Modeling Parkinson's disease in LRRK2 mice. Focus on synaptic dysfunction and the autophagy-lysosomal pathway

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Abstract

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are associated with familial and sporadic forms of Parkinson's disease (PD), for which the *LRRK2* locus itself represents a risk factor. Idiopathic and LRRK2-related PD share the main clinical and neuropathological features, thus animals harboring the most common LRRK2 mutations, i.e. G2019S and R1441C/G, have been generated to replicate the parkinsonian phenotype and investigate the underlying pathological mechanisms. Most LRRK2 rodent models, however, fail to show the main neuropathological hallmarks of the disease i.e. the degeneration of dopaminergic neurons in the substantia nigra pars compacta and presence of Lewy bodies or Lewy body-like aggregates of α-synuclein, lacking face validity. Rather, they manifest dysregulation in cellular pathways and functions that confer susceptibility to a variety of parkinsonian toxins/triggers and model the presymptomatic/premotor stages of the disease. Among such susceptibility factors, dysregulation of synaptic activity and proteostasis are evident in LRRK2 mutants. These abnormalities are also manifest in the PD brain and represent key events in the development and progression of the pathology. The present minireview covers recent articles (2018-2021) investigating the role of LRRK2 and LRRK2 mutants in the regulation of synaptic activity and autophagy-lysosomal pathway. These articles confirm a perturbation of synaptic vesicle endocytosis and glutamate release in LRRK2 mutants. Likewise, LRRK2 mutants show a marked impairment of selective forms of autophagy (i.e. mitophagy and chaperone-mediated autophagy) and lysosomal function, with minimal perturbations of nonselective autophagy. Thus, LRRK2 rodents might help understand the contribution of these pathways to PD.

Introduction

Parkinson's disease (PD) is a progressive and multisystemic movement disorder with a complex and incompletely understood etio-pathogenesis, where genetics play an increasingly recognized role (1). In fact, the genetic component accounts for approximately 22% of PD cases, either in the form of

mendelian (incomplete) inheritance or risk of developing the disease (2, 3). Among the strongest genetic contributors, autosomal dominant mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have been associated with 4% of familial and 1% of sporadic forms (4) while genomic variability in the *LRRK2* locus is a risk factor (2, 5). LRRK2 is a large multidomain protein encompassing a kinase and a GTPase domain surrounded by protein-protein interaction motifs (6) (Fig. 1). Most common pathogenic mutations occur in the kinase domain (p.G2019S) and in the GTPase domain (p.R1441C/G/H) (7). All the major pathogenic mutations confer higher kinase activity (8), highlighting the relevance of developing LRRK2 kinase inhibitors as disease modifying agents. Another major genetic determinant of PD is represented by mutations in the *GBA1* gene that encodes for the lysosomal hydrolase β-glucocerebrosidase (GCase) which metabolizes the glycosphingolipid glucosylceramide (GlcCer) into ceramide and glucose (Fig. 1). Indeed, heterozygous mutations of *GBA1* are the most common genetic risk factors of PD (9, 10). Main neuropathological hallmarks of PD are the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNpc) and the formation of Lewy bodies, mainly composed of α-synuclein, in surviving neurons (1). Several are the mechanisms leading to nigro-striatal dopamine (DA) neuron death. Increasing evidence points to synaptic dysfunction and impairment of the autophagy-lysosomal pathway (ALP) as key events in the development and progression of PD (11-15). Alterations in neurotransmission, synaptic vesicle (SV) trafficking, presynaptic autophagy, neurotransmitter release and reuptake, and cytosolic DA handling contribute to the synaptopathy that characterizes the PD brain (for recent reviews see (15-18). Likewise, alterations of autophagy and lysosomal markers have been found in the PD brain (for recent reviews see (19-22). Several LRRK2 models have been generated to mimic PD features (23-25) although disappointingly none proved to fully recapitulate the synaptopathy associated with PD. The present review covers recent articles (2018-2021) dealing with the contribution of LRRK2 models to the comprehension of PD pathogenesis, with a special focus on synaptic dysfunction and ALP dysregulation.

LRRK2 and synaptic dysfunction

Disruption of synaptic homeostasis has been reported after reduction of LRRK2 expression (26-28) or expression of G2019S and R1441G LRRK2 mutants (27, 29-33). One important pathway involved in synaptic homeostasis is SV endocytosis (SVE), a highly regulated process subserving the regeneration of synaptic vesicles from plasma membrane after neurotransmitter release. LRRK2 can modulate SVE via the interaction with numerous SV cycling proteins such as dynamin, EndophilinA

(EndoA), synaptojanin 1 (synj1), Auxilin, and Rab GTPases (i.e. Rab1, Rab5, Rab8, Rab10, Rab12, Rab29, Rab35, Rab43) (15, 34, 35).

