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LRRK-2 as a Key Molecule Bridging Inflammation to Parkinson's Disease

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Abstract. The pathogenetic mechanisms leading to typical Parkinson's Disease (PD), the second most common human neurodegenerative disorder remains unknown. Genetic variants of Leucine-Rich Repeat Kinase 2 (*LRRK-2*) are associated with a significantly enhanced risk for PD. The discovery that late-onset PD could be caused by the inheritance of a mutation in the *LRRK-2* gene leading to familial as well as sporadic forms of PD has provided researchers an opportunity to explore the pathophysiological events underlying this complex disease. Despite extensive research our understanding of *LRRK-2* biological function and regulation remains rudimentary. In this review, we give an insight into the role of *LRRK-2* in modulating inflammation in the central nervous system and we hypothesize that *LRRK-2* dysfunction may favor the neurodegenerative process observed in PD.

Keywords: Neuroinflammation, Parkinson's disease, *LRRK-2*, cytokines

INTRODUCTION ON LRRK-2

Leucine-rich repeat kinase 2 (*LRRK-2*) also known as dardarin is encoded by the PARK8 gene, located in the human chromosome 12 [1, 2]. *LRRK-2* is a 2257 amino-acid protein that belongs to the ROCO protein family, characterized by the following predicted domains: a Roc (Ras of complex proteins) domain with GTPase activity and a COR (C-terminal of Roc) domain. Also, a kinase domain with sequence similarities to RIPKs (receptor-interacting serine/threonine protein kinases) and MLKs (mixed lineage kinases), a subclass of the MAPKKK (mitogen-activated protein kinase kinase kinases) family, is located C-terminal to the COR domain. In addition, *LRRK-2* harbours several protein-protein interaction domains, such as a WD40 domain towards the N-terminal, leucine-rich repeats (LRRs), and an ankyrin domain towards the C-terminal [3–5].

The modular structure of *LRRK-2* suggests a role as a signalling protein, which can act as a kinase, GTPase, and/or scaffolding protein. *LRRK-2* forms dimers, and dimer formation appears to be essential for full catalytic activity [6–9]. *LRRK-2* shows a weak kinase activity *in vitro* mediating autophosphorylation and phosphorylation of generic substrates (i.e., myelin basic protein) or putative substrates, such as members of the ERM protein family (ezrin/radixin/moesin) that has been implicated in neurite outgrowth [10–17]. Increasing evidence suggests that GTPase activity but not the kinase is the main output of *LRRK-2* and that both activities are regulated reciprocally. In this sense, it has been demonstrated that the GTPase activity exerts regulation on the kinase activity that phosphorylates the Roc domain [4, 8, 18–21].

LRRK-2 is broadly detected in many organs, such as brain, heart, kidney, lung, and liver [22–24]. It is also expressed in mononuclear cells as monocytes, and B and T lymphocytes [25–27]. In the brain, *LRRK-2*

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expression is present in the cerebral cortex, medulla, cerebellum, spinal cord, putamen, and substantia nigra. *LRRK-2* protein has been shown to localise not only to neuronal populations in the brain, but also weakly to astrocytes and microglia [24].

Analysis of the subcellular localization of *LRRK-2* using transfected HEK 293 cells, has shown the protein partially associated to the microtubular cytoskeleton (β-tubulin) and inner cellular membranes, i.e. mitochondria, ER and Golgi [11]. *In vitro* studies using COS-1 and SH-SY5Y transfected cells, revealed that *LRRK-2* is associated with plasma membrane lipid rafts which are known to play important roles in cellular functions such as signal transduction, membrane trafficking and cytoskeletal organization [28, 29]. The observed affinity of *LRRK-2* for lipids or lipid-associated proteins and microtubules may suggest a potential role in the biogenesis and/or regulation of vesicular and membranous intracellular structures within the mammalian brain.

Several mutations in *LRRK-2* gene are associated with an increased risk of Parkinson's disease, Crohn's disease (CD), and leprosy [1, 30, 31]. Importantly, immune cell dysregulation plays a pivotal role in the pathogenesis of leprosy in which macrophages are infected by *Mycobacterium Leprae* and in Crohn's disease in which B-cells show upregulated gene transcription through an IFNγ mediated activation pathway [25, 28, 32]. Because all three disorders represent complex diseases with evidence of inflammation, it has been suggested a role for *LRRK-2* in immune cell functions.

