LRRK2 and Parkinson Disease

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**Objectives:** To review the molecular genetics and functional biology of leucine-rich repeat kinase 2 (LRRK2) in parkinsonism and to summarize the opportunities and challenges to develop interventions for Parkinson disease (PD) based on this genetic insight.

**Data Sources:** Publications cited are focused on LRRK2 biology between 2004 and March 2009.

**Study Selection:** Literature selected was based on original contributions, seminal observations, and thoughtful reviews.

**Data Extraction:** Unless stated otherwise, data was primarily abstracted from peer-reviewed literature appearing on PubMed.

**Data Synthesis:** Genetic mutations that predispose PD are diagnostically useful in early or atypical presentations. The molecular pathways identified suggest therapeutic interventions for Lrrk2 and idiopathic PD and the rationale and opportunity to develop physiologically relevant biomarkers and experimental models with which to test them.

**Conclusions:** Both affected and asymptomatic LRRK2 carriers now provide the opportunity to define the natural history of PD. This includes the frequency, penetrance, and rate of motor symptoms, nonmotor comorbidities, and their associated biomarkers.

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**Identification of PARK8 and LRRK2: The Predominant Genetic Risk Factor for PD**

In 2002, genetic linkage analysis of a Japanese family with dominantly inherited parkinsonism reminiscent of L-DOPA–responsive late-onset PD allowed Funayama and colleagues to map a novel PARK locus (PARK8) to chromosome 12q12. Linkage mapping localizes mutant genes based on the coinheritance of genetic markers and phenotypes in families over several generations. The PARK8 assignment was confirmed in Western Nebraska Family D and German-Canadian Family A, and pathogenic amino acid substitutions p.R1441C and p.Y1699C were subsequently identified in a novel gene, leucine-rich repeat kinase 2 (LRRK2; Lrrk2). Genetic linkage in other families with late-onset parkinsonism revealed other mutations including Lrrk2 p.R1441G in familial and seemingly sporadic PD from the Basque region of northern Spain and p.G2019S in families from Norway, Ireland, Poland, and the United States. Lrrk2 p.I2020T was found to explain disease in the original Sagamihara pedigree in Japan.

**LRRK2 and Leucine-Rich Repeat Kinase 2**

The genomic region comprised by LRRK2 is relatively large. The gene encompasses approximately 144 kilobases with 51 exons. It encodes a 2527–amino acid pro-
tein (286 KDa) termed leucine-rich repeat kinase 2 (Lrrk2) after 2 of its conserved domain structures, a leucine-rich repeat toward the N terminal of the protein and a kinase domain (MAPK) toward the C terminal. Several other conserved domain structures, including armadillo and ankyrin repeats, GTPase (Roc), and a C terminal of Roc spacer (COR) as well as WD40 repeats, have been recognized, and all are potentially associated with protein-protein interactions.12

The Lrrk2 kinase domain has been classified as a member of the tyrosine kinase–like subfamily whose members show sequence similarity to tyrosine and serine-threonine kinases.12 Serine or threonine residues now appear to be the major target for phosphorylation.13 Other members of this class initiate mitogen-activated protein kinase pathways where effector kinases activated in a multistep cascade ultimately execute a response to extracellular stimuli. In Lrrk2, the combination of Roc-COR-MAPK motifs encoding 2 distinct but physically linked enzymatic domains is highly conserved among vertebrates and shares homology with the ROCO protein family of receptor-interacting protein kinases. Receptor-interacting protein kinases are known for their role in cellular stress signaling and mediate their interactions through JNK, ERK, p38, and NF-kB pathways.14 Given the size, domain composition, and organization of Lrrk2, and the potential for protein-protein interactions, it is likely to be part of a higher-molecular-weight complex involved in cellular signaling. At a minimum, monomers of Lrrk2 protein dimerize similar to the conformation adopted by most protein kinases and Ras GTPases.15

The closest human homologue of LRRK2 is LRRK1 on chromosome 15q26 (70% homology of Roc, COR, and MAPK domains). The 2 proteins differ mostly in the organization of their N termini as Lrrk2 possesses several specific repeat structures absent in the Lrrk1 homologue. Phylogenetic analysis suggests that LRRK2 originated from gene duplication because Caenorhabditis elegans and Drosophila melanogaster possess only 1 LRR-1 gene.16 Thus, in vertebrates, Lrrk1 and Lrrk2 may have similar if not redundant functional activities. Intensive sequencing efforts have yet to reveal pathogenic LRRK1 mutations in familial parkinsonism.17

