 2006; Mata et al. 2006b).

WD40 domain

WD40 domains likely mediate protein-protein interactions. Interestingly, there are several examples in which WD40 domains are fused to domains that are predicted to have kinase activity, similar to the C-terminal domain arrangement of LRRK2 (Futey et al. 1995; Janda et al. 1996; Smith et al. 1999). The G2385R mutation lies within the WD40 domain (Mata et al. 2006b).

LRRK2 mRNA and Protein Expression

The expression pattern of *LRRK2* mRNA in mouse and rat brains differs markedly from the cellular expression patterns of other genes linked to PD to date. For example, *α-synuclein*, *parkin*, *UCHL-1*, *DJ-1*, and *PINK1* mRNA are all expressed in many, if not all, neurons of the brain, including dopamine neurons of the midbrain (Galter et al. 2007; Solano et al. 2000). Several recent reports, however, have found *LRRK2* mRNA expression in dopamine-innervated areas such as the striatum, cortex, and olfactory tubercle, but expression was devoid in dopamine synthesizing neurons of the SNpc (Galter et al. 2006; Higashi et al. 2007b; Melrose et al. 2006; Taymans et al. 2006).

LRRK2 protein has been shown to be ubiquitously expressed and relatively abundant in most regions of the mouse and rat brain. LRRK2 protein was found to localize throughout the nigrostriatal dopaminergic pathway, with highest levels detected in the striatum but also

at lower levels in the SNpc (Higashi et al. 2007b; Melrose et al. 2007). The apparent absence of *LRRK2* mRNA in the SNpc despite the detection of low to moderate protein levels in this region may be due to several possibilities. For example, *LRRK2* mRNA may have a short half-life or be transported to distal sites in nigral dopaminergic neurons. Alternatively, *LRRK2* protein may have a particularly long half-life in these neurons so that only a small number of mRNA copies are required to maintain protein levels. *LRRK2* protein expression was also significant in many non-dopaminergic areas such as the septal nuclei, hippocampus, and thalamus. *LRRK2* staining was also present in the subventricular zone, a region containing stem cells that give rise to both neurons and glia, suggesting a potential role for *LRRK2* in the regulation of neurogenesis (Melrose et al. 2007).

The expression of *LRRK2* protein in mouse and rat brains has been further studied at a subcellular level. Despite the large size of *LRRK2*, *in silico* analysis failed to indicate the presence of hydrophobic transmembrane domains or obvious organelle targeting sequences (West et al. 2005). *LRRK2* protein was found to localize throughout the cytoplasm of neuronal perikarya and dendritic processes where it associated with vesicular and membranous structures, the microtubule network, mitochondria, and other membrane-bound organelles (Biskup et al. 2006; Gandhi et al. 2008; Hatano et al. 2007). This is similar to the protein localization pattern found in *LRRK2*-overexpressing cell lines (Gloeckner et al. 2006; Greggio et al. 2006; Hatano et al. 2007; Smith et al. 2006; West et al. 2005). The R1441C and G2019S PD-associated mutations of *LRRK2* did not alter this localization pattern in cultured cells (West et al. 2005). In mouse and rat brains, *LRRK2* was specifically localized to Golgi transport vesicles, the plasma membrane, endosomes, lysosomes, mitochondria, synaptic vesicles, and microtubules (Biskup et al. 2006; Gandhi et al. 2008; Hatano et al. 2007). The membrane localization of *LRRK2* was resistant to solubilization by non-ionic detergent, indicating that *LRRK2* associates with lipid rafts, which are known to play important roles in signal transduction, membrane trafficking, and cytoskeletal organization (Brown and London 1998; Hatano et al. 2007). Taken together, these data suggest multiple possible roles for *LRRK2*: (1) a role in the biogenesis and/or regulation of vesicular structures throughout the neuron, (2) a role in vesicular transport, and (3) a role in membrane and protein turnover possibly through the lysosomal degradation pathway.