LRRK-2 AND PARKINSON'S DISEASE

PD is characterized by the degeneration and death of dopaminergic neurons in the substantia nigra pars compacta (SN) that project into the striatum, where specialized neuronal circuits control body movement. Other pathological hallmarks of the disease are the presence of intracellular alpha-synuclein (α-synuclein) containing aggregates termed Lewy bodies (LB) in surviving neurons [33]; autophagic stress [34, 35]; and mitochondrial dysfunction which drives neurons to energy exhaustion [36].

Despite intensive research the etiology of this neurodegenerative disease still stays unclear. About 10% of cases have clear genetic cause while the rest are of idiopathic origin. A number of risk factors have been identified including age, genetic predisposition, environmental cues, neuronal injury such as traumatic brain injury or stroke, and bacterial or viral infections [37–39]. The major environmental factors implicated in idiopathic PD are pesticides which have been shown to induce oxidative stress leading to increased lipid peroxidation, DNA damage, mitochondrial dysfunction and eventually dopaminergic neuronal dysfunction in the SN [40, 41]. Dopaminergic neurons in the SN are particularly susceptible to oxidative stress as they operate under high oxidant conditions due to their reduced levels of the anti-oxidant glutathione and increased iron content [42–44]. Oxidative stress is a key trigger of microglial activation, which subsequently leads to the generation of reactive oxygen species (ROS) increasing the inflammatory process that underlies the progression of the disease [45].

During the past 15 years, seven genes have been found carrying mutations associated to familial PD: autosomal dominant mutations SNCA, UCHL1, *LRRK-2*, and PARK3; and autosomal recessive mutations PARK2, PARK7, and PINK1 (Table 1).

LRRK-2 is an autosomal dominant gene mutation in families with late-onset Parkinson disease (the most common form of the disorder, which appears after age 50). Clinical and pathological features shown by patients with *LRRK-2* mutations are indistinguishable from idiopathic disease patients [46]. To date, more than 50 disease-associated mutations in *LRRK-2* have been identified in familial or sporadic cases. From these, at least six disease segregating mutations, all of which introduce a change of amino acids, have been identified in *LRRK-2*-linked families including the R1441C/G/H, Y1699C, G2019S and I2020T variants which cluster to the Roc, COR, and kinase domain [47, 48].

Depending on ethnicity and geography, the frequency of *bona fide* mutations in the *LRRK-2* gene can vary considerably. The R1441C/G/H mutation in the ROC domain is a relatively common cause of Parkinson disease in the Basque region, in Europe. Studies of several different populations from around the world revealed the *LRRK-2* gene G2019S mutation in 3 to 7 percent of familial PD cases. The incidence of the G2019S mutation in familial cases is highest among Arabs from eastern North Africa, and central European Jewish ancestry, and it is the lowest in Asian and North European populations. Studies in Chinese and Japanese populations have identified that the *LRRK-2* gene G2385R mutation occurs frequently in PD patients [49, 50]. This particular mutation has also been reported in 1 to 3 percent of sporadic PD cases, in which there is no family history of the disease [51–54].

R1441C/G/H is a mutation in the GTPase domain that causes accumulation of enlarged autophagic vacuoles in human HEK293 cells [55] leading to cellular

Table 1
Features of the genes that contribute to the genetic aetiology of PD

Protein	Gene	Mutation	Abundance	Protein features/function	Impact on PD	Reference
SNCA	PARK1	AR PM, deletion, duplication	rare	Typically found as a soluble protein in the cytoplasm or associated with lipid membranes. The exact biological function of SNCA in brain is still not fully understood, although there is evidence that SNCA may be involved in neurotransmitter release and vesicle turnover at the presynaptic terminals. Found in Lewy bodies	found in 2.5% of unrelated PD affected carriers	[133, 134]
UCHL1	PARK5	AD, PM	rare	*Ubiquitin C-terminal hydrolase* removes the post-translational modification from a substrate protein and regulates cellular processes as protein degradation localization and activation. Mutations on the gene have been associated to aberrant chaperone-mediated autophagy.	Found in familial and sporadic PD	[135]
Parkin	PARK2	AR, PM	50% of AR	E2-dependent *ubiquitin protein ligase*. The loss of parkin activity as consequence of mutations might disturb the ubiquitin-proteasome system, which allows unneeded proteins to accumulate and generates lead to cell death	the most frequent genetic risk factor for early-onset PD	[136]
DJ-1	PARK7	AR, PM, deletion	rare	Function of the protein is still unknown. Accumulating evidence which suggests that is involved in the regulation of mitochondrial dynamics and function in neuronal cells	Associated with early-onset PD	[137]
PINK1	PARK6	AR, PM	rare	*PTEN-induced putative kinase 1* is a protein with a transmembrane domain and a highly conserved serine/threonine kinase domain with homology to the calcium/calmodulin family. Most *PINK1* mutations have been described to impair its kinase activity or reduce the stability of the protein, in line with a loss of function	mutations in PINK1 account for 1–7% of **early onset PD** cases (depending on the ethnicity). The second most common cause of early-onset PD	[138]
LRRK-2	PARK8	AD, PM	10% of AD	Leucine-rich kinase 2 is a large multidomain kinase/GTPase expressed in immune cells, ie. B, T, and dendritic cells; glial cells and neurons. Function of *LRRK2* is not unravelled yet. Evidence suggests a role in neurite complexity, autophagy, mitochondrial	mutations along *LRRK-2* account for the single most common cause of late-onset PD	[46]