The influence of R1441C and G2019S LRRK2 mutations on SVE was studied in induced pluripotent stem cell (iPSC)-derived midbrain dopaminergic neurons using a transcriptomic and proteomic analysis (36). This study revealed that the endocytic and axon guidance pathways were most perturbed following LRRK2 mutations, with significant down-regulation of proteins involved in clathrin-mediated endocytosis such as endophilin I, dynamin 1 and clathrin, that was confirmed in 22-month-old R1441C and G2019S BAC transgenic rats. This is in agreement with the reduction of EndoA levels in iPSC-derived dopaminergic neurons from R1441G PD patients (31), although in contrast with the elevation of clathrin and endophilin levels observed post-mortem in the putamen of G2019S PD patients (36). This discrepancy might rely on the increased synaptic activity occurring in late stage of the disease to compensate for the loss of dopaminergic innervation (36).

Another aspect of LRRK2 biology recently re-examined in SH-SY5Y cells was the impact of LRRK2 G2019S to LRRK2 self-association, spatio-temporal distribution, and hetero-interactions (37). *In vitro* experiments highlighted the greater pathological propensity of G2019S LRRK2 to self-aggregate forming larger and intensely fluctuating clusters at the membrane, which was associated with higher interaction with EndoA1 and disrupted endocytosis. The Authors hypothesized that the altered oligomerization is due to the propensity of G2019S LRRK2 to self-associate combined with the inability to dissociate from cell membranes (37), and this might follow decreased GTPase activity conferred by the mutation (38-40).

In recent years, an increasing number of studies have investigated the function of LRRK2 in the development and homeostasis of excitatory synaptic networks (41-43). LRRK2 is associated with SVs (44) and interacts with SVE proteins that regulate neurotransmitter release, SV docking, fusion and recycling, such as Synapsin I (45) and N-ethylmaleimide sensitive fusion (NSF) (46). The WD40 domain was found to be essential for LRRK2 binding to Synapsin I and for the LRRK2-mediated Thr^{337} and Thr^{339} phosphorylation of Synapsin I central C domain (47). By measuring K^+-evoked $[^3H]$-D-Aspartate release from cortical synaptosomes and excitatory postsynaptic currents in cortical neurons of wild-type (WT) and G2019S BAC transgenic mice, and inhibitors of LRRK2 kinase activity (PF-06447475, IN-1 and GSK2578215A), these Authors showed that LRRK2 facilitates SV exocytosis and glutamatergic transmission through phosphorylation of Synapsin I, and G2019S further amplifies this phenomenon. This results in a reduced association of Synapsin I with SVs and actin filaments, promoting SV mobility from the reserve pool to the rapid releasable pool (47). A more recent study (48) demonstrated that the physiological activity of N-terminal domain of LRRK2 is required for SV dynamics, particularly for the fusion events and the interaction with SV proteins.

The E193K variant within the Armadillo domain induces structural modifications that change LRRK2 subcellular distribution, impair 14-3-3 binding and influence LRRK2 binding properties (48).
Another study in K^+-stimulated striatal and cortical synaptosomes from G2019S Knock-in (KI), LRRK2 Knock-out (KO) and kinase dead (KD) mice challenged with LRRK2 inhibitors IN-1 and GSK2578215A showed that presynaptic LRRK2 inhibits [^{3}H]-DA and facilitates endogenous glutamate (Glu) release (49). In the attempt to temporally correlate the effects on neurotransmitter release with inhibition of presynaptic LRRK2, a reduction of phosphoSerine935 (pSer935) LRRK2 levels (an indirect readout of kinase activity (50, 51) at 3 min but not 12 min after LRRK2 inhibitor application was observed, confirming that LRRK2 dephosphorylation is an early event. This analysis also revealed an unexpected reduction of endogenous LRRK2 levels in striatum but not cerebral cortex of G2019S KI mice (49). Increased levels of the excitatory transmitter aspartate was also observed in cerebral organoids derived from human iPSCs from a G2019S PD donor (52). This was associated with a reduction of DA levels in the medium, in keeping with the reduction of DA release observed with fast scan cyclic voltammetry (FSCV) from striatal slices of G2019S KI mice (53).
One important interactor of LRRK2 is Protein-Kinase A (PKA), a holoenzyme that regulates signaling pathways critical for neuron development and function. The specificity, efficacy, and distribution of PKA is regulated by the interaction between PKA regulatory subunits and A-Kinase Anchoring Proteins (AKAPs). It has been reported that LRRK2 acts as an AKAP-like regulator of the subcellular distribution of PKA in neurons, negatively modulating its activity in striatal projection neurons (SPNs) during synaptogenesis and in response to DA receptor activation (54). The same group of Authors (55) analyzed the impact of R1441C and G2019S mutations on direct and indirect pathway SPNs by crossing R1441C and G2019S KI mice with Drd1-Tomato and Drd2-eGFP reporter mice. Increased synaptic PKA activity, GluA1 receptor subunit phosphorylation and synaptic incorporation were found selectively in direct pathway SPNs (dSPNs) of mice carrying the R1441C mutation. Surprisingly, however, these changes translated into a reduction in mean frequency of miniature excitatory postsynaptic currents (mEPSCs) but no changes in mean EPSC amplitude, although a finer functional analysis revealed a fraction of excitatory synapses with stronger activity in dSPNs, offering a new mechanism through which mutant LRRK2 affects basal ganglia circuitry in PD (55).
To add to these studies, electrophysiological patch-clamp recordings of striatal MSNs in 6-month-old G2019S KI, LRRK2 KO, LRRK2 KD and WT mice revealed that stimulation of DA D2 receptors by quinpirole reduced the spontaneous and evoked EPSCs selectively in G2019S KI mice, in the absence of differences in electrical membrane properties and synaptic glutamatergic transmission across genotypes (56). This inhibitory effect was linked to D2 receptor coupling with the phospholipase C