The expression of *LRRK2* mRNA and protein has also been examined in neurologically normal human brains. Both mRNA and protein expression were found in brain regions of direct relevance to the pathogenesis of PD, including the cerebral cortex, caudate putamen, and SNpc (Biskup et al. 2006; Giasson et al. 2006; Greggio et al. 2006; Higashi et al. 2007a). *LRRK2* protein localized to neuronal perikarya and dendritic and axonal processes in all brain regions studied consistent with reports from cultured cells, primary neurons, and rodent brains (Biskup et al. 2006; Giasson et al. 2006; Gloeckner et al. 2006; Greggio et al. 2006; Smith, 2006#516; Higashi et al. 2007a; West et al. 2005).

There is evidence suggesting that *LRRK2* is localized to Lewy bodies in human PD brains. Several studies have demonstrated *LRRK2* immunostaining of α -synuclein-positive brainstem and cortical Lewy bodies, the pathological hallmarks of PD and dementia with Lewy bodies, respectively (Alegre-Abarrategui et al. 2008; Greggio et al. 2006; Perry et al. 2008; Zhu et al. 2006a; Zhu et al. 2006b). However, there are some reports that fail to detect

LRRK2 immunostaining in these pathological inclusions (Giasson et al. 2006; Higashi et al. 2007a; Melrose et al. 2007). This reported difference may be due to different epitopes recognized by LRRK2 antibodies used for immunohistochemistry, variations in preparation of brain tissues, and/or differences in brain pathologies of PD patients studied (Zhu et al. 2006a; Zhu et al. 2006b).

It remains to be seen if LRRK2 is essential to dopaminergic neurotransmission. Current evidence suggests that altered dopamine neurotransmission has no effect on LRRK2 protein expression. For example, no differences in *LRRK2* mRNA signal were observed in the striatum of PD patients where clear dopamine D2 receptor depletion was evident (Galter et al. 2006). Also, vesicular amine transporter deficient mice have unaltered levels of *LRRK2* mRNA expression despite extensive striatal dopamine depletion (Higashi et al. 2007b). The overall localization pattern of LRRK2 does, however, agree with a likely involvement in PD pathogenesis.

Putative Biologic Function of LRRK2 and Related Studies in Model Organisms

Dual enzymatic properties of LRRK2—Several groups have studied the kinase activity of the MAPKKK domain of LRRK2 and what effect PD-associated mutations may have on this enzymatic property. LRRK2 is a functional kinase, able to undergo both autophosphorylation and phosphorylation of the generic substrate myelin basic protein (MBP) (Gloeckner et al. 2006; West et al. 2005). One report, using bacterially expressed recombinant protein, demonstrated that autophosphorylation of LRRK2 is an inter-molecular reaction targeting two residues within the activation segment, T2031 and S2032, with the Ser residue of the G2019S mutation generating an additional autophosphorylation site (Luzon-Toro et al. 2007). In contrast, another study using mammalian expressed protein, demonstrated that dimeric LRRK2 undergoes intra-molecular autophosphorylation and that an intact C-terminus, but not N-terminus, is required for this activity (Greggio et al. 2008). There is also discrepancy in the literature regarding the effect PD-associated mutations have on LRRK2 kinase activity. For example, several studies demonstrated that the I1122V, R1441C, R1441G, R1514Q, Y1699C, G2019S, and I2020T familial PD-linked mutations of LRRK2 increased kinase activity as measured by autophosphorylation, suggesting a gain-of-function mechanism for LRRK2-linked disease (Gloeckner et al. 2006; Guo et al. 2007; Li et al. 2007; West et al. 2005; West et al. 2007). However, other reports found that only the G2019S mutation increased LRRK2 kinase activity, while all other mutations studied either did not influence or inhibited kinase activity (Jaleel et al. 2007; Lewis et al. 2007; Luzon-Toro et al. 2007). Interestingly, purified LRRK2 from transgenic mouse brain demonstrated kinase activity. Brain LRRK2 was associated with elevated kinase activity in comparison with that from transgenic lung or transfected cultured cells, which may help explain the brain-specific pathology in mutant LRRK2-induced PD (Li et al. 2007). As the physiological substrates of LRRK2 continue to be identified, the precise effect PD-associated mutations have on phosphorylation activity will likely be clarified.