AD: Autosomal dominant; AR: Autosomal recessive; PM: point mutation.

degeneration. It has been recently proven that variants located in or adjacent to the GTPase domain, including R1441C, R1441G and Y1699C, elevate the steady-state levels of GTP-bound *LRRK-2* [56].

Both G2019S and I2020T mutations occur in adjacent codons in the conserved activation loop of the kinase catalytic domain, and cause a dominant gain-of function effect that increases the kinase activity *in vitro* [10, 11, 57]. However, they display different pathological phenotypes. The I2020T mutation in the original Sagamihara kindred presents pure nigral degeneration without α-synuclein positive inclusions [58], whereas G2019S mutation exhibits classical IPD pathology which includes selective degeneration of neurons in the SN and Locus coeruleus, accompanied with α-synuclein positive LBs in the brainstem and limbic cortex [59].

G2019S is the most common variant that uniquely contributes to both familial and sporadic PD [53, 54, 60, 61]. Although there is evidence showing that expression of pathological *LRRK-2* mutations is sufficient to cause neurotoxicity [62, 63], transgenic *LRRK-2* mutant mice show little or no obvious degeneration of dopaminergic neurons [64–66]. Additionally, it has been shown that total elimination of *LRRK-2* gene in mice does not lead to loss of dopaminergic neurons [67]. Interestingly, the authors could not detect a difference on the number of TH neurons between control and *LRRK-2* KO mice upon challenge with a dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Thus, the *LRRK-2* protein is dispensable for the development and maintenance of the nigrostriatal pathway [67]. Therefore, suggesting that certain changes in the brain microenvironment in addition to genetic defects may be required to promote the development of PD.

Penetrance of the disease is highly variable and lower than expected for the most prevalent *LRRK-2* mutation (G2019S) (17–33% at age 50, and over 67% between ages 75 to 94) [53, 68, 69]. When the lifetime risk was estimated in populations not ascertain for familial history of the disease, the penetrance dropped to 17–32% [68, 70]. Furthermore, PD patients carrying *LRRK-2* mutations display significant variability of clinical phenotypes and pathological features across and within affected pedigrees [71], suggesting an important role of additional genetic and/or environmental factors in the development of the disease.

Comparison of G2019S carriers from the international *LRRK-2* Consortium with pathologically proven PD patients from the Queen Square Brain Bank showed their phenotype to be that of idiopathic PD, although some motor (disease severity, rate of progression, occurrence of falls and dyskinesia) and non-motor (cognition and olfaction) symptoms suggest that the disease is more benign in *LRRK-2* G2019S carriers than in patients with idiopathic PD [47]. Although, several studies have been performed [64, 66, 72–74] precise role of familial *LRRK-2* mutations *in vivo* related to the pathogenesis of PD remains unclear.

LRRK-2 MECHANISM OF ACTION IN PD: KINASE ACTIVITY, GAIN OR LOSS OF FUNCTION, IMMUNE REGULATION

LRRK-2 is a member of mixed lineage kinase subfamily of mitogen-activated protein kinase kinase kinases (MAPKKKs). *LRRK-2* mutants have been associated with apoptotic cell death when transfected into neuroblastoma SH-SY5Y cells [75, 76]. In line with this, an augmented kinase activity has been described as the cause of neurodegeneration and toxicity, the authors described upregulated mRNA expression of pro-apoptotic Bim and FasL, target genes of phospho-c-Jun^{Ser63}, and formation of active caspase-9, caspase-8 and caspase-3 were also observed in the SN of G2019S *LRRK-2* mice [77].