LRRK2 PATHOGENIC MUTATIONS AND POLYMORPHIC RISK FACTORS: FAMILIES AND FOUNDERS

More than 75 Lrrk2 coding substitutions have been described but not all contribute to the risk of parkinsonism, or to the same degree. Indeed, genetic evidence for pathogenicity is only proven for p.R1441C, p.R1441G, p.Y1699C, p.G2019S, and p.I2020T substitutions (by linkage and by association). Other sequence variants may be pathogenic, they might represent benign mutations or polymorphisms. This is a critical distinction in patient diagnosis and in interpreting Lrrk2 function. Lrrk2 p.G2019S appears to be the most frequent pathogenic substitution and directly affects the “activation hinge” of the MAPK domain (Figure). In terms of an odds ratio, carriers have more than a 20-fold increase in disease risk.18 In the United States and Europe, the mutation is found in 0.5% to 2.0% of seemingly sporadic PD and 5% of dominantly inherited familial parkinsonism.9 Family-based studies show disease penetrance in Lrrk2 p.G2019S carriers is age dependent, increasing from about 20% at age 50 years to 80% at age 70 years.10,19 Despite ascertainment bias in pedigree-based studies, the age-associated cumulative incidence of seemingly sporadic PD in clinic-based Lrrk2 carriers concurs.10,20 This is dramatically different from the incidence of PD in the general population, which is rare before age 50 years and may reach 4% in subjects older than 80 years.1 Historically, the Lrrk2 p.G2019S mutation is associated with 1 chromosome 12q12 haplotype inherited identical by descent from 1 ancestral founder; most patients with Lrrk2 p.G2019S are genetically related, although there are some exceptions.10,20 In North African Arab-Berbers, the major haplotype is shortest and most frequent, consistent with its origin in this ethnic group. Indeed, in this part of the world and possibly throughout the Maghreb, more than one-third of all patients diagnosed with PD are Lrrk2 p.G2019S carriers.18 Notably, about 15% of patients with Ashkenazi Jewish heritage have the same Lrrk2 p.G2019S mutation.21,22 In contrast, Lrrk2 p.R1628P and p.G2385R are the most common susceptibility variants, which double disease risk. Each is found in approximately 3% to 4% of healthy subjects and 6% to 8% of patients, and each appears to have been inherited identical by descent from an ancient founder.23,24 While the genetic association of Lrrk2 p.R1628P with parkinsonism appears restricted to

Figure. A “ribbon” model of the MAPK domain of Lrrk2. In catalytic domains of protein kinases, a small N terminal lobe and a larger C terminal lobe are connected to form a cleft in which adenosine triphosphate and the protein substrate may react. The Lrrk2 MAPK activation segment is a ribbon of protein from 2017 to 2042 amino acids of the large C terminal lobe (magenta). The region, defined by conserved tripeptide “hinges” DFYG and APE, serves to block substrate access to the catalytic site (yellow). Lrrk2 p.G2019S and 2020T side chains affect 1 hinge of the activation segment (blue), and the magnesium–adenosine triphosphate binding site is highlighted (turquoise). The majority of protein kinases, including Lrrk2, require phosphorylation of the activation segment for activity. On phosphorylation, the activation segment is believed to adopt an active conformation, enabling substrate access and catalysis to take place.
the ethnic Chinese population, Lrrk2 p.G2385R has been reported in clinical and community-based studies throughout Asia, including Taiwan, Singapore, Korea, and Japan.\textsuperscript{23-27} Lrrk2 p.R1629P is located within the COR domain that bridges the ROC and MAPK domains, whereas p.G2385R is located on the external surface of the C terminal WD40 “barrel.”