(PLC) pathway, leading to retrograde release of endocannabinoids (eCB), presynaptic CB1 receptor activation and reduction of Glu release. These data suggest that the G2019S mutation upregulates the D2 control over the PLC/eCB pathway in striatal SPNs, reverberating on the glutamatergic synapse (56). The same group also reported an increased sensitivity of striatal SPNs of G2019S KI mice to the complex I inhibitor rotenone possibly due to dysfunctional mitochondrial Ca^{2+} handling and elevated PKA activity (53). Interestingly, this effect was rescued by D2 receptor stimulation with possible implication for selective D2 agonist-based neuroprotective therapies in G2019S PD patients (53). Consistently, an *in vivo* study showed greater dopaminergic nigro-striatal degeneration in G2019S KI mice exposed to the complex I inhibitor MPTP, an effect reversed by LRRK2 kinase inhibitors MLi-2 and PF-06447475 (57).

LRRK2 and ALP

ALP is a complex and finely tuned process subserving proteostasis in cells. Main ALP subtypes are macroautophagy, chaperone-mediated autophagy (CMA) and microautophagy (20). *In vitro* studies failed to unequivocally prove whether LRRK2 inhibits or promotes macroautophagy (for recent reviews see (20, 58). Recent *in vivo* studies reported no major changes in macroautophagy markers in the cerebral cortex (59), striatum (60) or nigral dopaminergic neurons and cortical microglia (61) of G2019S KI mice, even at late ages (18-20 months). Despite in G2019S KI mice a reduction in key autophagy gene transcripts was observed (60), G2019S LRRK2 does not appear to have a major impact on autophagy (see also a recent *in vitro* study on fibroblasts of G2019S PD patients (62). Silencing of LRRK2 kinase activity, instead, was associated with an age-dependent impairment of the autophagic flux (60). This was inferred by the elevation of LC3-II and p62 levels and LC3B puncta in the striatum of 12-month-old LRRK2 KD mice and by the lack of further increase of LC3-II/LC3-I ratio after 3-day treatment with the lysosomotropic agent chloroquine that, instead, caused such expected elevation in WT mice (63). In contrast, 22-month-old hG2019S and hR1441C BAC transgenic rats showed an increase of LC3B puncta in SNpc dopaminergic neurons, which was interpreted as a consequence of macroautophagy impairment (64). The high level of transgene expression (3-5-fold over endogenous LRRK2) and the different cell-type examined (nigral dopaminergic neurons) might explain the discrepancy. Indeed, the effect of G2019S mutation on macroautophagy might be cell-type dependent (64).

Despite lack of evidence linking G2019S to nonselective autophagy, growing evidence support the view that G2019S impairs mitophagy (65), a specialized form of autophagy involved in the turnover of old, dysfunctional and damaged mitochondria (Fig. 1). Mitophagy impairment was observed in neuroepithelial stem cells (66) and fibroblasts (62, 67, 68) obtained from G2019S and/or R1441G PD

patients, which was rescued by LRRK2 kinase inhibitors (62, 67, 68). Although a recent study in G2019S PD fibroblasts drew the opposite conclusion (69), kinase-dependent defective mitophagy was reported in mouse embryonal fibroblasts (MEFs), nigral dopaminergic neurons, microglia, and peripheral organs (lung and kidney) from G2019S KI mice (61). Accumulation of ubiquitinated mitochondria and defective mitophagy were also observed in the striatum of 24-month-old R1441G KI mice (70) and fibroblasts from R1441G PD patients (62).