Several groups have also studied the GTPase activity of the Roc domain of LRRK2 and what effect PD-associated mutations may have on this enzymatic property. LRRK2 is a GTP-binding protein, as measured by specific binding to GTP-agarose and radiolabeled

GTP (Guo et al. 2007; Ito et al. 2007; Lewis et al. 2007; Smith et al. 2006). LRRK2 is also a functional GTPase, defined by the ability to bind and intrinsically hydrolyze GTP (Guo et al. 2007; Lewis et al. 2007; Li et al. 2007). Interestingly, purified LRRK2 from transgenic mouse brain also demonstrated GTPase activity (Li et al. 2007). According to one study, familial-linked mutations in LRRK2 within the Roc and COR domains (I1371V, R1441C, R1441G, and Y1699C) resulted in increased GTP-binding as measured by binding to GTP-agarose, while mutations outside these domains did not affect GTP-binding compared with wild-type LRRK2 (West et al. 2007). This is in contrast, however, to two reports that demonstrated that the Roc domain R1441C mutation did not increase GTP binding compared with wild-type LRRK2 (Guo et al. 2007; Lewis et al. 2007). Interestingly, though, the R1441C and R1441G mutations have a decreased rate of GTP hydrolysis compared with the wild-type protein, suggesting that these mutants spend more time in the activated GTP-bound state (Guo et al. 2007; Lewis et al. 2007; Li et al. 2007). This possibility is further corroborated by the structural studies of recombinant Roc and Roc-COR domains (Deng et al. 2008; Gotthardt et al. 2008).

A link exists between intrinsic GTPase activity and downstream kinase activity of LRRK2. Several studies have demonstrated that intact GTPase activity is required for LRRK2 kinase activity whereas GTPase activity functions independently of kinase activity (Guo et al. 2007; Ito et al. 2007; Smith et al. 2006; West et al. 2007). Also, GTP binding stimulates LRRK2 kinase activity, demonstrating intra-molecular regulation between the Roc and MAPKKK domains (Guo et al. 2007; Smith et al. 2006). Based on the newly appreciated dimeric structure of LRRK2, it is predicted that the dimeric Roc or Roc-COR domains act as binary switches to regulate kinase activation (Deng et al. 2008; Gotthardt et al. 2008). It is hypothesized that when in the GTP-bound conformation the dimerization of Roc or Roc-COR domains further induce self-association of the kinase domains, allowing for autophosphorylation and subsequent activation of downstream kinase activity (Deng et al. 2008; Gotthardt et al. 2008; Greggio et al. 2008).

PD-linked mutations of LRRK2 have been associated with neuronal cell toxicity. Several pathogenic mutations (I1122V, R1441C, Y1699C, G2019S, and I2020T) increased the tendency of LRRK2 to form inclusion bodies in multiple cell lines and rat primary neurons (Greggio et al. 2006). These pathogenic mutants of LRRK2 also caused neuronal degeneration, in both neuronal cell lines and primary neuronal cells, possibly through an apoptotic mechanism as measured by TUNEL staining (Greggio et al. 2006; Smith et al. 2006; Smith et al. 2005). The mitochondria-dependent apoptotic pathway, in which cytochrome c is released and caspase-3 is activated, is thought to mediate mutant LRRK2 toxicity in neuronal cells. This seems dependent on Apaf1, a scaffold protein participating in apoptosome formation (Iaccarino et al. 2007). Kinase-dead variants of LRRK2 blocked inclusion body formation and protected against neuronal cell death, suggesting that kinase activity is required for the dominant effects of mutant LRRK2 (Greggio et al. 2006; Smith et al. 2006; Smith et al. 2005). The G2019S pathogenic mutant of LRRK2 coupled with a GTP-binding defective alteration also reduced kinase activity and subsequent neuronal toxicity, further suggesting that GTPase activity regulates kinase activity, which in turn mediates the toxicity of mutant LRRK2 (Smith et al. 2006; West et al. 2007). Therefore,

inhibiting activity of either the Roc or MAPKKK domains may serve as a potential therapeutic approach for combating LRRK2-mediated PD.

