Mutations in *LRRK2* as a Cause of Parkinson's Disease

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Key Words

α -Synuclein • Kinase • *LRRK2* • *PARK8* • Parkinson's disease

Abstract

Mutations in leucine-rich repeat kinase 2 (*LRRK2*) are the most common known cause of late-onset Parkinson's disease (PD). Clinical and pathological studies have demonstrated that in the majority of cases *LRRK2* mutations lead to PD with classical clinical and pathological features. However, in some patients the pathological features can be distinct and/or more extensive than typically seen in PD. Collectively, these findings provide important clues into the mechanisms by which *LRRK2* mutations can lead to demise of dopaminergic neurons. The understanding of *LRRK2* protein function and its gene regulation and the consequences of mutations are still at their infancy, but scientific findings are progressing at a rapid pace. Although more detailed information on *LRRK2* is still needed in the quest for therapeutic intervention that could halt or slow the progression of disease, here we summarize the current information on the biological and pathological properties of *LRRK2*.

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Introduction

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene (also known as *PARK8*) resulting in amino acid substitutions are collectively the single most common known

cause of late-onset Parkinson's disease (PD). The protein product of *LRRK2* has also been termed dardarin. PD is the second most common neurodegenerative disease in the developing world and is characterized by bradykinesia, resting tremor, cogwheel rigidity and postural instability [1, 2]. These major clinical features of PD are associated with the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta [3, 4]. In addition, postmortem analysis of the majority of clinically diagnosed PD patients reveals intracytoplasmic inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) in some of the remaining dopaminergic neurons. The presence of these inclusions is a criterion used to differentiate PD from other disorders associated with parkinsonism [5, 6]. LBs and LNs are formed as the result of aberrant polymerization of the normally soluble, presynaptic protein α -synuclein (α -syn) and α -syn inclusions can also form in other brain regions in PD and in other related disorders [5, 7, 8].

LRRK2 Structure, Expression and Function

LRRK2 is a large, 2,527-amino-acid protein with several discrete domains (fig. 1). A region with many leucine-rich repeats and a putative ankyrin-like domain are present in the N-terminal half. A Ras (renin-angiotensin system) of complex (ROC), which belongs to the Ras GTPase superfamily, followed by a C-terminal of ROC domain is present in the middle. A kinase domain and a

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1424–862X/08/0161–0099\$24.50/0

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Fig. 1. Predicted structure of LRRK2. Major domains that have been established or predicted [30, 31]: ANK = ankyrin-like, LRR = leucine-rich repeat, ROC = Ras of complex, COR = C-terminal of ROC. Mutations that are considered definitely pathogenic are identified in red font above. Mutations that can act as risk factors or are likely disease causing, but could not be established with certainty, are identified in orange. Mutations that have been identified in PD patients, but for which evidence for cosegregation is limited, DNA from relative is unavailable or pathogenicity has been questioned, are labeled in blue.

WD40 domain are present towards the C-terminal end.

Initial studies on the expression profile of *LRRK2* mRNA by RT-PCR [9] and Northern blot analysis [10] showed that *LRRK2* mRNA is expressed in various regions of the neuroaxis including the whole brain, spinal cord, cerebellum, SN and striatum. However, it is also expressed at substantial levels in many peripheral organs including the heart, placenta, lung, kidney, skeletal muscle and pancreas [9–11].

LRRK2 is predominantly a cytoplasmic protein, but it can be associated with various organelles [11–15]. At the protein level, LRRK2 expression has been demonstrated in the brain as well as in peripheral organs [14, 16]. LRRK2 is expressed and localized to a variety of neuronal populations in human and rodent brains [15, 17–23]. In terms of specific neuronal populations implicated in PD, all published studies have shown that LRRK2 is highly expressed in the dopaminergic regions of the mammalian brain such as the caudate-putamen and frontal cerebral cortex [15, 17–19, 22]. Some studies showed negligible LRRK2 mRNA levels in human SN dopaminergic neurons [22], but others have reported clearly detectable expression in these neurons [17]. Similarly, in rodent brains, several studies have reported the absence or extremely low levels of *LRRK2* mRNA in the SN [18, 19, 22], while others have reported detectable levels [21, 23]. These conflicting reports may reflect biological variation, or simply differences in the sensitivity of the analytical methods used. Immunofluorescence analysis has re-

vealed LRRK2 in some dopaminergic neurons of the SN [15, 18, 20].

LRRK2 functions as a serine/threonine kinase that can undergo autophosphorylation [11, 24, 25]. In vitro it can phosphorylate the generic kinase substrate myelin basic protein [11]. Several other likely more physiological substrates such as moesin, ezrin and radixin have been identified [26].