Limited evidence that LRRK2 mutation disrupts another form of specialized autophagy, i.e. chaperone-mediated autophagy (CMA), has been presented (71). CMA removes proteins carrying the KFERQ pentapeptide motif recognized by the Hsc70 chaperone. R1441G KI mice develop CMA dysfunction, accompanied by higher levels of soluble and amyloid-like synuclein oligomers. Lysosomal dysfunction was confirmed by the perinuclear redistribution of lysosomes in striatal neurons and by the increased levels of LAMP2A, Hsc70 and the CMA substrate GAPDH in the membrane fraction of striatal lysates of 18-month-old R1441G KI mice (71). These data add to a growing body of evidence that aberrant lysosomal morphology and function is a feature of LRRK2-related PD and LRRK2 models, and that LRRK2 contributes to preserve normal lysosomal function (for recent reviews see (34, 72).

In keeping with the view that removal of LRRK2 improves lysosomal function, an age-dependent increase in lysosomal proteases was revealed by proteomic screen of kidneys of LRRK2 KO mice (73). Here, an increase colocalization of cathepsin D and its mannose-6-phosphate receptor was found, possibly suggesting an increase trafficking to lysosome (73). Consistently, proteomic screens revealed an increase of the glycosylated form of the late-endosomal/lysosomal marker LAMP1 in the kidney of LRRK2 KO mice (8). Instead, G2019S KI mice showed elevation of the non-glycosylated form of LAMP1 that was normalized by chronic in-diet administration of MLi-2, suggesting LRRK2 kinase-dependent inhibition of lysosomal function (8). In both proteomic studies (8, 73), however, no changes in lysosomal protein patterns were observed in the brain. Nonetheless, cortical neurons obtained from LRRK2 KO mice showed an increase of basal LAMP1 staining along with resistance to pre-formed fibril (PFF)-induced α-synuclein pathology, again supporting the view that LRRK2 deletion improves lysosomal function (74). In fact, cortical neurons from G2019S KI mice displayed increased lysosome counts, aberrant lysosomal morphology and lysosomal alkalinization associated with accumulation of insoluble α-synuclein and α-synuclein release, that were rescued by LRRK2 inhibitors (59) (Fig. 1). Higher lysosomal pH and dysregulated lysosomal calcium dynamics were also found in primary cortical neurons from R1441C transgenic mice due to the disruption of LRRK2 binding to the subunit a1 of the vacuolar ATPase (vATPase) proton pump (64). LRRK2 regulates transcription factor EB (TFEB) activation, thus autophagy induction, in immune cells modulating

calcium dynamics. In fact, B-lymphocytes from G2019S KI mice exhibited increased TFEB activity due to an exaggerated NAADP-dependent calcium signal (75). Likewise, G2019S recombinant cell lines showed impaired lysosomal proteolytic activity paralleled by an accumulation of endogenous α-synuclein, that were ameliorated by LRRK2 kinase inhibition (76). An impairment of lysosomal proteolytic activity associated with reduced endolysosome number/size and α-synuclein internalization/degradation was reported in striatal astrocytes from G2019S KI mice (77). Consistently, overexpression of G2019S LRRK2 in HEK293T cells represses the activity of Cathepsin B and L (78, 79) independent of LRRK2 kinase activity (78). Finally, downregulation of the lysosomal *LAMP2* gene was reported in the striatum of 12-month-old G2019S KI mice, suggesting that the increased LRRK2 kinase activity affects lysosomal homeostasis also at transcriptional level (60).

Upon lysosomal damage, LRRK2 is recruited on the lysosomal membrane along with and a number of interacting Rabs which, then, become phosphorylated (72). If the insult is limited, like short exposure to the lysosomotropic agent L-leucyl-l-leucine methyl ester (LLOME), LRRK2 recruits and phosphorylates Rab8a that, in turn, participates in repairing the damaged membrane via endosomal sorting complex required for transport (ESCRT) intervention (80). If the insult is more severe, Galectin 3 (Gal3) mediates the ubiquitination of different lysosomal proteins initiating lysophagy by the recruitment of Unc-51 like autophagy activating kinase 1 (ULK1) and autophagy related 16 like 1 (ATG16L1) to the lysosomal membrane (81). Consistently, Chloroquine (CQ) treatment induces lysosomal stress in HEK293T and RAW264.7 cells, promoting the Rab29-mediated recruitment of LRRK2 at the stressed lysosomal membrane (82). Upon translocation, LRRK2 phosphorylates Rab10 and Rab8a, which in turn recruit their effectors (EHBP1 and EHBP1L1) to mediate lysosomal exocytosis and release of non-degraded cargo in the extracellular matrix (82). However, the mechanisms of action of LLOME and CQ are different, and changes in lysosomal pH is not sufficient to induce LRRK2 translocation to the lysosomes, as suggested by the lack of effects on LRRK2 translocation upon Bafilomycin A treatment *in vitro* (82).