LRRK2 phosphorylation substrates—Kinase activity is clearly a critical component of LRRK2 function, however, little is known about the physiological substrates of LRRK2 and if aberrant phosphorylation of these substrates is involved in PD pathogenesis. Moesin, a member of the ERM (ezrin/radixin/moesin) family of proteins that functions to anchor the actin cytoskeleton to the plasma membrane, was recently identified as a potential substrate of LRRK2 (Bretscher et al. 2002; Jaleel et al. 2007). Moesin was efficiently phosphorylated by the G2019S variant of LRRK2 at Thr⁵⁵⁸, a previously characterized physiologically relevant phosphorylation site, which when phosphorylated enables moesin binding to F-actin (Huang et al. 1999; Jaleel et al. 2007). Moesin and radixin have been implicated in regulating neurite outgrowth (Paglini et al. 1998). Recent studies also demonstrated that overexpression of the G2019S LRRK2 mutant impedes neurite length and branching (MacLeod et al. 2006; Plowey et al. 2008). Taken together these data suggest that deregulation of moesin phosphorylation by mutant LRRK2 might contribute to the early loss of dopaminergic axon terminals in PD. The ERM-CD44 system has also been implicated as a GDI dissociation stimulator for the Rho family of Ras-related small GTPases. RhoGDI, the Rho subfamily specific GDI, directly interacts with ERM, initiating activation of the Rho GTPase by reducing RhoGDI activity (Takahashi et al. 1997). It is interesting to speculate that ERM may play a similar role for the Roc domain of LRRK2.

A second, weaker LRRK2 substrate was also identified: collapsing response mediator protein-2 (CRMP-2) (Jaleel et al. 2007). CRMP-2 is involved in regulation of growth cones and microtubule dynamics (Stenmark et al. 2007). CRMP-2 also interacts with Numb, an endocytosis-related protein that controls cell number in neurogenesis, and is found in active neurogenesis areas including the hippocampus and olfactory bulb (Johnson 2003; Stenmark et al. 2007). Localization of LRRK2 to these brain regions further suggests a role for LRRK2 in the regulation of neurogenesis (Melrose et al. 2007).

LRRK2 interacting proteins and related signaling cascades—Protein phosphorylation plays a pivotal role in MAPK-linked signal transduction pathways in both normal and pathologic states (Kyosseva 2004). The JNK and p38 MAPKs are two important branches of the MAPK signaling cascade that modulate stress and inflammatory responses and are activated during age-associated neurodegeneration (Ho et al. 2005; Silva et al. 2005). A recent study showed that activation of various MAPK signaling proteins (Src, Hsp27, and JNK) was reduced in patients with G2019S-associated PD, non-symptomatic G2019S carriers, and patients with sporadic PD compared with healthy controls (White et al. 2007). These results suggest that changes in MAPK-signaling may be common to PD pathophysiology, regardless of etiology. Another report demonstrated a reduction in LRRK2(G2019S)-induced neuritic autophagy and neurite shortening in SH-SY5Y cells, a dopaminergic neuroblastoma cell line, in response to an ERK inhibitor, U0126, further implicating involvement of MAPK-related signaling in LRRK2 function (Plowey et al. 2008). It still waits to be determined if various MAPK signaling proteins are direct substrates for LRRK2.

In addition to MAPK-related signaling, LRRK2 has been predicted to be involved in several other cellular pathways. For example, utilizing a combination of LRRK2-mediated protein precipitation and tandem mass spectrometry, several potential LRRK2 binding partners have been identified and subgrouped into various functional cellular pathways, including (1) chaperone pathway and cellular stress, (2) proteins associated with the cytoskeleton and trafficking, and (3) phosphorylation and kinase activity (Dachsel et al. 2007). Rab5b has been demonstrated to be a LRRK2-interacting protein and is suspected to play an important role in synaptic function by modulating the endocytosis of synaptic vesicles, further supporting a role for LRRK2 in trafficking (Shin et al. 2008). In addition, one study monitored changes in expression profiles in SH-SY5Y cells that were depleted of *LRRK2* by RNAi and found that the differentially expressed genes could be grouped into signaling networks involved in axonal guidance, nervous system development, cell cycle, cell growth, cell differentiation, cell communication, MAPK cascades, and Ras signal transduction (Habig et al. 2008). Further characterization of these pathways is necessary to decipher the main functional roles of LRRK2.

