university of groningen

University Medical Center Groningen

University of Groningen

The multifaceted role of LRRK2 in Parkinson's disease

Oun, Asmaa; Sabogal-Guaqueta, Angelica Maria; Galuh, Sekar; Alexander, Anastasia; Kortholt, Arjan; Dolga, Amalia M

Published in:
Neurobiology of Disease

DOI:
10.1016/j.nbd.2022.105837

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Oun, A., Sabogal-Guaqueta, A. M., Galuh, S., Alexander, A., Kortholt, A., & Dolga, A. M. (2022). The multifaceted role of LRRK2 in Parkinson's disease: From human iPSC to organoids. *Neurobiology of Disease*, *173*, [105837]. https://doi.org/10.1016/j.nbd.2022.105837

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

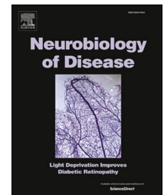
Download date: 20-11-2022

Neurobiology of Disease 173 (2022) 105837

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

The multifaceted role of LRRK2 in Parkinson's disease: From human iPSC to organoids

Asmaa Oun [a,b,c], Angelica Maria Sabogal-Guaqueta [a], Sekar Galuh [a], Anastasia Alexander [a], Arjan Kortholt [b,d,*], Amalia M. Dolga [a,**]

[a] Department of Molecular Pharmacology, Faculty of Science and Engineering, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Groningen, the Netherlands
[b] Department of Cell Biochemistry, Groningen Institute of Biomolecular Sciences & Biotechnology (GBB), University of Groningen, Groningen, the Netherlands
[c] Department of Biotechnology, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt
[d] YETEM-Innovative Technologies Application and Research Centre Suleyman Demirel University, Isparta, Turkey

ARTICLE INFO

Keywords:
Parkinson's disease
LRRK2
Mitochondria
Inflammation
iPSC

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting elderly people. Pathogenic mutations in Leucine-Rich Repeat Kinase 2 (*LRRK2*) are the most common cause of autosomal dominant PD. LRRK2 activity is enhanced in both familial and idiopathic PD, thereby studies on LRRK2-related PD research are essential for understanding PD pathology. Finding an appropriate model to mimic PD pathology is crucial for revealing the molecular mechanisms underlying disease progression, and aiding drug discovery. In the last few years, the use of human-induced pluripotent stem cells (hiPSCs) grew exponentially, especially in studying neurodegenerative diseases like PD, where working with brain neurons and glial cells was mainly possible using postmortem samples. In this review, we will discuss the use of hiPSCs as a model for PD pathology and research on the LRRK2 function in both neuronal and immune cells, together with reviewing the recent advances in 3D organoid models and microfluidics.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNPc) (Lees et al., 2009). The prevalence of the disease increases sharply with age (Hirsch et al., 2016). Although most PD cases are idiopathic, particular genes are linked to familial PD among which Leucine-Rich Repeat Kinase 2 (*LRRK2*) gene is involved in autosomal dominant PD (Zimprich et al., 2004). LRRK2 is a large multidomain protein with enzymatic, i.e., kinase and GTPase (ROC-COR domains), and protein-protein interacting domains (LRR, ARM, ANK, and WD40) (Mata et al., 2006). In humans, LRRK2 is highly expressed in the lungs, peripheral immune cells, kidneys, and the central nervous system (CNS). LRRK2 proteins are involved in several cellular processes, including autophagy, cytoskeleton dynamics, inflammation, innate immunity, and mitochondrial function (Wallings et al., 2015).

LRRK2 carries multiple pathogenic mutations that reside mainly in the kinase or GTPase domains, with G2019S, I2020T, R1441G and R1441C being the most characterized, and all were shown to result in enhanced kinase activity of LRRK2 proteins (Lis et al., 2018; Shu et al., 2016). Interestingly, the increase in kinase activity of LRRK2 is also a shared feature in idiopathic PD (Di Maio et al., 2018), making LRRK2 of crucial importance to understand PD pathology. Recent studies have shown that human-induced pluripotent stem cells (hiPSCs) from patients carrying *LRRK2* genetic mutations and variant risk alleles can be used as a model for both familial and sporadic PD (Marrone et al., 2018; Tran et al., 2020).