LRRK2 phosphorylation of Rab10 and Rab35 at the lysosomal membrane is required to recruit the motor adaptor protein Jip4 (JNK-interacting protein 4). This protein binds to motor proteins (such as kinesins and dyneins) that mediate lysosomal tubulation sorting (83). Consistently, the recruitment of Jip4 to the autophagosomes was increased in cortical neurons from G2019S KI mice, leading to an increased kinesin activity and disruption of transport of autophagic vacuoles (AVs) (84). The fusion with lysosomes and, hence, acidification of AVs was impaired, an effect rescued by genetic and pharmacological inhibition of LRRK2 kinase activity (84). Interestingly, kidney Jip4 levels were reduced in LRRK2 KO mice or G2019S KI mice treated with MLi-2 (8). Consistent with LRRK2

kinase-dependent mistrafficking of lysosomal proteins in LRRK2 mutants, LRRK2 regulation of retrograde and anterograde transport by scaffolding Golgi-associated retrograde protein (GARP) complex at the *trans*-Golgi network (TGN) was proven in G2019S models (85). As a result, Cathepsin D transport from the TGN to lysosomes was disrupted by LRRK2 mutations and reversed by LRRK2 kinase inhibitors (85).

Quite a few new studies (Tab. 1, Fig. 1) also focused on the role of LRRK2 in the regulation of GCase activity. LRRK2 inhibits GCase activity (for a recent review see (86), as also confirmed by a recent study in LRRK2 KO and KD mice (60). In fact, in line with that observed in LRRK2 KO astrocytes (87), striatal GCase activity was enhanced in LRRK2 KO mice at 12 months and in LRRK2 KD mice already from 3 months of age onwards (60). This points to a crucial role of LRRK2 kinase activity (although only a trend to reduction was observed in G2019S KI mice) (60). In fact, LRRK2 kinase-dependent reduction of GCase activity was observed in iPSCs and fibroblasts obtained from G2019S and R1441G/C PD patients (31, 88, 89). Consistently, MLi-2 reversed GCase enzymatic deficit and attenuated the accompanying PD-like phenotype, i.e. accumulation of oxidized dopamine and pSer129-α-synuclein (88). MLi-2 also rescued some lysosomal deficits in *D409V GBA1* astrocytes and *GBA1* heterozygous (HET)-null human iPSCs differentiated to induced cortical neurons (iNs) without however affecting their ability to degrade α-synuclein (90, 91).

LRRK2-mediated phosphorylation of auxilin has been hypothesized to underlie the reduction of GCase activity associated with PD-like features. In fact, restoring physiological levels of phosphorylated auxilin in patient-derived R1441G LRRK2 dopaminergic neurons, rescued the impaired GCase function and PD-like phenotype (31). Interestingly, similar therapeutic rescue was achieved treating G2019S iPSC-derived dopaminergic neurons with the antipsychotic quetiapine (92). Different from what reported by (88) in iPSC-derived dopaminergic neurons and patient fibroblasts, no reduction of GCase activity was observed in the striatum of G2019S KI mice at 3, 6 or 12 months (60, 93). Actually, after normalization of GCase levels, lysosomal GCase activity resulted to be elevated in peripheral blood mononuclear cells (PBMCs), plasma, fibroblasts and iPSC-derived neurons expressing G2019S LRRK2 (93), which is in line with that found in dried blood spots from G2019S PD patients (94) but in contrast with studies on human and animal samples (60, 87, 88).

Concluding remarks

Research into the physiological functions of LRRK2 and their alterations in PD has advanced at remarkable velocity in the last decade. In this review, we focused on the most recent developments in the processes of synaptic transmission and autophagy. These are linked to the presymptomatic

stage and the full-blown, late neuropathological picture of the disease, respectively. Importantly, these critical neuronal functions have been very recently linked, with autophagy occurring locally at the presynaptic terminal and regulating neurotransmission (95, 96). Indeed, they also share common molecular machinery, such as Synaptojanin1 and EndophilinA (96). Of note, the latter is regulated by LRRK2 (97), which thus appears to be well located at the nexus of these functions and could represent a molecular link in the pathogenesis of PD. Consistently, synaptic/axonal dysfunction is thought to be causative of neuronal demise (12).