Other studies have also identified an interaction of LRRK2 with specific chaperone proteins. For example, LRRK2 was found to interact, via the MAPKKK domain, with Hsp90 and its co-chaperone p50^{cdc37}, which forms a chaperone system that has been shown to interact with several kinases, including the MAPKKKs Raf-1 and MLK-3 (Gloeckner et al. 2006; Grammatikakis et al. 1999; Zhang et al. 2004). In both instances, the Hsp90/p50^{cdc37} proteins do not serve as substrates but rather associate as chaperones assisting in both proper folding and activation of the kinases. It has been shown that inhibition of Hsp90 disrupts the association of this chaperone with LRRK2 and leads to proteosomal degradation of LRRK2, suggesting that Hsp90 inhibitors may be useful therapeutically to limit mutant LRRK2-mediated toxicity in neurons (Wang et al. 2008b).

Parkin, an E3 ubiquitin ligase, is mutated in recessive familial forms of PD and several of its substrates and interaction partners are also relevant in disease pathogenesis. Recently, wild-type and mutant forms of LRRK2 overexpressed in cultured cells were found to interact with parkin, but not with other PD-related proteins such as α -synuclein, DJ-1, or tau (Smith et al. 2005). The COR domain of LRRK2 interacts preferentially with the C-terminal R2 ring finger domain of parkin and while LRRK2 can be ubiquitinated, it is not a substrate for parkin. Co-overexpression of LRRK2 and parkin increased cytoplasmic protein aggregates containing LRRK2 and enhanced the ubiquitination of these aggregates (Smith et al. 2005). Parkin also associates with and ubiquitinates synphilin-1, a protein that interacts with α -synuclein, via the C-terminal R2 ring finger domain, the same domain necessary for interaction with LRRK2 (Chung et al. 2001). Similar to the results found with LRRK2, parkin also stimulates the formation of ubiquitinated aggregates when α -synuclein and synphilin-1 are co-overexpressed (Chung et al. 2001). Parkin is also highly enriched in Lewy bodies (Schlossmacher et al. 2002). To date, six heterozygous *LRRK2* and *parkin* mutation carriers with parkinsonism have been identified (Dachsel et al. 2006; Lesage et al. 2006; Paisan-Ruiz et al. 2005). Compared with individuals carrying a single *LRRK2* or *parkin* mutation, no significant variations regarding disease age at onset or severity of disease were observed. Although the clinical findings do not support a synergistic effect of

LRRK2 and *parkin*, the modulating effects of other genes might conceal a potential genetic interplay.

LRRK2 orthologs in model organisms—Model organisms, both invertebrate and vertebrate, have been used to generate *in vivo* models of neurodegenerative diseases. An ideal *in vivo* model of LRRK2-linked pathogenesis should recapitulate key phenotypes of PD such as dopaminergic dysfunction and neurodegeneration, as well as cognitive and locomotive impairments. Thus far, only a few studies of LRRK2 and its orthologs in model organisms have been published.

LRRK is the *Drosophila melanogaster* ortholog of human LRRK2 and transgenic and loss-of-function mutants have been created in the fruit fly. One report revealed that a LRRK truncation mutant exhibited severely impaired locomotive activity and degeneration of dopaminergic neurons as seen by a reduction in tyrosine hydroxylase immunostaining and a shrunken morphology, demonstrating an endogenous role of LRRK2 in the maintenance of dopaminergic neurons (Lee et al. 2007). However, a subsequent study failed to confirm the neurodegenerative feature but instead showed increased sensitivity to oxidative stress in the same LRRK-deficient flies (Wang et al. 2008a). As this mutant line may still harbor a truncated protein with both LRR repeats and a Roc domain, further studies on additional LRRK-null mutants will be necessary to clarify the normal function of LRRK. A more recent study showed that overexpression of wild-type or LRRK2 (G2019S) in *Drosophila* resulted in selective loss of dopaminergic neurons, locomotor dysfunction, and early mortality. Interestingly, levodopa treatment improved locomotor impairment but did not prevent neuronal degeneration (Liu et al. 2008). This work provides an important *in vivo* gain-of-function model that recapitulates several features of human PD.