Within the last two decades, the majority of LRRK2 research was performed using immortalized cell lines and/or transgenic PD animal models. Despite the fact that such models helped to shed more light on the potential role of LRRK2 in PD pathology, they suffer main limitations

* Corresponding author at: Department of Cell Biochemistry, Groningen Institute of Biomolecular Sciences & Biotechnology (GBB), University of Groningen, Groningen, the Netherlands.
** Corresponding author at: Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Groningen, the Netherlands.
E-mail addresses: a.kortholt@rug.nl (A. Kortholt), a.m.dolga@rug.nl (A.M. Dolga).

https://doi.org/10.1016/j.nbd.2022.105837
Received 7 May 2022; Received in revised form 21 July 2022; Accepted 6 August 2022
Available online 11 August 2022
0969-9961/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

such as discrepancies in the regulation of gene expression and sensitivity to and metabolism of toxins compared to humans (Potashkin et al., 2011). hiPSCs provide a valuable tool in research since i) Human neuronal and glial cells required for studying neurodegenerative diseases like PD were largely studied from postmortem material. ii) Findings derived from the cell lines have to subsequently be assessed at physiologically relevant expression levels to validate their applicability to the human PD condition. iii) hiPSCs provide appropriate controls using genome-editing technology that allows studying cell populations from patients vs. isogenic controls (gene correction) or controls vs. inserted mutation(s). By using the isogenic approach, variation in the genetic and epigenetic settings between individuals is eliminated.

This review summarizes the main findings of recent studies on LRRK2 functions using hiPSCs and highlights the importance of using different models of hiPSCs in PD research (Figs. 1 and 2).

2. LRRK2 in iPSC-derived dopaminergic neurons

2.1. LRRK2 mutations alter neuronal morphology and differentiation

PD is characterized by progressive neuronal loss in specific brain areas, including the substantia nigra and striatum. The most vulnerable neurons affected early in PD pathology are the dopaminergic neurons located in the substantia nigra. Therefore, many studies have focused on the differentiation of dopaminergic neurons from iPSC cells derived from PD patients reviewed in (Bose et al., 2022). Recently, many studies included differentiated human dopaminergic neurons as a model to investigate *in vitro* how LRRK2 affects human neurons. Neuronal morphology and differentiation are known to be affected by pathogenic mutations in *LRRK2*. In hiPSC models, *LRRK2* G2019S mutation consistently displays shortened neurite length (Borgs et al., 2016; Chang et al., 2021; Korecka et al., 2019; Lin et al., 2016; Qing et al., 2017; Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schwab and Ebert, 2015; Tran et al., 2020). The shortened neurons carrying *LRRK2* mutations may resemble the selective neuronal loss in the SNPc. The exact role of LRRK2 in dopaminergic neurons remains to be determined. However, one study correlated neurite shorting in PD hiPSC-derived dopaminergic neurons to alteration in the autophagic system in long-time cultures (Sánchez-Danés et al., 2012). Another study found that gene correction of *LRRK2* G2019S rescued the length of neurites in hiPSC derived midbrain dopaminergic neurons (Reinhardt et al., 2013). The neurotoxic effects observed in LRRK2 G2019S neurons were attributed to the higher kinase activity of LRRK2, resulting in increased phosphorylation of extracellular signal-regulated kinase 1/2 (ERK) compared to the isogenic control neurons. Kinase inhibition of LRRK2 (by LRRK2-IN1) or inhibition of ERK were able to ameliorate the observed neurotoxic effects in the mutation-carrying neurons (Reinhardt et al., 2013).