Common morphological changes in neurites have been associated with for the kinase-active mutant G2019S *LRRK-2*, i.e., neurite shortening due to G2019S *LRRK-2* expression in differentiated SH-SY5Y cells [78, 79] and in cultured neurons derived from G2019S *LRRK-2* transgenic mice [72], as well as markedly reduced neurite complexity of cultured dopaminergic neurons from brains of aged G2019S *LRRK-2* transgenic mice [72].

In a recent study, it was demonstrated the direct interaction of *LRRK-2* with tau, a microtubule-associated protein found predominantly in the central nervous system and expressed mainly in neuronal axons [80]. Tau drives neurite outgrowth by promoting the assembly of microtubules, which is critical for the establishment of neuronal cell polarity [81]. Highly phosphorylated Tau forming a paired helical filament has been frequently found in a number of neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [82]. Also, it has been described, from *in vitro* studies, that *LRRK-2* directly phosphorylates tubulin-associated tau, but not free tau [83]. Thus, *LRRK-2* plays an important role as a physiological regulator for phosphorylation-mediated dissociation of tau from microtubules, which is an integral aspect of

microtubule dynamics involved in neurite outgrowth and axonal transport [83]. Interestingly, PD-associated *LRRK-2* mutations, G2019S and I2020T, have shown elevated degree of tau-phosphorylation suggesting tubulin-associated tau as a direct substrate for *LRRK-2* kinase activity.

While the pathways involved in *LRRK-2* kinase signalling are yet to be fully elucidated, several lines of evidence link *LRRK-2* to MAPK signalling cascades [12, 78, 84–87]. In a recent report, Carballo-Carbajal and col. have shown that PD-associated mutants G2019S and R1441C, induce ERK phosphorylation to the same extent but in a slower manner than wild type *LRRK-2*. Furthermore, induction of the ERK module by *LRRK-2* was associated with a small but significant induction of α-synuclein [88], which has been demonstrated to have a role in both inherited and idiopathic PD as major constituent of LB [89].

Accumulating information links *LRRK-2* to neurotoxicity, cellular stress, cytoskeletal dynamics and vesicular transport. Furthermore, alterations in the activity of kinases acting downstream of mixed linage kinases (MLKs), like MKKs and JNK, have been associated with PD as well as other neurodegenerative diseases [90, 91]. Interestingly, *LRRK-2* variants R1441C, I2020T, and G2019S have been found to phosphorylate mitogen-activated protein kinase kinases (MAPKK), including MKK3 -4, -6 and -7, in a system using HEK293 transfected cells [12]. MKKs act upstream of the MAPK p38 and JNK mediating oxidative cell stress, neurotoxicity and apoptosis. In particular, mutant G2019S of *LRRK-2* has been associated to the phosphorylation of the JNK upstream activators MKK4, and MKK7 at their canonical residues; and to the phosphorylation of a non-canonical site of MKK6, activating the MAP kinase p38 [12]. Thus, MKKs represent a group of potential targets that could link neuronal toxicity of PD-associated *LRRK-2* mutations to a molecular mechanism signalling cellular stress.

IMMUNE REGULATION AND PD

Neurodegeneration in PD is still incompletely understood but involves oxidative stress, mitochondrial dysfunction and impairment of the ubiquitin–proteasome system. Activation of microglia and neuroinflammatory processes are also prominent PD-associated features [92]. Furthermore, data from human PD post-mortem specimens and animal models suggest that the blood–brain barrier becomes impaired at some point during the evolution of this disease [93, 94] allowing the entrance of leukocytes to the cerebral parenchyma which finally locate near dopaminergic neurons [95]. Thus, suggesting a potential contribution of leukocyte infiltration, inflammation and immune-mediated mechanisms to the cascade of events leading to neurodegeneration.

Since the demonstration of the presence of activated microglia in the SNpc of a PD patient (McGeer et al., 1988) abundant clinical and animal studies support the role of activated microglia and increased levels of inflammatory mediators such as cytokines, chemokines and ROS in the pathology of PD [96–109].

Proinflammatory cytokines, such as IL-1, TNFα, and IFN-γ coordinate the action of microglia and PD patients have been found to possess elevated levels of TNFα and IFN-γ in cerebrospinal fluid and postmortem brain tissue [106, 110–114]. Moreover, interleukin-1β (IL-1) and tumor necrosis factor α (TNF) have been implicated as main effectors of neuroinflammation and neurodegeneration in different PD animal models (reviewed by Leal et al. [115] and Roca et al. [116]).