**LRRK2 IN THE BRAIN**

LRRK2 messenger RNA expression is found in most regions, including nuclei affected in PD. Messenger RNA is especially abundant in dopamine-innervated areas and A9 midbrain dopaminergic neurons of the substantia nigra and is found in peripheral organs, including the heart, liver, kidney, lung, and leukocytes.\textsuperscript{28} However, the specificity of antibodies and different fixation techniques has hampered studies of protein expression (with fixation, Lrrk2 antigens may be masked, their molecular interactions perturbed, or the complex may simply fall apart). Nevertheless, overexpression of tagged protein expression in FLAG-Lrrk2 wild-type protein and a 2- to 10-fold increase of immunostaining described earlier, it remains unclear whether Lrrk2 protein is a constituent of Lewy bodies and clear whether Lrrk2 protein is a constituent of Lewy bodies.\textsuperscript{31} With the caveat that antibodies and different fixation techniques have hampered studies of protein expression (with fixation, Lrrk2 antigens may be masked, their molecular interactions perturbed, or the complex may simply fall apart), nevertheless, overexpression of tagged protein expression in FLAG-Lrrk2 bacterial artificial chromosome mice provides a guide.\textsuperscript{29} Both Lrrk2 protein and messenger RNA levels appear high in the striatum, cortex, and cerebellum. LRRK2 messenger RNA and protein levels also appear high in the subventricular zone and dentate gyrus, with an active role in adult neurogenesis (H. Melrose, PhD, oral and written communication). Imaging studies suggest the in vivo neurochemical phenotype of Lrrk2 mutations is comparable with idiopathic PD. However, the ability to assess asymptomatic carriers provides the most remarkable window into disease onset and progression.\textsuperscript{30} Pathologically, more than 80% of autopsy-examined cases with Lrrk2 parkinsonism present with typical Lewy body disease consistent with a postmortem diagnosis of “definite” PD.\textsuperscript{31} With the caveats for immunostaining described earlier, it remains unclear whether Lrrk2 protein is a constituent of Lewy bodies.\textsuperscript{28} Intriguingly, some carriers reveal pathological changes reminiscent of other neurodegenerative disorders. These include tau-positive neurofibrillary tangles reminiscent of argyrophilic grains disease\textsuperscript{32,33} and pure nigral degeneration and gliosis without Lewy body pathology but with multiple ubiquitin and TDP-43 immunoreactive cytoplasmic and nuclear inclusions.\textsuperscript{34} “Pleomorphic” pathology can occur among Lrrk2 carriers with the same pathogenic mutation and even within the same family.\textsuperscript{8} Hence, Lrrk2 has been dubbed the “Rosetta stone” of parkinsonism, perhaps providing a common link between these neuropathologies.

**FINDINGS ON LRRK2 FUNCTION**

Several biochemical studies have shown kinase activity for Lrrk2 wild-type protein and a 2- to 10-fold increase of intramolecular and intermolecular phosphorylation for the p.G2019S mutant.\textsuperscript{15,39,40} Whether enhanced kinase activity in vivo represents a characteristic feature shared by all pathogenic Lrrk2 variants remains controversial. Regulation of Lrrk2 kinase activity is complex, requiring intermolecular and perhaps intramolecular phosphorylation, and in vitro several groups have shown Lrrk2 GTP binding and GTPase activity has a modifying effect.\textsuperscript{29,36,37} The Roc-COR domain also requires intramolecular dimerization for optimal GTPase activity.\textsuperscript{15,39,40}

A further limitation is that most assays focus on in vitro autophosphorylation or artificial substrates, as few authentic Lrrk2 substrates have been nominated. These include threonine 558 of moesin, a member of the ERM/martin family that links plasma membrane receptor complexes to the microfilament cytoskeleton,\textsuperscript{41} and threonine 37/46 of 4E-BP, a negative regulator of elf4E-mediated protein translation that is critical in stress response and dopaminergic neuronal maintenance.\textsuperscript{42} However, validation in the brain is required and relevance to PD has not been established.

Lrrk2 kinase activity seems to be the culprit in several cellular models where overexpression of Lrrk2 (wild type or mutants) leads to aggregation and cellular toxicity.\textsuperscript{31,41} Transfection of a Lrrk2 variant with deficient kinase activity (kinase “dead”) blocks the formation of aggregates and seems to delay cellular death. While it remains unclear if Lrrk2 aggregation is a feature of PD, or a transfection artifact, the data suggest protein aggregation and/or clearance may be regulated by Lrrk2 kinase activity.\textsuperscript{45,46}

In differentiated neuroblastoma or primary neuronal cultures, and in the intact rodent central nervous system, overexpression of Lrrk2 mutants induces a progressive reduction in neuritic length and branching.\textsuperscript{47-49} In contrast, Lrrk2 knock down induced by RNA interference, or overexpression of a Lrrk2 variant with deficient kinase activity, confers increased neuritic length and branching.\textsuperscript{37,30} In culture systems, overexpression of pathogenic Lrrk2 variants linked to PD may also lead to phospho-tau immunopositive inclusions and apoptosis.\textsuperscript{40,37}

