Presynaptic dysfunction in Parkinson’s disease: a focus on LRRK2

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Abstract

PD (Parkinson’s disease) is a common neurodegenerative disease clinically characterized by bradykinesia, rigidity and resting tremor. Recent studies have proposed that synaptic dysfunction, implicated in numerous studies of animal models of PD, might be a key factor in PD. The molecular defects that lead to PD progression might be hidden at the presynaptic neuron: in fact accumulating evidence has shown that the majority of the genes linked to PD play a critical role at the presynaptic site. In the present paper, we focus on the presynaptic function of LRRK2 (leucine-rich repeat kinase 2), a protein that mutated represents the main genetic cause of familial PD described to date. Neurotransmission relies on proper presynaptic vesicle trafficking; defects in this process, variation in dopamine flow and alteration of presynaptic plasticity have been reported in several animal models of LRRK2 mutations. Furthermore, impaired dopamine turnover has been described in presymptomatic LRRK2 PD patients. Thus, given the pathological events occurring at the synapses of PD patients, the presynaptic site may represent a promising target for early diagnostic therapeutic intervention.

PD (Parkinson’s disease) and LRRK2 (leucine-rich repeat kinase 2)

Early in 1817, James Parkinson described in his monograph Essay on the Shaking Palsy the most representative clinical features of a illness that at present is recognized as the second most diffuse neurodegenerative disease in aging populations and the second most common movement disorder. PD (OMIM 168600) affects 2% of the population over 60 years of age and occurs at an incidence of 16–19 in 100000 individuals per year [1]. The clinical phenotype of PD is characterized by tremor at rest, rigidity, postural instability, bradykinesia (slowness of movements), flexed posture and freezing (motor blocks) [2]. Two pathological events define the disease: a progressive loss of dopaminergic neurons in the SNpc (substantia nigra pars compacta) of the basal ganglia, accounting for the motor symptoms, and the formation of Lewy bodies in the surviving neurons [3]. However, it is not only the SNpc that is targeted by PD; instead PD is emerging as a pan-neuronal disease: in fact, other brain areas are affected at different times of the pathology, as reviewed by Hawkes et al. [4]. Accordingly, some of the earliest changes in PD are olfactory dysfunction, sleep disturbances and cardiac sympathetic denervation [5].

Mutations in seven genes have been identified in various forms of familial parkinsonism. Two autosomal-dominant genes [SNCA (coding for α-synuclein) and LRRK2] and three autosomal-recessive genes [PARK2 (encoding parkin), PARK7 (encoding DJ-1) and PINK1 (phosphatase and tensin homologue deleted on chromosome 10-induced putative kinase 1)] have been definitively associated with Mendelian-inherited PD (reviewed in [6]). Genetic studies have identified other candidate genes putatively associated with PD, such as GIGYF2 (growth-factor-receptor-bound protein 10-interacting GYF domain protein 2), ATP13A2 (ATPase type 13A2), HTRA2 (encoding Omi/HtrA2), UCHL1 (ubiquitin C-terminal esterase L1), SNCAIP (encoding synphilin 1) and NR4A2 (nuclear receptor subfamily 4, group A, member 2), but their confirmation is still awaited (reviewed in [7]). Mutations in LRRK2 (PARK8; OMIM 609007) are linked to late-onset autosomal-dominant PD, accounting for up to 13% of familial PD cases compatible with dominant inheritance [8] and 1–2% of sporadic PD patients, suggesting that LRRK2 is the most significant player in PD pathogenesis identified to date [9].

From the clinical and pathological prospective, the features of LRRK2-associated parkinsonism are often indistinguishable from idiopathic PD, although pathological variability exists even within the same LRRK2 kindred. This pleomorphic pathology ranges from pure nigral degeneration to general neuronal loss with α-synuclein, ubiquitin or tau inclusions (reviewed in [10]). The neuropathological examinations of post-mortem brain often revealed the presence of synucleinopathies and occasionally tauopathy.
in patients with LRRK2 mutations, suggesting a role for LRRK2 that is upstream of protein aggregation pathways [11]. Although different studies show little agreement regarding the level of LRRK2 mRNA/protein in the SNpc, LRRK2 protein expression has been demonstrated in tyrosine-hydroxylase-positive neurons of the SNpc and in the striatum [12]. Cortical regions affected in dementia associated with PD, pyramidal neurons of the cerebral cortex and of Ammon’s horn also present from moderate to high levels of LRRK2 expression [12]. At the subcellular level, previous studies have shown that LRRK2 is mainly associated with mitochondria, endoplasmic reticulum and with multiple vesicular structures, including SVs (synaptic vesicles) [13]. Biochemical analysis indicates that LRRK2 possesses a molecular mass of approximately 280 kDa and contains several domains including a ROC (Ras of complex proteins)/GTPase-like domain, a COR (C-terminal of ROC) domain, a kinase (similar to mitogen-activated protein kinase kinase kinases) domain and a WD40 domain (reviewed in [14]).