Sustained expression of IL-1β in the SN at pro-inflammatory levels causes irreversible and pronounced dopaminergic neuronal loss in the SN. Long lasting expression of IL-1β achieved by the delivery of an adenoviral vector encoding for IL-1β either directly to the SN [117], or by transducing neuronal axons located in the striatum which retrogradly transport the vector to the neuronal bodies located in the SN [118]. Moreover, progressive neurodegeneration of dopaminergic cells in the SN, motor symptoms and microglial activation, provoked using this paradigm, were attenuated by an anti-inflammatory treatment with dexamethasone [119].

Another well-described mediator of the inflammatory response is TNF-α. Chronic expression of TNF-α in the SN causes progressive neurodegeneration of dopaminergic cells and motor impairments, together with leukocyte infiltration and increased number of activated microglia/macrophages in rats [120]. In the same direction, in a study designed to address the effect of low and chronic TNF-α expression in the SN of adult mice Chertoff and coll. showed a transient neuroprotective effect against 6-OHDA toxicity in the SN and in the striatum when low levels of TNF were expressed, and cell toxicity when high levels of TNF were present. The expression of TNF was controlled by the endogenous engrailed promoter and it was achieved by the transduction of the SN of hypomorphic mice with an adenovirus vector encoding for Cre recombinase [121]. Interestingly, blockade of the soluble form of

the TNF receptor using a dominant-negative TNF, was found to attenuate the death of dopaminergic neurons in 6-hydroxydopamine (6-OHDA)-lesioned rats [122]. Thus, chronic upregulated expression of TNF resulted in a progressive loss of DA neurons accompanied by the recruitment of monocytes/macrophages.

Although, most of the research done leads to the conclusion that IL-1β and TNF-α are toxic for the dopaminergic neurons in the SN, it has been also found neuroprotection exerted by these two cytokines. A number of groups including ours, have found that the final effect of IL-1β and TNF-α on the viability of dopaminergic neurons depend on variables like concentration, duration of the expression and state of activation of neighboring cells [99, 117, 123, 124]. Taken together, these findings suggest that in the clinical scenario co-morbidities present in the patient before the neurodegenerative stimulus starts ie., bacterial or viral infections and immune condition, could be crucial for determining the extent of the PD lesion.

It has been shown that *LRRK-2* is a positive regulator of brain inflammation [125]. Recent experimental findings point to a clear role for *LRRK-2* in the regulation of the immune system during the course of peripheral immune responses. Analysis of *LRRK-2* mRNA expression in different tissues indicates that the highest levels of expression occur in immune cells, particularly in B cells, macrophages, and dendritic cells, with lower levels in T cells [26, 27, 32]. *LRRK-2* has been found in macrophages recruited near pathogens during bacterial infection [26], and upregulated upon exposure to microbial and viral particles [27, 126]. *LRRK-2* deficiency impairs ROS production during phagocytosis in a transfected mouse RAW 264.7 macrophage cell line [32]; and is associated with elevated pro-inflammatory molecules as IL-1-β, IL-6, TNF-α [125, 127].

Moreover, *LRRK-2* expression is significantly induced upon INF-γ stimulation in peripheral blood mononuclear cells [32], and primary microglia from an R1441G transgenic *LRRK-2* mouse [126]. Elevated INF-γ levels are pathological hallmark of CD [128]. INF-γ is a cytokine that coordinates a variety of cellular programs through transcriptional regulation of immunologically relevant genes [129]. Another recent study has also suggested that *LRRK-2* can modulate inflammatory cytokine secretion by promoting cytoplasmic localization of the Nuclear Factor of Activated T-cells (NFAT) transcription factor, through a mechanism that does not involve regulation of NFAT phosphorylation [130].

Toll-like receptors (TLR) signalling induces production of pro-inflammatory cytokines and upregulation of co-stimulatory molecules, which result in the activation of the adaptive immune system [131]. Lastly, *LRRK-2* mRNA has been found up regulated in murine bone marrow derived macrophages upon *in vitro* stimulation with the corresponding ligands for TLR4, TLR7 and TLR9; and down regulated after TLR2 stimulation [27]. A recent report showed activation of the MyD88-dependent pathway by TLR agonists induced phosphorylation of *LRRK-2* at Ser935 in bone marrow derived macrophages, which may lead to the induction of Nuclear Factor-KappaB (NF-kB) dependent genes such as IL-6 and TNF-α [132]. Thus, suggesting that *LRRK-2* may play a role modulating the innate immune signalling.