The ability of LRRK2 and more specifically its ROC domain to bind GTP has been reproducibly demonstrated in several studies [16, 24, 25, 27, 28]. However, the intrinsic ability of LRRK2 to hydrolyze GTP is more controversial. Studies using cultured cells reported a lack of GTPase activity [28] or low levels thereof [16, 27]. In contrast, LRRK2 purified from transgenic mouse brain expressing human LRRK2 demonstrated robust GTPase activity comparable to Rac1 [16]. These discrepancies could be due in part to experimental differences and/or the presence of an LRRK2-specific GTPase-activating protein in mouse brains. GTP binding stimulates LRRK2 kinase activity and binding of GTP/GDP appears to be required for kinase activity such that specific artificial mutations that disrupt the GTP/GDP binding site abolish kinase activity [24, 25, 28].

Although the physiological functions of LRRK2 remain mostly unknown, it may play some role in regulating neurite outgrowth, such that decreased expression in cultured cells results in increased neurite length and branching [29].

Fig. 2. Lewy pathology in a PD patient with the G2019S mutation in *LRRK2*. Immunocytochemistry using an antibody specific for α -syn in the SN of a patient with the G2019S mutation. **a** Classical LBs (arrows) in dopaminergic neurons containing neuromelanine. **b** An LN (arrow) and neuroaxonal spheroids (arrowheads) staining for α -syn. Scale bar = 50 μ m.

LRRK2 Disease-Causing Mutations

To date, 5 missense mutations in *LRRK2* associated with autosomal dominantly inherited PD are considered definitely pathogenic (R1441C, R1441G, Y1699C, G2019S, I2020T) [30, 31], but many additional *LRRK2* genetic variations have been identified by numerous groups and are being evaluated for conclusive pathogenicity or increased risk for PD (fig. 1). The most common mutation p.G2019S (c.6055G>A) is reportedly responsible for 2–8% of hereditary PD [32–37]. In addition, the G2019S mutation has been observed in 0.6–1.6 % of sporadic PD cases [33, 35, 38]. The frequency of the *LRRK2* G2019S mutation has been found to be much higher in some ethnic groups, for example in North African Arabs and Ashkenazi Jews; the G2019S mutation is found in 22–41% of sporadic and 30–41% of familial PD cases [39–41]. However, in Asian populations this mutation is much less common [42, 43]. In addition, penetrance for this mutation increases with age from 15–17% at the age of 50 years to 32–100% at the age of 80 years, depending on the cohort reported and the method used for calculation [35, 39, 44].

Most patients with an *LRRK2* mutation exhibit clinical and neurochemical features, including responsiveness to carbidopa/levodopa, that are indistinguishable from typical sporadic PD patients [32–34, 45–48]. Mutations in *LRRK2* are not associated with other primary neurodegenerative diseases as demonstrated by the paucity of *LRRK2* mutations in patients with Alzheimer's disease (1 carrier of 2,889 patients screened), multiple system atrophy (none of 194 patients screened) or progressive supranuclear palsy (none of 527 patients screened) [36, 49–54].

For the majority of cases with *LRRK2* mutations for which neuropathological analyses were performed, LB

pathology typical of PD was demonstrated. However, different pathologies including 'pure' nigral degenerative pathology in the absence of LBs, neuronal loss with only ubiquitin-positive inclusions, and nigral degeneration with tau-positive-only pathology have also been reported. Even in patients carrying the same mutation, the pathological findings can be diverse. Of the 20 autopsies reported for PD patients with the G2019S mutation, 17 patients showed classical nigral degeneration with nigral LBs (fig. 2) [14, 38, 53, 55], although 3 of them had pathological changes indicating concurrent Alzheimer's disease [14, 53]. Two PD patients with the G2019S mutation presented with nigral degeneration without LB pathology [14, 56]. One of these patients presented with only rare tau inclusions [14], while the other contained occasional tau inclusions and Marinesco bodies [56]. An additional patient with parkinsonism carrying the G2019S mutation was also reported to have a paucity of LB pathology, but pathologically exhibited a tauopathy with features suggestive of progressive supranuclear palsy and early Alzheimer's disease-type pathology [57].

For the patients reported so far with the p.I2020T (c.6059T>C) mutation, the clinical and pathological features appear to be more uniform. All 4 individuals from a kindred (known as Sagami-hara) carrying this mutation were reported to be afflicted by pure nigral degeneration without LB pathology [58, 59].

Patients with other *LRRK2* mutations have been reported with clinical and pathological features that are more extensive and diverse. Patients with the p.R1441C (c.4321C>T) mutation have a classical PD phenotype [9, 60–62], but only 2 of 4 individual autopsies of patients with this mutation demonstrated classical α -syn-positive LB and LN pathology [9, 60]. One patient was found to have tau pathology reminiscent of progressive supranuclear palsy and the other was described to have asym-

metric ubiquitin-positive cytoplasmic inclusions [9, 60].