LRRK2 kinase inhibitors are currently in clinical trials in both familial (LRRK2-related) and idiopathic PD (98, 99). They have been reported to modulate synaptic and autophagic alterations in LRRK2 models *in vitro* and *in vivo* (18, 61, 76, 100, 101) and to reverse neuropathological changes in models of PD (57, 102-105), albeit not consistently (106-109). Therefore, these prospective therapeutics might provide disease modification, if administered early enough in the course of the disease, modulating neuronal physiology in two of its most critical and interconnected aspects. Elucidating the temporal relationship between these dysfunctions, and identifying suitable biomarkers or human imaging correlates, could also open novel possibilities for early diagnosis.

Perspectives

- Rodents harboring pathogenic mutations of the LRRK2 gene show dysregulation of synaptic activity and autophagy-lysosomal pathway, that are key events in the pathogenesis of Parkinson's disease. LRRK2 rodents model the presymptomatic/premotor stages of the disease showing increased susceptibility to parkinsonian toxins and triggers.
- LRRK2 mutations perturb synaptic vesicle trafficking, synaptic dopamine handling and neurotransmitter release through the interaction with key synaptic proteins such as synapsin I and NSF. Likewise, LRRK2 mutants inhibit forms of selective autophagy, such as mitophagy and chaperone-mediated autophagy, and lysosomal function, with less or indirect impact on nonselective macroautophagy.
- Identifying novel LRRK2 interactors along the endolysosome and autophagy-lysosomal pathways might provide novel druggable targets in the therapy of Parkinson's disease. LRRK2 kinase inhibitors might rescue synaptic and autophagy dysfunction and slow/stop the degeneration associated with Parkinson's disease.

Authors contributions

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Conflicts of interests
The Authors declare no conflict of interest

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Abbreviations

AKAP	A-kinase anchoring protein
ALP	autophagy-lysosomal pathway
ATG16L1	autophagy related 16 like 1
AVs	autophagic vacuoles
BAC	bacterial artificial chromosome
CMA	chaperone-mediated autophagy
CQ	chloroquine
dSPNs	direct pathway striatal projection neurons
DA	dopamine
eCBs	endocannabinoids
EndoA	endophilin A
EPSCs	evoked excitatory postsynaptic currents
ESCRT	endosomal sorting complex required for transport
Gal3	galectin 3
GCase	glucocerebrosidase
GARP	Golgi-associated retrograde protein
GlcCer	glucosylceramide
Glu	glutamate
HET	heterozygous
IHC	immunohistochemistry
iPSC	induced pluripotent stem cell
Jip4	JNK-interacting protein 4
KI	knock-in
KO	knock-out
KD	kinase dead
LC-MS	liquid chromatography-mass spectrometry
LLOME	l-leucyl-l-leucine methyl ester
LRRK2	leucine-rich repeated kinase 2
mEPSCs	miniature evoked excitatory postsynaptic currents
MEFs	mouse embryonal fibroblasts
4-MU	4- methylumbelliferyl-β-D-glucopyranoside
NSF	N-ethylmaleimide sensitive fusion
PBMCs	peripheral blood mononuclear cells
PD	Parkinson’s disease
PFB-FDGlu	5-(Pentafluorobenzoylamino)Fluorescein Di-β-D-Glucopyranoside

PFFs	pre-formed fibrils
PKA	protein-kinase A
PLC	phospholipase C
RT-qPCR	real time-quantitative polymerase chain reaction
SNpc	substantia nigra *pars compacta*
SPNs	striatal projection neurons
SV	synaptic vesicle
SVE	synaptic vesicle endocytosis
Synj1	synaptojanin 1
TEM	transmission electron microscope
TNG	*trans*-Golgi network
ULK1	Unc-51 Like Autophagy Activating Kinase 1
WB	Western blotting
WT	wild-type