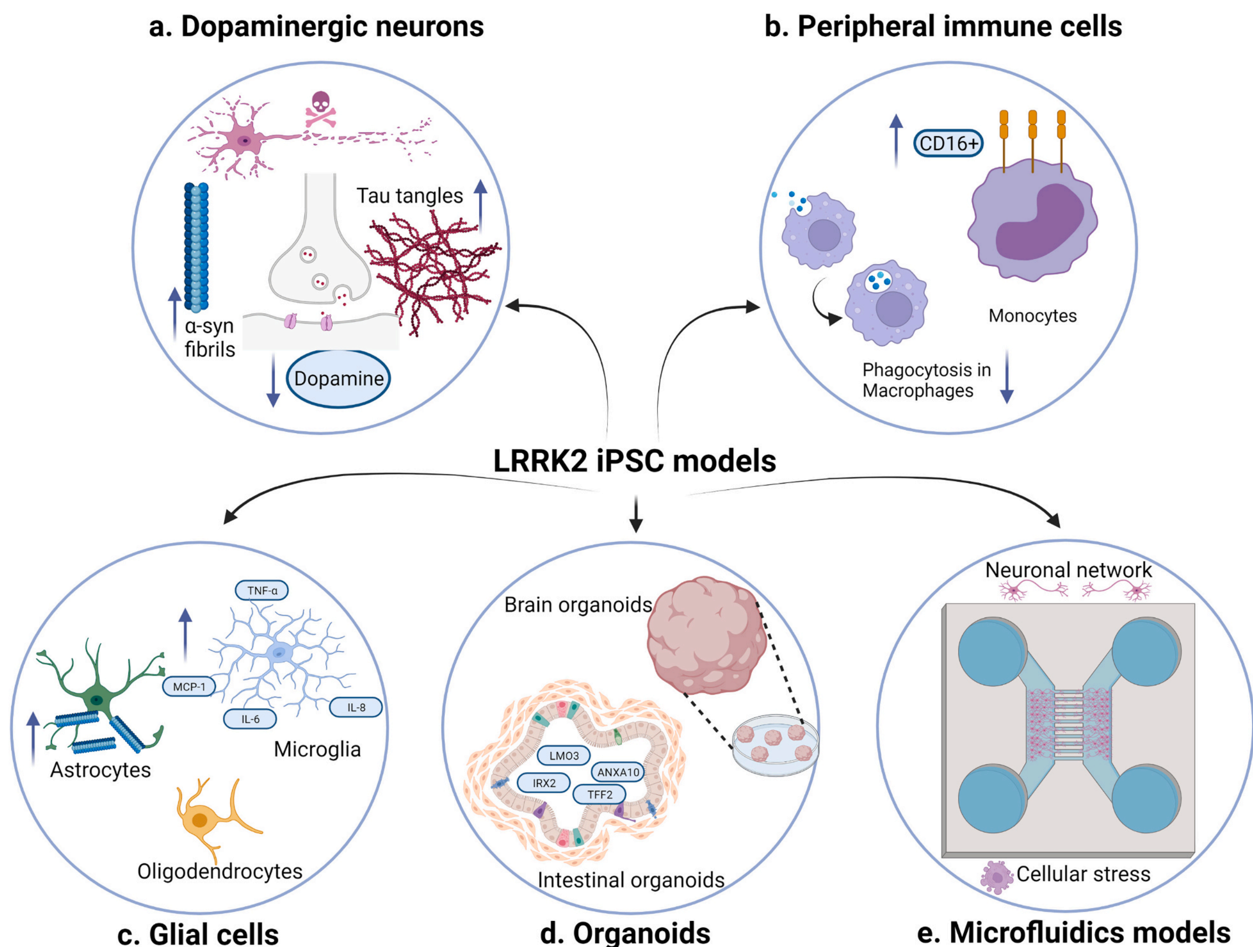


Fig. 1. An overview of the different LRRK2 iPSC models.a. Dopaminergic neurons have been assessed intensively, revealing that *LRRK2* mutations are associated with increased α-synuclein fibrils, increased Tau tangles, reduced dopamine release, neurite destruction as well as neuronal death. **b.** Peripheral immune cells revealed increased CD16^{+} monocytes besides reduced macrophage phagocytic capacity in *LRRK2* mutations. **c.** Glial cells have shown increased cytokine release in microglia, increased α-syn accumulation in astrocytes and increased relevance of oligodendrocytes. In terms of 3D models (**d.**), brain organoids recapitulate the increased complexity of LRRK2 effects on the brain. In intestinal organoids, LMO3, IRX2, ANXA10, and TFF2 seem to be important genes affected in intestinal organoid-PD models. **e.** Microfluidics models are studied to show neuronal networks and neural responses to stress.