Results from D melanogaster models show loss of dLRRK (CG5483, the single orthologue of human LRRK1 and LRRK2) may induce degeneration of dopaminergic neurons with a consequent locomotor deficit.\textsuperscript{41} However, the findings are controversial because a kinase-null dLRRK has been reported to have negligible effects on the development, life span, or survival of dopaminergic neurons.\textsuperscript{52} Similarly, overexpression of human wild-type LRRK2 complementary DNA (cDNA) and p.G2019S mutant proteins in flies, using a panneuronal driver, have been reported to result in the selective loss of dopaminergic neurons and a locomotion deficit.\textsuperscript{53} However, in other models of transgenic cDNA overexpression, or with the knock in of equivalent human mutations in the endogenous gene, there have been no pathologic effects.\textsuperscript{51} The reason for these disparities is unclear. Most recently, dLRRK was shown to regulate dopamine neuronal function and maintenance; aged dLRRK-null flies were found to have normal dopaminergic neurons, albeit with elevated brain dopamine levels, and wild-type transgenic expression had no effect, whereas mutant overexpression demonstrated relatively specific dopaminergic neuronal toxicity.\textsuperscript{52}

In C elegans, the nematode worm, LRK-1 localizes to the Golgi and determines the polarized sorting of synaptic vesicle proteins to axons by excluding them from the dendrite-specific transport machinery.\textsuperscript{55} Much of the biology cited on WormBase preceded the discovery of pathologic LRRK2 mutations (http://www.wormbase
LRK-1–null and knock-down worms have an experimentally useful chemosensory deficit. Reduced LRK-1 expression also appears to make worms more susceptible to rotenone-induced oxidative stress, although overexpression of wild-type and mutant Lrrk2 appears to confer neuroprotection. Most recently, in C elegans, a dominant mutation in Lrk-1 and loss of PINK-1 (PARK6) have been shown to act antagonistically in stress response and neurite outgrowth.

While the concept of Lrrk2 kinase activity as a key player in dopaminergic function and parkinsonism has been reasonably well established in man, the majority of phenotypes described in model organisms have yet to be validated in vertebrate models under more physiological conditions. Several overexpression, bacterial artificial chromosome transgenic, murine knockout, and knock-in mouse lines have been developed but publications to date have focused on their biochemical utility to study Lrrk2 and its protein interactions. Four independent mouse models of LRRK2 have recently been published, 2 based on human or murine LRRK2 bacterial artificial chromosome integration, 1 based on human cDNA overexpression in an α-synuclein overexpression cross, and 1, a murine knock-in. Dopaminergic release deficits are consistently reported, with profound neuropathology in human bacterial artificial chromosome and cDNA animals. Which phenotypes will prove philosophically insightful in human disease remain to be elucidated.

A better understanding of Lrrk2’s physiological function is required to elucidate Lrrk2’s signaling pathways. Alterations in MAPK signaling have previously been associated with parkinsonism and PD, and the discovery of Lrrk2’s downstream effector kinases is likely to identify additional targets for future therapeutic interventions. While more effort is needed, preliminary steps have been undertaken to identify pathways central to Lrrk2 biology. These include a screen for alterations in the phosphorylation level of MAPK signaling cascades in human subjects, microarray expression studies in a Lrrk2 knockdown model, and identification of Lrrk2 protein interactions, as well as Lrrk2 kinase inhibitors.

TOWARD A PARSIMONIOUS MOLECULAR MODEL OF LRRK2 ACTIVITY

Converging genetic and functional approaches will help elucidate the mechanisms leading to LRRK2-associated parkinsonism. A central problem is how different pathogenic mutations in Lrrk2, within its many domains (Roc, COR, MAPK, and WD40), may be reconciled with 1 phenotype. Wild-type protein may not normally be an active kinase, although p.G2019S may confer enzymatic activity in vitro. I2020T adjacent to p.G2019S appears to have lower activity (in most assays) than wild-type protein but clearly leads to the same disease. Not all proteins that possess a kinase domain defined by their amino acid sequence serve to phosphorylate substrates. The presence of several protein–protein interaction domains at the N and C terminal ends suggests Lrrk2 may primarily act as a regulatory scaffolding protein. In such a model, different mutations that impair adequate binding of partner proteins might lead to a dominant negative effect and result in a similar phenotype. Although the p.G2019S mutation may increase kinase activity, it may drastically modify its specificity with very different consequences for the phosphorylation of a genuine substrate(s).