LRK-1 is the *Caenorhabditis elegans* ortholog of human LRRK2 and transgenic and deletion mutants have been created in the worm. It was reported that in LRK-1 deletion mutants, synaptic vesicle proteins mis-localized to both presynaptic and dendritic endings in neurons and this aberrant localization was dependent on the AP-1 μ 1 clathrin adaptor, UNC-101, which is involved in polarized dendritic transport. This study suggests that LRK-1 is involved in determining polarized sorting of synaptic vesicle proteins to axons by excluding these proteins from the dendritic specific transport machinery in the Golgi (Sakaguchi-Nakashima et al. 2007). Another study showed that overexpression of wild-type and LRRK2 (G2019S) in *C. elegans* was protective against rotenone toxicity while knockdown of endogenous LRK-1 by RNAi potentiated toxicity, suggesting a role for LRRK2 in mitochondrial regulation (Wolozin et al. 2008).

Prevalence and Pathological Features of LRRK2 Disease-Associated Mutations

To date, about 30 disease-associated variants are found throughout the LRRK2 gene (Fig. 1). The G2019S mutation, located in the MAPKKK domain, is by far the most common and was identified by several groups as a cause of both familial and sporadic PD. The frequency of this mutation in PD varies greatly across populations (Bonifati 2006). Studies from large series in the United States estimated a mutation frequency of ~3% in familial and ~1% in

sporadic cases (Clark et al. 2006; Kay et al. 2006). The G2019S variant seems to be present at a lower frequency in patients from Northern Europe, than in those from Southern Europe such as Italy (~5% of familial and ~1% of sporadic cases), Spain, and Portugal (~6–18% of familial and ~3–6% of sporadic cases) (Aasly et al. 2005; Bialecka et al. 2005; Bras et al. 2005; Carmine Belin et al. 2006; Gaig et al. 2006; Goldwurm et al. 2005; Goldwurm et al. 2006; Infante et al. 2006; Mata et al. 2006a; Pchelina et al. 2006; Williams-Gray et al. 2006). However, an extremely high prevalence is found among Arab patients from North Africa (~37% of familial and ~41% of sporadic cases) and among Ashkenazi Jewish patients (~29% of familial and ~13% of sporadic cases) (Lesage et al. 2006; Ozelius et al. 2006). Therefore, a north to south gradient seems to exist for increasing frequency of the G2019S mutation. In contrast, the G2019S mutation has not been identified in three large series of Chinese patients and is rarely found in Indian, Japanese, and Greek patients (Fung et al. 2006a; Kalinderi et al. 2007; Lu et al. 2005; Punia et al. 2006; Spanaki et al. 2006; Tan et al. 2005; Tomiyama et al. 2006; Xiomerisiou et al. 2007; Zabetian et al. 2006b).

The G2019S mutation has been suggested to arise from at least three haplotypes. Most patients carrying this mutation and living in disparate countries in Europe and America share a common, ancient founder haplotype, which likely originated from North Africa or the Middle East ~2000 years ago or earlier (Goldwurm et al. 2005; Kachergus J 2005; Lesage et al. 2005; Zabetian et al. 2006b). A second haplotype has been detected in a few patients of European ancestry, while a third haplotype was found in Japanese patients (Zabetian et al. 2006a; Zabetian et al. 2006b). The occurrence of the G2019S mutation in at least three haplotypes suggests either an extremely old founder or a mutational hot spot.

The penetrance of the LRRK2 G2019S variant appears to be age-dependent, increasing from ~17% at age 50 to ~85% at age 70 (Kachergus J 2005). Recent estimates of the lifetime penetrance of this mutation in large series of PD patients from the United States and Europe yielded values of ~24–33% (Clark et al. 2006; Goldwurm et al. 2007; Ozelius et al. 2006). This incomplete penetrance may explain the high prevalence of the G2019S mutation among patients with sporadic PD and its rare occurrence in controls. It is important to note, however, that penetrance may vary across different ethnic populations.