From a phylogenetic analysis, the LRRK2 kinase domain belongs to the TKL (tyrosine-like kinase) family and presents relatively high similarity to MLKs (mixed-lineage kinases) and RIPKs (receptor-interacting protein kinases) [15]. From different studies, it is clear that LRRK2 is a serine/threonine kinase. *In vitro* assays have suggested the nature of some LRRK2 substrates, including LRRK2 itself, moesin, 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1), MKK (mitogen-activated protein kinase kinase) members (MKK3, MKK6 and MKK7), tubulin-β and α-synuclein [16–20], but the physiological relevance of many of these modifications has not yet been confirmed. Interestingly, several independent groups have observed that LRRK2 exists predominantly as a dimer under native conditions and that autophosphorylation might orchestrate the monomer–dimer equilibrium [21,22].

Several missense mutations have been identified in LRRK2, covering all functional domains, but only five mutations robustly segregate with PD in large family studies (reviewed in [23]). Disease-segregating mutations in LRRK2 are located in the kinase domain (G2019S, I2020T), in the ROC domain (R1441C/G) and in the COR domain (Y1699C) (reviewed in [24]). Robust evidence in the literature indicates that the G2019S mutation in the kinase domain increases LRRK2 kinase activity up to 3-fold, whereas mutations in the ROC domain appear to decrease GTPase activity and affect protein dimerization (reviewed in [25]). The G2019S mutation has also been identified in PD patients with no family history of disease, suggesting that it also acts as a risk factor [26]; other LRRK2 variants appear to be important risk factors as suggested by independent genome-wide association studies of sporadic PD [27].

Taken together, all these data support a strong association of LRRK2 dysfunction with PD and suggest that understanding the consequences of LRRK2 mutations represents a unique opportunity for the identification of the molecular pathways leading to PD. But, despite its predominance in PD, the physiological function of LRRK2 is not clear, and therefore its precise role in the aetiology of PD is far from being understood.

**PD and synaptic activity**

Synaptic dysfunctions are emerging as one of the early and major neurobiological events in a number of neurological diseases leading to severe cognitive dysfunction [28]. Accumulating evidence has pointed out how genes involved in PD play a role in the regulation of presynaptic activity. Studies on neurotransmitter release in α-synuclein-KO (knockout) mice have lent support to the hypothesis that α-synuclein plays a pivotal role in the regulation of presynaptic neurotransmitter vesicle pools (reviewed in [29]). Intriguingly, DJ-1-, PINK1- and parkin-KO mice also exhibit presynaptic defects. Specifically, DJ-1-, PINK1- and parkin-KO mice exhibit reduced dopamine overflow and impaired striatal synaptic plasticity [30–32]. Severe neurotransmission defects have been observed in different LRRK2 models [33–35]. The R1441C LRRK2 homozygous knockin mice and the R1441C LRRK2 BAC (bacterial artificial chromosome) transgenic mice display impairments in nigrostriatal dopaminergic innervation and degeneration of the nigrostriatal projections [34,35]. Furthermore, chromaffin cells from R1441C.LRRK2 knockin mice exhibit a reduction in catecholamine release [35]. Interestingly, the G2019S BAC transgenic mice show deficiencies in striatal dopamine release and increased striatal tau immunoreactivity without dopaminergic neuron loss in the SNpc [33,36]. Previous studies have highlighted that LRRK2 may have a direct impact on the secretory and endocytic molecular machinery. Shin et al. [37] showed that overexpression of wild-type, mutant G2019S or kinase-dead LRRK2 (K1906M), as well as siRNA (small interfering RNA) knockdown of LRRK2 modulates SV endocytosis rates in primary rat hippocampal neurons. Similar evidence was reported by Xiong et al. [38], who showed reduced rates of SV endocytosis and exocytosis in hippocampal neurons overexpressing wild-type LRRK2. Finally, it has been suggested that electrophysiological properties as well as proper vesicular trafficking and spatial distribution in the presynaptic pool depend on the presence of LRRK2 as an integral part of the presynaptic protein complex [39].

But what are the molecular links between LRRK2 and the presynaptic environment?