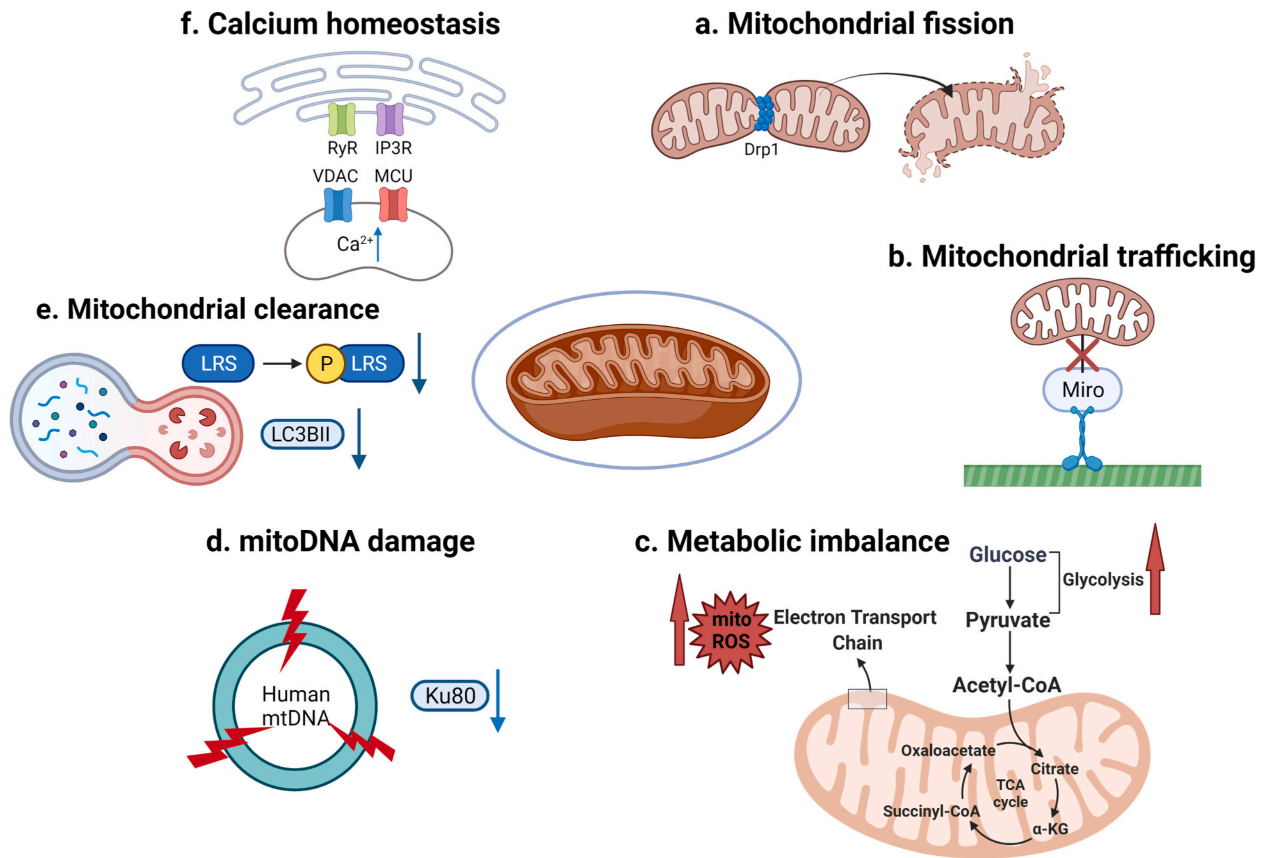


Fig. 2. ***LRRK2*** **mutation induced mitochondrial damage.** **a.** *LRRK2* mutations increase mitochondrial fragmentation, pointing towards disruption in mitochondrial fission, mostly through Drp1. **b.** Due to disturbance in complex formation with Miro in *LRRK2* mutations, trafficking is dysfunctional. **c.** *LRRK2* mutations shift respiration from oxidative phosphorylation to aerobic glycolysis and increase mitoROS, resulting in altered mitochondrial respiration. **d.** *LRRK2* mutations increase mitochondrial DNA damage and decrease Ku80 expression. **e.** Reduction in LRS phosphorylation and LC3BII expression were observed in *LRRK2* mutations, pointing towards reduced mitophagy. **f.** Calcium homeostasis is affected by *LRRK2* mutations via influencing calcium channels RyR, IP3R, VDAC, and MCU, increasing mitochondrial calcium levels.

Investigation of neuronal differentiation revealed an increase in neuritic tree complexity at the early developmental stage in differentiating LRRK2 G2019S neural stem cells (NSCs) to dopaminergic neurons compared to the control (Borgs et al., 2016). Despite the increase in neurite arborization at this early stage of differentiation, the total neurite length at day 5 of differentiation was significantly reduced, which aligns with the reduced neurite length observed for mature LRRK2 G2019S dopaminergic neurons (Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schwab and Ebert, 2015). Further analysis is necessary to better understand the role of LRRK2 G2019S in neurite arborization since the measurements of the neurite length have been performed at early stages of differentiation, which do not reflect the typical maturation time for cultured neurons.

Increased neuritic arborization might be a developmental defect causing the neuron to be more susceptible to oxidative damage due to a higher demand of ATP and increased ROS production (Borgs et al., 2016). In addition, LRRK2 R1441G iPSC-derived NSCs showed impaired differentiation compared to the wildtype NSCs cells. This might be due to the downregulation of Let-7a and miR-9 in LRRK2 R1441C NSCs, two genes involved in the process of neuronal differentiation (Bahnassawy et al., 2013). On the other hand, LRRK2 deficient NSCs showed upregulated differentiation with unchanged expression of Let-7a and miR-9 compared to wildtype NSCs. A potential explanation for the increased differentiation of KO-LRRK2 NSCs is represented by an immature neuronal phenotype as KO-LRRK2 NSCs are often positive for the progenitor CNS cell marker Nestin, which suggests differentiation may be more rapid yet incomplete (Bahnassawy et al., 2013). Differentiation capacity and clonal expansion were reduced in LRRK2 G2019S NSCs following extensive cell passaging, suggesting that these effects might emerge as a result of aging or senescence (Liu et al., 2012). These data refer to the important role of LRRK2 in modulating neuronal differentiation of NSCs.

2.2. LRRK2 association with α-synuclein and Tau

Several studies pointed towards a potential link between LRRK2 and both α-synuclein (α-syn) (Bieri et al., 2019; Nguyen et al., 2011; Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schapansky et al., 2018; Skibinski et al., 2014; Zhao et al., 2020) and Tau proteins (Ohta et al., 2015; Reinhardt et al., 2013). Emerging evidence demonstrated that LRRK2 and α-syn influence each other in PD pathogenesis. The *LRRK2* G2019S mutation was reported to increase α-syn accumulation in hiPSC-derived neurons (Bieri et al., 2019; Chang et al., 2021; Nguyen et al., 2011; Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schapansky et al., 2018; Skibinski et al., 2014). In line with this finding, G2019S mutation increases the aggregation level of α-syn compared to isogenic control and KO LRRK2 hiPSC-derived neurons using an α-syn preformed-fibril induction assay (Bieri et al., 2019). Additionally, α-syn is affecting neuronal viability in *LRRK2* mutant neurons as the removal of α-syn in *LRRK2* G2019S mutant hiPSC-derived neurons significantly increases neuronal survival (Skibinski et al., 2014). LRRK2 kinase inhibitors alleviate the accumulation of α-syn in LRRK2 G2019S hiPSC-derived neurons, indicating that the accumulation of α-syn in mutant *LRRK2* is kinase-dependent (Schapansky et al., 2018; Zhao et al., 2020).