The prevalence of various other LRRK2 amino acid substitutions is in general lower than that of the G2019S mutation but may also vary depending on ethnicity. For example, the R1441C and R1441G mutations, located in the Roc domain, were initially identified in several families (Paisan-Ruiz C 2004; Zimprich A 2004). The R1441G mutation has been associated with an apparently high incidence among Spanish and Basque PD patients, with a prevalence of up to 8% (Paisan-Ruiz C 2004). Another study on sixty European families with autosomal PD found a prevalence of 3.4% for the R1441C mutation (Di Fonzo A 2006). The R1628P and G2385R variants, located in the COR and WD40 domains respectively, have been identified as common risk factors for sporadic PD in Chinese and Asian populations, each with an attributable risk of ~2–4% (Di Fonzo et al. 2006; Farrer et al. 2007; Fung et al. 2006b; Mata IF 2005; Ross et al. 2008; Tan et al. 2008; Tan et al. 2007b; Tomiyama et al. 2006). Other putative mutations, located throughout the multi-domain structure of LRRK2 (R1067Q, I1122V, S1228T, R1441H, Y1699C, M1869T, and I2020T) have been reported with a frequency of < 1% (Punia et al. 2006). It is important to

note that the majority of studies have focused on screening for mutations in exons 31 and 41, which encode the Roc and MAPKKK domains respectively, and, therefore, may prevent identification of novel variants in other regions of *LRRK2*. While some studies have done comprehensive mutation screening of all 51 exons of *LRRK2*, the continuation of such widespread studies will be crucial to the further identification and understanding of *LRRK2* variants (Clarimon et al. 2008; Di Fonzo A 2006; Johnson et al. 2007; Nichols et al. 2007; Paisan-Ruiz et al. 2006; Paisan-Ruiz et al. 2008; Skipper et al. 2005).

There is a wide pathological spectrum associated with *LRRK2* mutations. While age at disease onset ranges from ~40–80 years and clinical features are similar to those found in sporadic PD, some cases show various degrees of pathological heterogeneity. Presumably, mutations in different domains of *LRRK2* could possibly result in different disease phenotypes.

Patients with the common G2019S mutation in the MAPKKK domain show no difference in the average age at disease onset and duration compared with those of sporadic cases (Goldwurm et al. 2005). Clinical and positron emission tomography (PET) studies have confirmed that the overall phenotype associated with the G2019S mutation is similar to that of idiopathic PD (Hernandez D 2005). Some patients with this mutation demonstrate classic PD with Lewy bodies, however, others are characterized by parkinsonism without Lewy bodies but demonstrate dystrophic neurites in the SNpc that stain for *LRRK2* (Giasson et al. 2006).

Typical parkinsonism with nigral degeneration has also been reported in patients with other *LRRK2* mutations, but in some cases the clinical and pathological features are more diverse. The I2020T mutation, located in the MAPKKK domain, is responsible for parkinsonism in a large Japanese family, the Sagamihara kindred, and these patients are afflicted with pure nigral degeneration without α -synuclein-positive Lewy body and Lewy neurite pathology (Funayama et al. 2005). Patients with the Y1699C mutation, located in the COR domain, are mainly from a large British kindred and displayed dementia and amyotrophy, in addition to parkinsonism (Khan NL 2005; Zimprich A 2004). Upon pathological examination, a patient with this mutation was found to display a few α -synuclein-positive Lewy body and Lewy neurite pathologies but had more extensive ubiquitin-positive cytoplasmic and nuclear inclusions (Zimprich A 2004). In patients with the Roc domain R1441C mutation, some individuals had classic α -synuclein-positive Lewy body and Lewy neurite pathology, while others had tau pathology reminiscent of progressive supranuclear palsy (PSP) and some patients did not have any Lewy bodies or Lewy neurites, but displayed asymmetric ubiquitin-positive inclusions (Haugarvoll et al. 2008; Zimprich A 2004). Moreover, it has been noted that in patients with the Y1699C and R1441C mutations, degeneration is not solely restricted to dopaminergic neurons of the SNpc, however, PET scan analysis indicated that the *in vivo* phenotype associated with these two mutations is indistinguishable from that of sporadic PD despite the pathological heterogeneity (Adams et al. 2005). Interestingly, mutations in *LRRK2* have not been found in other primary neurodegenerative diseases such as Alzheimer's disease (AD), PSP, or frontotemporal dementia (FTD) (Hernandez D 2005; Tan et al. 2007a; Toft et al. 2005). The diverse pathologies associated

with *LRRK2* mutations strongly suggest that this protein may be involved in multiple cellular processes in neurons.