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Figure legend

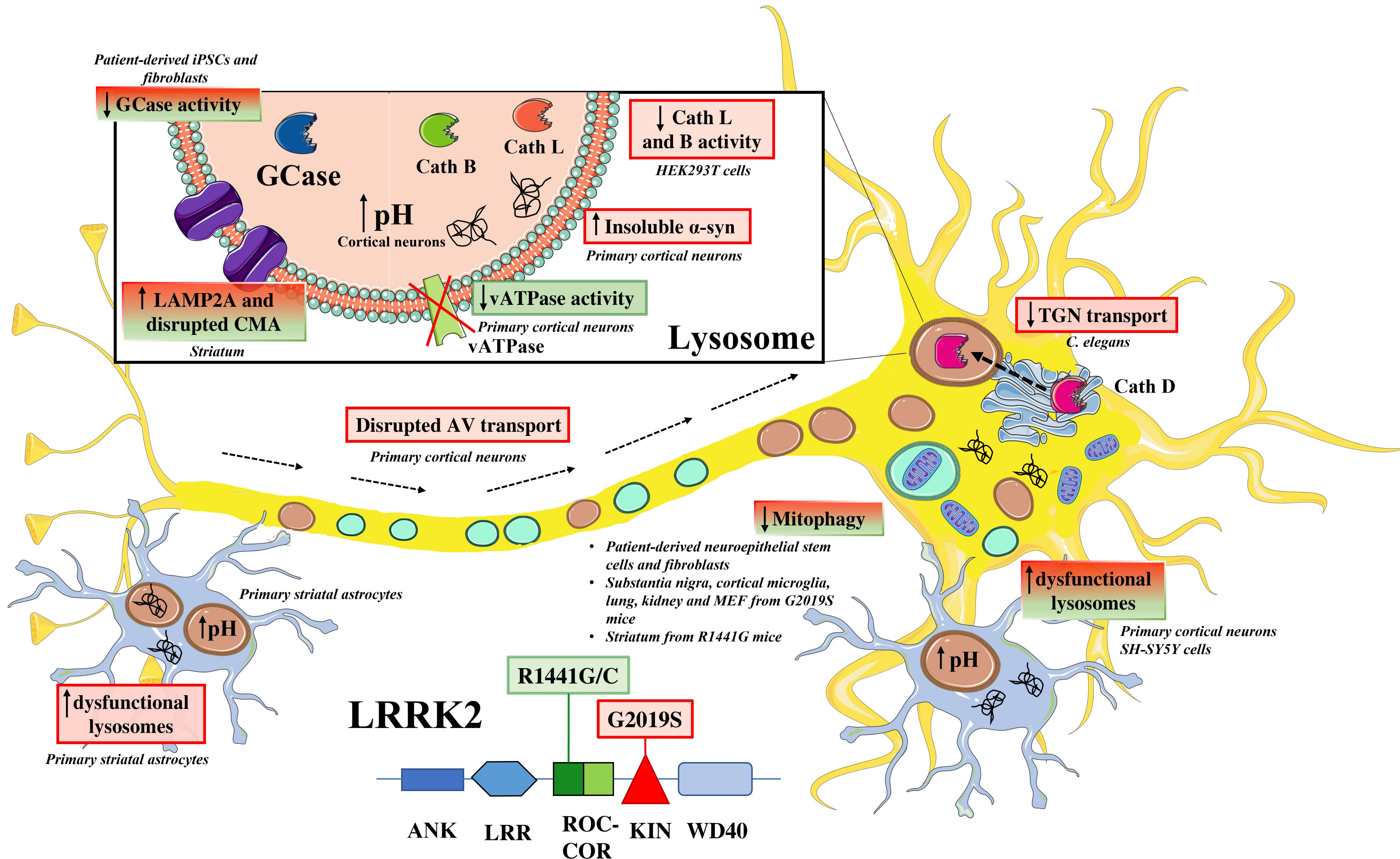
Fig. 1 Impact of LRRK2 pathogenic mutations on forms of selective autophagy and lysosomal homeostasis in neurons and astrocytes. LRRK2 structure with domains (ANK, LRR, ROC, COR, KIN, and WD40) and most frequent and studied pathogenic mutations, i.e. G2019S and R1441G/C. The effects reported to be associated with the G2019S and the R1441G/C mutations are shown in red and green boxes, respectively, whereas those linked to both mutations are represented in boxes with both colors. α-synuclein (α-syn), autophagic vacuoles (AV), Cathepsin B (Cath B), Cathepsin L (Cath L), Cathepsin D (Cath D), Chaperone-mediated autophagy (CMA), Glucocerebrosidase (GCase), vacuolar ATPase (vATPase).