Tau, a microtubule-associated protein, becomes hyperphosphorylated and its aggregates form neurofibrillary tangles that are associated with neurodegenerative tauopathies like PD and Alzheimer's disease (AD). Under physiological conditions, Tau is found in a soluble, unfolded form, is mainly expressed in the neurons and contributes to microtubule stabilization and axonal transport. Following hyperphosphorylation, Tau proteins aggregate and form insoluble neurofibrillary tangles that disrupt axonal trafficking and neuronal communication (Pan et al., 2021; Zhang et al., 2018). Tau hyperphosphorylation was observed in LRRK2 I2020T iPSC-derived neurons due to a decrease in Akt phosphorylation which in turn increases glycogen synthase kinase-3β (GSK-3β) phosphorylation compared to control neurons. Akt modulates Tau phosphorylation via GSK-3β (Ohta et al., 2015). Interestingly, treatment of LRRK2 I2020T iPSC-derived neurons with LRRK2 kinase inhibitors lowered Tau phosphorylation level indicating that I2020T mutation might affect Tau phosphorylation in a kinase-dependent manner (Ohta et al., 2015). In addition, Tau hyperphosphorylation has been observed in LRRK2 G2019S iPSC-derived neurons (Schapansky et al., 2018), indicating potential involvement of LRRK2 in Tau hyperphosphorylation process, and of LRRK2 kinase inhibitors in PD therapy by targeting Tau hyperphosphorylation.

2.3. LRRK2 and endocytosis

Dysfunctional endocytosis has been shown to be associated with the pathology of PD, especially in dopaminergic neurons. In LRRK2 R1441C dopaminergic neurons, less functional and more disrupted synaptic vesicles were observed (Nguyen and Krainc, 2018). Consequently, oxidized dopamine levels were significantly increased in LRRK2 R1441C/G and G2019S iPSC-derived dopaminergic neurons (Nguyen and Krainc, 2018). Furthermore, it was found that the increased oxidized dopamine was caused by auxilin dysfunction, linking it to improper synaptic vesicle endocytosis. Auxilin is an enzyme involved in the regeneration of synaptic vesicle pools via clathrin-dependent synaptic vesicle endocytosis (Nguyen and Krainc, 2018).It is responsible for the recruitment of hsc70 to remove the clathrin lattice from the newly formed vesicle to produce a synaptic vesicle that can be packaged with neurotransmitters (Nguyen and Krainc, 2018). Thus, LRRK2-mediated endocytosis impairment via auxilin dysfunction reduces the synaptic vesicle pool leading to the accumulation of oxidized dopamine as the latter cannot be packaged into vesicles but remains in the cytosol, leading to dopamine toxicity. LRRK2-mediated disruption of endocytosis was reproduced in LRRK2 G2019S iPSC-derived dopaminergic neurons. Transcriptomics and proteomics also revealed the downregulation of essential endocytic proteins, including endophilin I-III, dynamin-1, and Rab proteins (Connor-Robson et al., 2019). Another study involving LRRK2 I2020T iPSC-derived neurons revealed reduced dopamine release that cannot be associated with low cellular levels of dopamine or DOPAC production, as these were similar to the control neurons (Ohta et al., 2015). Instead, the low dopamine release was related to low oligomerized SNARE complex and SNARE protein levels, which are both linked to synaptic exocytosis (Ohta et al., 2015). This concludes that dopamine lifecycle alteration could be associated with PD pathology, and *LRRK2* mutations can affect this pathway.

3. LRRK2 and mitochondrial function in hiPSC- derived neurons

The link between mitochondrial function and PD pathogenesis was revealed when MPTP and rotenone (mitochondrial complex I inhibitors) were shown to induce parkinsonian-like symptoms in humans. Numerous studies have shown that mitochondria indeed contribute to the development of PD pathogenesis (Chen et al., 2019; Eldeeb et al., 2022). Furthermore, it has been suggested that LRRK2 can directly interact with mitochondria, which implies a possible influence on mitochondria functions (Singh et al., 2019). The observation of mitochondrial impairment in PD linked to *LRRK2* mutations has been extensively studied in various cellular models, including iPSC. Here, we discuss the irregularity of mitochondrial functions in hiPSC-derived dopaminergic neurons carrying *LRRK2* mutations. Mitochondrial alterations studied in LRRK2 iPSC-derived neurons are summarized in Table 1.