Conclusions

PD is a devastating neurodegenerative disorder and despite nearly 200 years since its initial description, a definitive understanding of disease etiology and a preventive therapeutic approach are still missing. The discovery of *LRRK2* mutations in PD has proven to be monumental to the field because, for the first time, gene mutations have been found to be a frequent determinant of both familial and sporadic PD. The pleomorphic pathology associated with *LRRK2* mutations places this protein further upstream in disease-linked signaling, compared with previously identified PD-related genes. The identification of a vast number of mutations found throughout *LRRK2* also suggests an upstream role in neuronal signaling. Because of the potential upstream role *LRRK2* likely plays in neurons, therapeutic strategies aimed at this protein may be effective in combating a broad spectrum of PD. The unique multi-domain organization of *LRRK2* may provide multiple avenues for therapeutic intervention, such as inhibiting GTP binding activity through the Roc domain or inhibiting kinase activity through the MAPKKK domain. It is still too early, but is certainly much desired, to reconcile the roles of *LRRK2* and other disease-linked proteins (encoded by various *PARK* loci, Table 1) into overlapping neurodegenerative pathways. It is clear that further knowledge of *LRRK2* is necessary to fully appreciate the role this multi-domain, dual enzyme plays in both normal neuronal function and the pathogenesis of PD.

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DOMAIN	PREDICTED FUNCTIONAL FEATURES
LRR	Role in protein-protein interactions
Roc	GTPase domain functioning as molecular switch to regulate kinase domain activity
COR	LRRK2 dimerization domain; molecular hinge between Roc and MAPKKK domains
MAPKKK	Kinase responsible for activation of mitogen activated protein kinase signaling
WD40	Role in protein-protein interactions

Fig. 1. Schematic representation of the predicted LRRK2 domain structure

LRRK2 is composed of 13 LRRs, followed by the Roc, COR, and MAPKKK domains, as well as seven WD40 repeats at the C-terminus. Mutations that are considered definitively pathogenic are shown in red. Amino acid substitutions that can act as risk factors for disease and are putatively pathogenic are shown in green. Mutations that have been identified in PD patients but for which pathogenicity is uncertain are shown in blue. The amino acid residue numbers beneath each domain indicates the estimated domain boundaries. Predicted functions of each of the LRRK2 domains are listed. The GenBank accession number for human LRRK2 is AAV63975.

Parkinson's Disease-Associated Genes

Table 1

Locus	MOI	Chromosomal Location	Protein Name	Mutations	Protein Function	Age at Onset
<i>PARK1/4</i>	AD	4q21	α -synuclein	dominant A30P, E46K and A53T; genome duplication/triplication	DA transmission, SV dynamics	30–60s
<i>PARK2</i>	AR	6q25	parkin	numerous recessive homozygous/compound heterozygous missense, truncating, exon deletion/duplication/triplication	E3 ubiquitin ligase	20–40s
<i>PARK5</i>	AD	4p14	UCH-L1	heterozygous I93M	ubiquitin hydrolase	30–50s
<i>PARK6</i>	AR	1p35-37	PINK1	various recessive missense, exon deletion	mitochondrial kinase	30–50s
<i>PARK7</i>	AR	1p38	DJ-1	various recessive homozygous/compound heterozygous missense, exon deletion	oxidative stress response	20–40s
<i>PARK8</i>	AD	12q12	LRRK2	numerous dominant missense	protein kinase and GTPase	40–60s
<i>PARK11</i>	AD	2q21.2	GIGYF2	several dominant missense	insulin signaling	40–70s

Abbreviations: MOI, mode of inheritance; AD, autosomal dominant; AR, autosomal recessive; UCH-L1, ubiquitin C-terminal hydrolase; PINK1, phosphatase and tension homolog (PTEN)-induced putative kinase 1; LRRK2, leucine-rich repeat kinase 2; GIGYF2, GRB-10-interacting GYF protein 2; DA, dopamine; SV, synaptic vesicle. Age of onset reported in years.