Tab.1 Studies focused on the interaction between LRRK2 and GCase in preclinical models

	Models	Area	Methods	Readout	LRRK2 inhibition
(87)	Male LRRK2 KO mice (12 mos)	Whole brain	LC-MS	↓ Ceramide/total lipids ↓ SM/total lipids ↓ Sulfatides/total lipids ↑ Gly-Cer/total lipids	
			WB	↓ GCase levels	
			4-MU assay	↑ GCase activity*	
(31)	iPSC-derived DA neurons from G2019S, R1441C/G PD patients		4-MU assay	↓ GCase activity	
(102)	Male Lewis rats (8-10 mos) treated with subacute rotenone	Substantia Nigra	Fluorescence IHC	↓ lysosomal GCase puncta	Rescued by PF-360 (10mg/Kg p.o. b.i.d.)
			4-MU assay	↓ GCase activity	
(88)	iPSC-derived DA neurons from G2019S, R1441C/G PD patients		PFB-FDGlu assay	↓ GCase activity	MLi-2, LRRK2-IN-1: - ↑ basal GCase activity in WT iPSC - rescue GCase activity in LRRK2 mutants
	Fibroblasts from G2019S PD patients				
(90)	Astrocytes from GBA1 D409V KI mice		High-content analysis	↓ lysosomes/cell	MLi-2 rescues only lysosomal pH
			LysoSensor assay	↑ lysosomal pH	
			4-MU assay	(↓) GCase activity	
(91)	GBA1 HET-null human iPSC		WB	↓ GCase levels	
			4-MU assay	↓ GCase activity#	
	GBA1 HET-null human iPSC differentiated to iNs		RT-qPCR	↓ GBA1 mRNA	MLi-2 only and partially rescues lysosomes/cell and lysosomal pH
			WB	↓ GCase levels	
			High-content analysis	↓ lysosomes/cell	
			LysoSensor assay	↑ lysosomal pH	
(60)	LRRK2 KD mice (3-12 mos)	Striatum	4-MU assay	↑ GCase activity	
	LRRK2 KO mice (12 mos)			↑ GCase activity	
	G2019S KI mice (3-12 mos)			No change	
	LRRK2 KD mice (12 mos)		RT-qPCR	No change	
	LRRK2 KO mice (12 mos)			No change	
	G2019S KI mice (12 mos)			(↓) GBA1 mRNA	

(93)	Male G2019S KI mice (6 mos)	Midbrain	WB	↓ GCase levels	
			4-MU assay	↑ GCase activity*	
	PBMCs or plasma from G2019S PD patients		4-MU or BCA assay	↑ GCase activity	
	Fibroblasts from G2019S PD patients		TEM	↑ electron dense or lamellar structures	
			WB	(↓) GCase levels	
			4-MU assay	(↑) GCase activity*	
	iPSC-derived DA neurons from G2019S PD patients		4-MU assay	↑ GCase activity	
	G2019S HEK293 cells treated with MLi-2		WB	(↑) GCase levels	
			4-MU assay	↓ GCase activity*	
	G2019S HEK293 cells	Lysosomal fraction	4-MU assay	↑ GCase activity*	Rescued by LRRK2 KD mutant
	LRRK2 KO RAW 264.7 macrophages	Lysosomal fraction	WB	↓ GCase levels	
			4-MU assay	↓ GCase activity*	
			PFB-FDGlu	(↑) GCase activity	
(92)	Quetiapine-treated iPSC-derived DA neurons from G2019S PD patients		4-MU assay	↑ GCase activity	
			WB	↑ GCase levels	
	HET GBA1 D409V KI mice (17-19 mos) treated with subacute Quetiapine	Hippocampus	4-MU assay	↑ GCase activity	

(↓) (↑) indicates a tendency
* = normalized to GCase levels
# = normalized to whole cell lysates
LC-MS = liquid chromatography-mass spectrometry
4-MU = 4- methylumbelliferyl-β-D-glucopyranoside
PFB-FDGlu = 5-(Pentafluorobenzoylamino)Fluorescein Di-β-D-Glucopyranoside
WB = western blotting
IHC = immunohistochemistry
RT-qPCR = real time-quantitative polymerase chain reaction
TEM = transmission electron microscope


Patient-derived iPSCs and fibroblasts
↓GCase activity
GCase
Cath B
Cath L
↓Cath L and B activity
HEK293T cells
↑pH
Cortical neurons
↑Insoluble α-syn
Primary cortical neurons
↑LAMP2A and disrupted CMA
Striatum
↓vATPase activity
Primary cortical neurons
vATPase
Lysosome
↓TGN transport
C. elegans
Cath D
Disrupted AV transport
Primary cortical neurons
↓Mitophagy
• Patient-derived neuroepithelial stem cells and fibroblasts
• Substantia nigra, cortical microglia, lung, kidney and MEF from G2019S mice
• Striatum from R1441G mice
Primary striatal astrocytes
↑pH
↑dysfunctional lysosomes
Primary striatal astrocytes
↑dysfunctional lysosomes
Primary cortical neurons
SH-SY5Y cells
↑pH
LRRK2
R1441G/C
G2019S
ANK
LRR
ROC-COR
KIN
WD40