3.1. LRRK2 in mitochondrial fission

Fusion and fission processes are crucial for maintaining healthy and functional mitochondria. Excessive mitochondrial fission and fragmentation, caused by an imbalance in the mitochondrial fission and fusion ratio, point towards impaired mitochondrial functioning. Fragmented mitochondria were observed in iPSC-derived neuroepithelial stem cells (NESCs) from PD patients carrying *LRRK2* G2019S mutation compared to LRRK2 wildtype cells (Walter et al., 2019). Similarly, LRRK2 G2019S iPSC-derived dopaminergic neurons have less mitochondria and showed more mitochondrial fragmentation than the wildtype neurons (Su and Qi, 2013).

Interestingly, LRRK2 G2019S iPSC-derived dopaminergic neurons have reduced mitochondrial content and showed an impaired distribution, while the G2019S mutation does not have an effect on iPSC-derived glutamatergic and sensory neurons. This selective effect of the G2019S mutant reflects the higher vulnerability of dopaminergic neurons (Schwab et al., 2017). Mitochondria in LRRK2-G2019S NESCs displayed greater sphericity, less elongation and increased mitochondrial fragmentation (Walter et al., 2019). However, the mitochondria from LRRK2 G2019S iPSC-derived neurons were similar in size to the neuronal mitochondria from healthy subjects (Cooper et al., 2012). The difference in mitochondrial morphology between LRRK2 G2019S NESCs and iPSC-derived neurons might be related to the influence of LRRK2 G2019S on the differentiation process and neuronal maturation. Furthermore, LRRK2 R1441C iPSC-derived neurons contained 20% shorter mitochondria in the proximal axons compared to mitochondria from neurons derived from healthy subjects. The decrease in length of LRRK2 R1441C neuronal mitochondria is associated with altered dynamics, pointing towards an increased susceptibility to cellular stress and cell death (Cooper et al., 2012). Therefore, it is clear that different *LRRK2* mutations might affect mitochondrial dynamics in distinct manners; however, the mechanisms underlying these alterations are yet to be discovered.

3.2. LRRK2 in mitochondrial trafficking

The above described selective disruption of mitochondrial morphology and fragmentation in iPSC-derived dopaminergic neurons are also reflected in mitochondrial trafficking. Mitochondrial trafficking/movement is an essential cellular process to distribute mitochondria in order to meet the local energy demand throughout the neuronal cell body, dendrites and axon. Static mitochondria are necessary for supplying local ATP in high-energy demanding long axons of dopaminergic neurons. Newly generated mitochondria are distributed along the axon from the neuron cell body (soma) towards the synaptic terminal in the anterograde direction; meanwhile, the damaged mitochondria return to soma in retrograde direction for further degradation (Zheng et al., 2019). As the majority (70–80%) of static mitochondria exist in the CNS, higher mitochondrial mobility disrupts the balance of mitochondrial energy supply and demand in the axon (Sheng, 2017).

Mitochondrial velocity and mobility in iPSC-derived dopaminergic neurons harboring *LRRK2* G2019S and R1441C mutations were significantly increased compared to control neurons (Cooper et al., 2012). In a different study, the increase in mitochondrial velocity in LRRK2 G2019S iPSC-derived neurons was found to be specific for dopaminergic neurons, and was not observed in glutaminergic or sensory neurons. However, mitochondrial mobility was shown to be increased only in the retrograde direction, indicating that mitochondria move towards the neuron cell body. This correlated well with the reduced mitochondrial

Table 1
Mitochondrial alteration in hiPSCs derived neuronal cells.