A number of presynaptic proteins described previously as key elements for proper SV trafficking such as NSF (N-ethylmaleimide-sensitive factor), AP-2 (adaptor protein 2) complex subunits, SV2A (synaptic vesicle protein 2A), synapsin 1A, syntaxin 1, dynamin-1, clathrin [39] and Rab5b [37], as well as actin [40], have been found to interact, at least *in vitro*, with LRRK2. But we know that LRRK2 not only is characterized by functional domains allowing protein–protein interactions, such as the N-terminal leucine-rich repeat domain and the C-terminal WD40 domain, but also contains an active kinase domain. Therefore it is tempting to speculate that LRRK2 might affect SV trafficking at different levels according to the functions of its proposed presynaptic
The Figure illustrates four steps of SV trafficking where LRRK2 may play a role: (1) SV motility; (2) priming of SVs to be competent for Ca\(^{2+}\)-evoked exocytosis; (3) disassembling the SNARE complex; and (4) endocytosis/recycling of SV. (1) Actin and synapsin are two proteins involved in the motility of SVs; in particular, synapsin tethers SVs to actin filaments and this function is finely regulated by synapsin phosphorylation/dephosphorylation. LRRK2 kinase activity might modulate the motility of SV acting on these two proteins. (2) LRRK2 interacts with SV2A, which renders SVs competent to Ca\(^{2+}\)-triggered release. Since phosphorylation of SV2A increases its binding to synaptotagmin, a SV integral protein which acts as a Ca\(^{2+}\) sensor, LRRK2 kinase activity may modulate SV Ca\(^{2+}\)-evoked release. (3) NSF is the protein implicated in disassembling the SNARE complex and it has been characterized as an LRRK2 interactor. Hypothetically, LRRK2 could affect the disassembling of the complex by phosphorylating NSF. (4) LRRK2 is implicated in SV recycling as indicated by defects in several LRRK2 models. LRRK2 has been reported to interact with dynamin-1, AP-2 complex, clathrin and Rab5. t-snare, target SNARE; v-snare, vesicle SNARE.

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targets: mobilization and priming of SV, disassembling of SNARE (soluble NSF-attachment protein receptor) complex and recycling of SV (Figure 1).

The mobilization of SVs is finely tuned by phosphorylation/dephosphorylation of synapsin 1A, which regulates the tethering of SVs to actin filaments (reviewed in [41]). Given the proposed interaction among actin, synapsin 1A and LRRK2, LRRK2 may modulate the mobilization of SVs by acting on these two proteins via its kinase activity. Actin phosphorylation inhibits the elongation of the filament in *Dictyostelium* [42]. Hence LRRK2 kinase activity could control the mobilization of SVs by regulating either actin filament stability and/or SV tethering. In support of this hypothesis, silencing LRRK2 in cortical neurons increases the mobility of SVs, suggesting a role for this protein in controlling the trafficking of SVs [39].

Another proposed LRRK2 interactor is SV2A, a protein specific to neurons and endocrine cells involved in Ca\(^{2+}\)-stimulated exocytosis [43]. Interaction of SV2A with synaptotagmin is modulated negatively by Ca\(^{2+}\) and positively by the phosphorylation state of SV2A [44]. One possibility is that LRRK2 phosphorylates SV2A, thus increasing its binding to synaptotagmin and, in turn, favouring SV fusion to the presynaptic membrane. Moreover, LRRK2 interacts with NSF, an AAA (ATPase associated with various cellular activities) protein responsible for the disassembling of the SNARE complex [35]. NSF phosphorylation prevents its interaction with α-SNAP (soluble NSF-attachment protein α) [45], an essential co-factor crucial for NSF-dependent disassembling of the SNARE complex (reviewed in [46]). Thus LRRK2 may regulate the assembly–disassembly equilibrium of SNARE complexes by modulating the NSF phosphorylation state.

LRRK2 has been demonstrated to interact with the AP-2 complex, clathrin and dynamin-1 [39]; all of these proteins are implicated in the first steps of SV endocytosis and are regulated by phosphorylation (reviewed in [47]). Phosphorylation of dynamin-1 reduces the binding with their interacting partners (amphiphysin and clathrin respectively) and impairs the formation of clathrin-coated vesicles [48]. Clathrin light and heavy chains and the AP-2 complex are themselves targets of phosphorylation with putative regulatory effects on membrane binding [49]. Hence LRRK2 might tune endocytosis acting on the phosphorylation state of these interactors. Finally, Rab5, a key regulator of vesicle endocytosis, has been demonstrated to interact with...
Figure 2 | LRRK2, α-synuclein and autophagy