CONCLUSION

Idiopathic Parkinson's disease (PD) is characterized by a complex interaction between the inherent vulnerability of the nigrostriatal dopaminergic system, potentially increased by a genetic predisposition, and exposure to environmental cues including inflammatory triggers as viral or bacterial infections.

Increasing information on *LRRK-2* mutations suggest that *LRRK-2* neurodegenerative effect on PD is performed through enhancing the inflammatory response. *LRRK-2* has been found associated with a number of pathologic features that coincidentally are strongly associated with diseases in which inflammation has a critical role as CD and leprosy. *LRRK-2* has been found to be upregulated by IFN-γ during bacteria or CD inflammation, and *LRRK-2* is an activator of the NF-κB pathway, a first responder to harmful cellular stimuli. Known inducers of NF-κB activity are highly variable and include ROS, TNF-α, IL-1β, and bacterial lipopolysaccharides, all of them recognized participants of the neuroinfammatory puzzle in PD. This effect can be delivered directly with *LRRK-2* acting on neurons, increasing their sensitivity to pro-inflammatory stimuli or by increasing the expression of neurotoxic inflammatory mediators; or indirectly with *LRRK-2* mediating an exacerbated inflammatory response seen in microglia/macrophages, which in turn exerts a toxic effect on neurons.

A number of cellular defects have been described associated to the different *LRRK-2* mutations described, although the expression of *LRRK-2* found in the nervous system is not as wide as it has been found in the immune system. The pathological mutants seem to induce defects including cytoskeletal organization, neurite shortening, accumulation of LB,

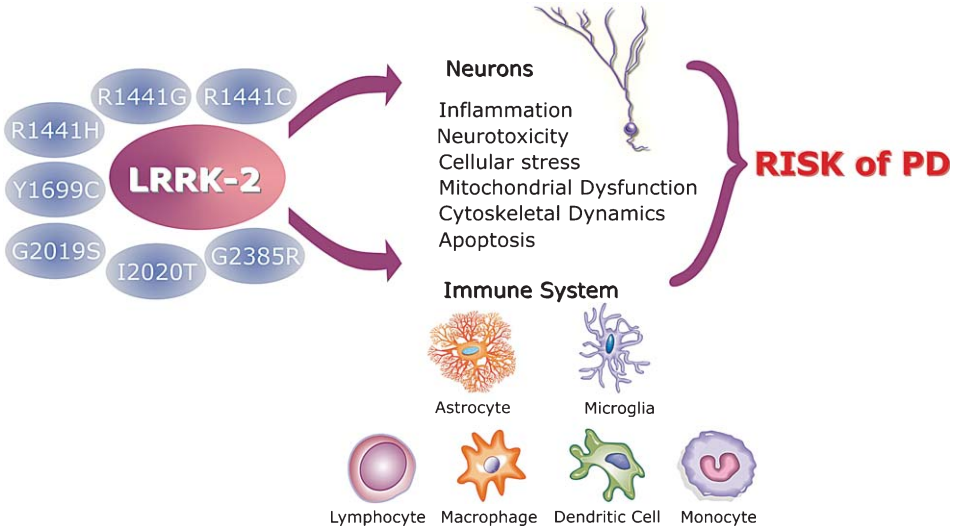

Fig. 1. Schematic illustration depicting the potential role of *LRRK-2* mutations influencing the different components of the CNS and immune system. LRRK-2 mutations (R1441C/G/H, Y1699C, G2385R, G2019S, and I2020T) are found associated with increased risk for PD. Although, the mechanisms are not clear yet, several lines of study include inflammation, neurotoxicity, cellular stress, apoptosis, cytoskeletal and mitochondrial dysfunctions. During neurodegeneration found in PD dopaminergic neurons are the target cells, while members of the immune system, i.e. astrocytes, microglia, lymphocytes, macrophages, dendritic cells and monocytes are thought to be the critical participants.

autophagy and mitochondrial dysfunctions. Still more work is needed to uncover how *LRRK-2* exerts its modulatory action on the central nervous system upon a neurodegenerative insult (Fig. 1).

Clarifying the pathological role of *LRRK-2* in PD will not only allow us to shed light on the mechanisms of toxicity that contribute to neuroinflammation and the clinical decline of patients carrying this debilitating disease, but also supplement the ground for the development of new therapeutic strategies.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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