Parameters	Models	*LRRK2* mutations	Results	References
Mitochondria morphology	iPSC-derived neuroepithelial stem cells	G2019S	Increase in the number of mitochondria	(Walter et al., 2019)
			Less elongated and more sphere mitochondria	(Walter et al., 2019)
			Increase in mitochondrial volume and total surface area	(Walter et al., 2019)
	iPSC-derived dopaminergic neuron	G2019S	Less mitochondria and longer distance between them	(Su and Qi, 2013)
			Shorter length mitochondria	(Su and Qi, 2013)
			Diminished mitochondrial content and distribution	(Cooper et al., 2012)
		R1441C	Shorter length mitochondria	(Cooper et al., 2012)
Mitochondria fragmentation	iPSC-derived neuroepithelial stem cells	G2019S	Increased level of mitochondrial fragmentation	(Walter et al., 2019)
	iPSC-derived dopaminergic neuron	G2019S	Increased mitochondrial fragmentation	(Su and Qi, 2013)
Mitochondrial trafficking	iPSC-derived dopaminergic neuron	G2019S	Increase in mitochondrial mobility	(Cooper et al., 2012; Schwab et al., 2017)
			Decrease in mitochondrial mobility due to delayed Miro removal	(Hsieh et al., 2016)
		R1441C	Increase in mitochondrial mobility	(Cooper et al., 2012)
Mitochondrial respiration	iPSC-derived neuroepithelial stem cells	G2019S	Decrease in basal and maximal respiration	(Walter et al., 2019)
	iPSC-derived dopaminergic neuron	G2019S	Reduced basal, ATP-linked, maximal, and spare respiration	(Cooper et al., 2012; Schwab et al., 2017)
		R1441C	Reduced basal respiration	(Cooper et al., 2012)
Mitochondrial ROS and oxidative stress	iPSC-derived neuroepithelial stem cells	G2019S	Increased mitochondrial ROS	(Sim et al., 2020a; Walter et al., 2019)
			Increased expression of genes related to oxidative stress	(Sim et al., 2020a)
	iPSC-derived dopaminergic neuron	G2019S	Increased mitochondrial ROS	(Su and Qi, 2013)
			Increased mtDNA damage	(Sanders et al., 2014; Sim et al., 2020a)
			Increased sensitivity to oxidative stress	(Nguyen et al., 2011; Reinhardt et al., 2013; Sim et al., 2020a)
			Increased sensitivity to mitochondrial toxin rotenone	(Reinhardt et al., 2013)
			Impaired NF-κB signaling	(López de Maturana et al., 2016)
		R1441C	Increased mtDNA damage	(Sanders et al., 2014)
			Impaired NF-κB signaling	(López de Maturana et al., 2016)
Mitochondrial membrane potential (MMP)	iPSC-derived neuroepithelial stem cells	G2019S	Lower MMP in LRRK2 G2019S – expressing NESCs compared to LRRK2 WT	(Walter et al., 2019)
	iPSC-derived dopaminergic neuron	G2019S	Lower MMP	(Su and Qi, 2013)
Mitochondrial clearance	iPSC-derived neuroepithelial stem cells	G2019S	Reduced mitophagic clearance via lysosomes	(Walter et al., 2019)
			Reduced autophagosomal – lysosomal pathway	(Walter et al., 2019)
			Reduced autophagosome transport	(Boecker et al., 2021)
	iPSC-derived dopaminergic neuron	G2019S	Increase in lysosomal hyperactivity and autophagic flux	(Su and Qi, 2013)
			Mitophagy delayed	(Hsieh et al., 2016)
			Disrupted mitophagy via delayed Miro removal	(Hsieh et al., 2016)
			Delay in autophagosome formation	(Hsieh et al., 2016)
			Impaired autophagy	(Ho et al., 2018; Sánchez-Danés et al., 2012)
			Reduced basal autophagy and increased number of autophagosomes	(Reinhardt et al., 2013)
Ca^{2+} homeostasis	iPSC-derived neurons	G2019S	Decrease in the basal level of ER Ca^{2+}	(Korecka et al., 2019)
			Decrease mRNA expression of regulator ER Ca^{2+}	(Korecka et al., 2019)
			Decrease intracellular Ca^{2+} buffer capacity	(Korecka et al., 2019)
			Increase Ca^{2+} influx	(Kim et al., 2020b)
			Increase of cytosolic intracellular Ca^{2+}	(Kim et al., 2020b)

Ca^{2+}: Calcium, ER: Endoplasmic reticulum, iPSC: Human-induced pluripotent stem cells, mtDNA: Mitochondrial DNA, MMP: Mitochondrial membrane potential, ROS: Reactive oxygen species.

content in the distal neurite as the mitochondria move towards the neuronal cell body (Schwab et al., 2017).

Further research is needed to understand how *LRRK2* mutations affect mitochondrial movement. However, the observed increase in mitochondrial dynamics is most likely linked to mitochondrial damage that is followed by mitophagy. Another critical player in mitochondrial trafficking is the protein Miro which associates with motor proteins in the cell to form the mitochondrial motor/adaptor complex. LRRK2 binds to Miro to form a complex that promotes Miro removal from damaged mitochondria in iPSC-derived neurons (Hsieh et al., 2016). Furthermore, antimycin A, a mitochondrial complex III inhibitor, was shown to trigger mitophagy and reduce mitochondrial trafficking in LRRK2 G2019S neurons. LRRK2 G2019S delays Miro removal and mitochondrial trafficking due to a loss of association with Miro (Hsieh et al., 2016). Thus, reduced mitochondrial trafficking observed in LRRK2 G2019S iPSC-derived neurons upon mitophagy induction could result from the inability to form a complex with Miro. However, such a causal relationship is yet to be determined.