LRRK2 may influence α-synuclein function/dysfunction by acting on two levels: (1) α-synuclein phosphorylation and (2) autophagy. (1) LRRK2 might phosphorylate α-synuclein, regulating its function. This possibility is represented by the broken arrow, because, to date, there has been only one study (by Qing et al. [20]) showing this. Phosphorylated α-synuclein is found in Lewy bodies of PD patients, but the role of this post-translational modification in physiological and pathological conditions is not clear. (2) LRRK2 has been linked to autophagy and α-synuclein is degraded mainly via the autophagic pathway. It has been shown recently that LRRK2 partially co-localizes with LC3, a marker of autophagosomes, which is regulated by phosphorylation. Hence it will be intriguing to determine whether the autophagy pathway would be modulated by LRRK2 acting with LC3. At this point, it is clear that an impairment of this pathway caused by LRRK2 mutations will reduce the ability of the cell to eliminate aggregated α-synuclein, favouring the pathological condition.

LRRK2 [37]. Interestingly, overexpression of Rab5 rescues the endocytosis defects caused by LRRK2 overexpression [37].

Taken together, these findings place LRRK2 as a pivotal regulator of SV recycling/endocytosis and suggest that the presynaptic site represents a pathological target in PD.

There are several indications linking LRRK2 and α-synuclein clearance at the presynaptic site via autophagy (Figure 2). First, it has been shown that overexpression of LRRK2 in HEK (human embryonic kidney)-293T cells induces an increase of α-synuclein expression [50]. One study reported that LRRK2 can directly phosphorylate α-synuclein at Ser129 [20]. Since phosphorylated α-synuclein is a major component of Lewy bodies [51], an intriguing possibility is that pathological LRRK2 kinase activity abnormally increases the levels of phosphorylated α-synuclein with negative outcomes [20]. The same authors also reported that LRRK2 and α-synuclein co-immunoprecipitate both from brain and cellular lysates [52]. However, no additional studies have confirmed these findings, calling for caution in interpreting these data. Recently, a role for LRRK2 in the autophagy process has been observed both in vitro and in LRRK2 genetic models [53–55]. Alegre-Abarrategui et al. [56] have shown that LRRK2 co-localizes with LC3 (light chain 3), a marker of the autophagosomes [56]. The phosphorylation level of LC3 is tightly linked to autophagy efficiency [57]; hypothetically, LRRK2 might modulate autophagic processes acting on the phosphorylation state of LC3. Autophagy has been suggested to be an important route for clearing α-synuclein aggregates [58] and a possibility is that impairment of autophagy as a consequence of mutant LRRK2 aberrant function may result in increased α-synuclein accumulation. Given the role played by α-synuclein at the presynaptic terminus, SV trafficking might result in one of the first mechanisms targeted by α-synuclein aggregation. Taken together, these findings place LRRK2 as a pivotal regulator of SV recycling/endocytosis and suggest that the presynaptic site represents a pathological target in PD.

Conclusions

The link described recently between LRRK2 function and SV trafficking, the intersecting pathways between LRRK2 and α-synuclein [10,50], the impact of α-synuclein on SV recycling [59,60] and finally the functional interplay of DJ-1, parkin, PINK1 and α-synuclein [61,62] point towards a common pathological mechanism: the impairment of SV release from the dopaminergic terminals might constitute one of the main biological pathways compromised during PD onset. Notably, increased dopamine turnover has been observed in presymptomatic mutant LRRK2 carriers [63]. Increased turnover might represent a compensatory mechanism to counteract dopaminergic neuron loss [64], but it has also been suggested that increased dopamine turnover might by itself...
contribute to disease progression secondary to dopamine-associated toxicity [65]. The molecular mechanisms underlying dopamine synaptic transmission defects, however, remain largely unknown. Dopamine release at the nerve terminal is mediated by the SNARE complex-dependent fusion of SVs, and is triggered by Ca\(^{2+}\) binding to synaptotagmins. SVs undergo high-frequency trafficking cycles mediated by the presence of an extremely specialized machinery, which finely controls neurotransmitter release in response to transient increases in intracellular Ca\(^{2+}\) concentration triggered by action potential membrane depolarization. In the last decade, much insight has been gained on the molecular actors orchestrating neurotransmitter release, with several hundreds of proteins described to function at the presynaptic nerve terminal and to participate in exo/endo-cytosis. The process depends on the interaction proteins expressed on SV membranes and proteins expressed on the presynaptic terminal membranes [66]. This complex interactome is plastically modulated by post-translational modifications, particularly by phosphorylation [67] and protein degradation [68]. One intriguing possibility is that LRRK2 modulates SV trafficking via modification of presynaptic proteins.

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