Of the 4 individuals from the original Canadian family A described with the p.Y1699C (c.5096A>G) mutation, all had parkinsonism, but in addition 2 individuals presented with amyotrophy [9]. The autopsies of 2 individuals from this kindred demonstrated SN degeneration without LB pathology, but with ubiquitin-positive cytoplasmic inclusions [9]. One of these patients also had concurrent AD pathology. These findings could lead to the suggestion that these atypical features may be due to the nature of this particular mutation. However, a more recent report on another well-characterized large family (Lincolnshire kindred) with the Y1699C mutation revealed typical PD with LB-positive pathology in the brain and olfactory bulb [63]. Similarly, the only PD patient with the putatively pathogenic LRRK2 mutation p.I1371V (c.4111A>G) that has been autopsied so far showed classical LB-positive PD [48].

Effects of LRRK2 Disease-Causing Mutations

In vitro expression of several mutant LRRK2 proteins (R1141C, Y1699C, G2019S and I2020T) has been shown to cause toxicity in cultured cells [13, 24, 25, 29, 64] and some of the LRRK2 protein variants reportedly resulted in the formation of aggregates within the cultured cells [13], suggesting that perhaps these mutations can promote misfolding of LRRK2. An active kinase domain has been shown to be required for the toxic effect of mutant LRRK2 expressed in cells [13, 24, 25]. Expression of these pathogenic LRRK2 mutants in cultured cells also resulted in decreased neurite outgrowth and branching [29].

Biochemical studies of culture cells transfected to express LRRK2 have consistently demonstrated an increase in kinase activity, although modest (0.5- to 3-fold), for the G2019S mutation [11, 13, 25, 26]. It is not surprising that this mutation has an effect on kinase activity, since residue G2019 is located within the conserved Asp-Tyr-Gly-Mg²⁺-binding motif in the VII subdomain of the kinase domain. However, intuitively, the opposite effect would have been predicted, that is to say that this substitution should impair kinase activity since this residue is universally conserved in kinases.

The effects of other *LRRK2* mutations on kinase activity are more controversial. The R1441C mutation has been demonstrated to increase kinase activity in some studies [11, 25], but others have reported no significant change [13, 26]. The I2020T mutation has been docu-

mented to either modestly increase [12, 25] or decrease kinase activity [26]. No change in kinase activity was reported for the Y1699C mutation [13, 26], while another study reported increased activity for this mutation [25]. A modest increase in kinase activity was reported for the R1441G mutant [25], but this was not observed in another study [26]. The putatively pathogenic mutations G2385R and I1371V do not appear to affect kinase activity [25], while the I2012T variant appears to decrease kinase activity [25, 26]. The explanation for some of the discrepancies may be analytical, that is the use of autophosphorylation versus myelin basic protein or meosin as the kinase substrate, as well as the means used to express or purify the kinase. Use of different tags to purify the kinase or the expression of full-length versus truncated proteins may also contribute to some of the conflicting results. Nevertheless, the effects of mutations on LRRK2 kinase activity are not unequivocal and these findings suggest that changes in kinase activity alone may not be the only noxious effect of *LRRK2* mutations.

The mutations R1441C and R1441G in the ROC domain have been reported to decrease GTP hydrolysis activity [16, 27] or increase GTP binding [25]. Interestingly, the I1371V and Y1699C mutations that are outside the ROC domain also appear to increase GTP binding [25]. Consequently, a predicted consequence of the mutations in this region of LRRK2 has been to influence the downstream kinase activity through perturbation of the GTPase domain.

Conclusion

In just the last few years since the discovery that mutations in *LRRK2* can cause PD, much information has been generated; however, many important issues remain unanswered. For example, what is the normal function of LRRK2? How can the contrast between the widespread expression of LRRK2 in the brain and the selective, although not exquisite, vulnerability of specific populations of neurons associated with PD be reconciled? How can only modest changes in LRRK2 activity and/or perhaps structure changes lead to the demise of these neurons? The role of increased kinase activity in disease is further confounded by the finding that patients heterozygous or homozygous for the G2019S mutation display similar clinical phenotype and range of disease onset [65], suggesting that incremental increase in kinase activity does not appear to enhance neuronal injury. It is like-

ly that the normal function of LRRK2 is protective from cell death and that mutations in LRRK2 bring neurons to dysfunction and increased vulnerability to other adverse insults that can result in neuronal degeneration with or without α -syn aggregation. The latter inclusions may act to further accrue insults to these neurons. It is clear that a better understanding of how LRRK2 is regulated, the cellular pathways that are influenced by this kinase, its precise substrates and the effects of its gene mutations

and variants is needed to provide the clues that will help unravel the pivotal role that this enzyme plays in the pathobiology of PD.

Acknowledgements

This work was funded by grants from the National Institute on Aging (AG09215 and AG17586) and the Ellison Medical Foundation (AG-NS-0331-06).

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