Alternatively, the discrepancy among kinase activities of different Lrrk2 mutations may simply reflect a limitation of in vitro enzymatic activity assays. Recombinant Lrrk2 protein might not be in the correct physiologic context or there may be a shortage of cofactors necessary for activation and efficient phosphorylation. Biochemically, it has been shown that the Roc domain can act as an intrinsic regulator of Lrrk2 kinase activity and potentially Lrrk2 must adopt a dimeric conformation. A model is conceivable where efficient phosphorylation of heterologous Lrrk2 substrates requires a high-molecular-weight Lrrk2 complex, first activated by GTP binding and intramolecular and intermolecular autophosphorylation. Pathologic activation of a Lrrk2 complex via a mutation is consistent with the finding that patients with heterozygous or homozygous p.G2019S mutations do not differ in the severity of symptoms, age at onset, or disease progression.

Lastly, more emphasis might be placed on Lrrk2’s Ras GTPase activity as a binary switch, cycling between GTP-bound and GDP-bound forms. Monomeric GTPases are typically regulated by GTPase-activating proteins and guanine nucleotide exchange factors, but Lrrk2’s GTPase may also be regulated through the COR-MAPK domain. Related Ras, Rho, and Rab family members activate multiple effector-mediated signaling pathways and are involved in many biological functions, including gene expression, cytoskeletal rearrangement, vesicle trafficking, cellular proliferation, and oncogenesis. Identifying Lrrk2’s signaling network mediated by the Roc-COR-MAPK domains, comprehending its regulation and functional consequences, may prove as complicated.

The hypothesis that Lrrk2 might function as a multisubunit complex underlines the need for physiologic animal models; simple overexpression of partial or full-length wild-type or mutant Lrrk2 under control of a heterologous promoter may not be stoichiometric. Preserving the Lrrk2 multisubunit complex may be required for interaction studies and Roc-COR-MAPK assays, although methodologically more challenging. More insight into Lrrk2 function might be achieved with a variety of knockout and knock-in mutations (including kinase and/or GTPase dead); LRRK2 bacterial artificial chromosome transgensics and constitutive and inducible complementary DNA expression may be less optimal.

CONCLUSIONS

ably via the same pathogenic mechanism, whereby 1 mutant Lrrk2 molecule is sufficient to change the properties of the entire complex, leading to a dominant effect.

The frequency of pathogenic LRRK2 mutations among patients with PD highlights the importance of a genetic predisposition in this seemingly sporadic neurodegenerative disorder. Nevertheless, PD should still be considered a multifactorial syndrome influenced by a variety of genetic, environmental, and stochastic factors, for which age remains a major determinant. Disease risk may have been most precisely estimated for Lrrk2 p.G2019S but remains a probability rather than a certainty. Penetrance is also variable; indeed, some very elderly Lrrk2 p.G2019S carriers appear to have escaped disease.73 Thus, widespread genetic screening for LRRK2 mutations in asymptomatic subjects is not warranted. In contrast, genetic screening in patients may help provide a definite clinical diagnosis in early and difficult presentations. While many amino acid substitutions have been identified in the Lrrk2 protein, the majority are rare orphan changes and only 8 are confirmed pathogenic (http://www.mg-esten.org). Additional studies of Lrrk2 variant frequency, prevalence in disease, penetrance, and independent and joint effects (in cis or trans) are required.

On a clinical research basis, pathogenic LRRK2 mutations now provide a relatively homogeneous background to elucidate the natural history of parkinsonism, from the earliest signs and symptoms in drug-naive, asymptomatic carriers, to the relationship of other comorbidities, including depression and dementia in affected subjects. Biomarker identification and validation, specific to trait, stage, and progression, will become imperative if neuroprotection trials are to be feasible. On this background, epidemiological studies of environmental exposures may be informative. Meanwhile, molecular genetic insights provide the tools and rationale to develop model systems and novel molecular therapeutics aimed at halting disease progression, not just symptomatic benefit. If enhanced kinase activity proves to be a major pathogenic event in Lrrk2 parkinsonism and perhaps idiopathic PD, then kinase inhibitors given sufficiently early may prevent disease and in effect provide a cure. A great deal of academic and pharmaceutical interest is now focused on this issue. While the remaining challenges are considerable, the opportunity to successfully diagnose and develop novel molecular therapeutics for Lrrk2 parkinsonism and PD has never been greater.

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