3.3. *LRRK2 in mitochondrial respiration and DNA damage*

LRRK2 kinase activity and mutations can affect mitochondrial

respiration (Cooper et al., 2012; Rosenbusch et al., 2021; Schwab et al., 2017). The mitochondrial respiratory chain enables the activity of ATP synthase via oxidative phosphorylation (OxPHOS). In pathological conditions, mitochondrial respiration is much lower in iPSC-derived dopaminergic neurons with LRRK2 G2019S (Cooper et al., 2012; Schwab et al., 2017) and R1441C mutations (Cooper et al., 2012) compared to control iPSC-derived dopaminergic neurons. Furthermore, NF-κB signaling, which is also involved in regulating the balance between glycolysis and mitochondrial respiration (Mauro et al., 2012), was impaired in iPSC-derived neurons with the *LRRK2* G2019S and R1441G mutation (López de Maturana et al., 2016). Inhibition of this important pathway shifts the energy metabolism balance towards aerobic glycolysis rather than mitochondrial respiration, thereby inducing necrosis in neurons following dramatic glucose lack (Mauro et al., 2012). This shift from oxidative phosphorylation to aerobic glycolytic respiration was also observed in LRRK2 G2019S iPSC-derived astrocytes (Ramos-Gonzalez et al., 2021). These emerging pieces of evidence indicate that the unbalanced mitochondrial energy may disturb the mitochondrial ATP-linked respiration in cells with *LRRK2* G2019S mutations.

Another key parameter of mitochondrial functionality is the mitochondrial membrane potential ($\Delta\Psi_m$), as it reflects the process of electron transport chain and OxPHOS. In iPSC-derived NESCs expressing LRRK2 G2019S, both mitochondrial respiration (OCR) and $\Delta\Psi_m$ were found to be decreased compared to the control NESCs. In addition, total and mitochondrial ROS levels were elevated in NESCs expressing LRRK2 G2019S (Walter et al., 2019). Similarly, in another study, an increase in mitoROS production and overexpression of genes related to oxidative stress were displayed in LRRK2 G2019S iPSC-derived NSCs (Sim et al., 2020a). *LRRK2* G2019S mutation has similar effects in iPSC-derived dopaminergic neurons. A reduction of $\Delta\Psi_m$ with an increase in mitoROS level (Su and Qi, 2013) and increased sensitivity to oxidative stress (Reinhardt et al., 2013) have been reported in iPSC-derived NESCs and dopaminergic neurons harboring *LRRK2* G2019S mutation.

Excessive oxidative stress is harmful to mitochondrial DNA (mtDNA). Compared to control iPSC-derived neurons, the damage of mtDNA is increased in iPSC-derived neurons carrying *LRRK2* G2019S and R1441C mutations (Sanders et al., 2014). Furthermore, an isogenic line in which the G2019S mutation was corrected using Zinc finger nuclease-mediated precision genome editing did not show mtDNA damage, indicating that gene correction is protective (Sanders et al., 2014). Furthermore, downregulation of Ku80 (A DNA damage repair protein encoded by the *XRCC5* gene) was observed in LRRK2 G2019S pNSCs along with increased autophosphorylation of ATM serine/threonine kinase (ATM) as well as upregulation of H2A histone family member X (H2AFX) and p53. Together, these findings support previous results showing iPSC-derived dopaminergic neurons carrying mutant G2019S being more sensitive to oxidative stress (Nguyen et al., 2011) and mitochondrial toxin rotenone (Reinhardt et al., 2013) compared to healthy dopaminergic neurons.

3.4. LRRK2 in mitochondrial clearance

Mitochondrial clearance is initiated by mitophagy. As described above, Miro proteins are involved in mitochondrial trafficking preceding the step of mitochondrial clearance. LRRK2 significantly regulates mitochondrial removal by binding to the Miro complex to start the clearance process for damaged mitochondria. However, *LRRK2* G2019S mutation causes delayed removal of Miro in the damaged mitochondria of iPSC-derived dopaminergic neurons, which led to mitophagy disruption (Hsieh et al., 2016).

Abnormal lysosomal hyperactivity and autophagic flux are also described in the iPSC-derived dopaminergic neurons with *LRRK2* mutations (Su and Qi, 2013). Interestingly, lysosomal activity and autophagy correlate with higher mitochondrial fission or fragmentation (Su and Qi, 2013). A study using iPSC-derived dopaminergic neurons carrying *LRRK2* G2019S reported impaired autophagy (Sánchez-Danés et al., 2012). Impaired autophagy was dependent on LRRK2-mediated leucyl-tRNA synthetase (LRS) phosphorylation since increased LRS phosphorylation activity was mediated by *LRRK2* G2019S mutation (Ho et al., 2018). LRS senses leucine for mTORC1 signaling and activates mTORC1 (Han et al., 2012). Differentiated dopaminergic neurons derived from LRRK2 G2019S iPSCs were shown to have reduced basal autophagy and increased autophagosomes compared to the control (Reinhardt et al., 2013). Likewise, iPSC-derived NESCs display a lower basal level of the autophagic flux in the mutant *LRRK2* G2019S cells compared to wildtype NESCs (Walter et al., 2019). Additionally, the number and size of lysosomes were low in LRRK2 G2019S cells compared to the LRRK2 wildtype. Moreover, LRRK2 G2019S cells showed a reduced colocalization of LAMP2 puncta, a lysosomal membrane protein, with the mitochondrial TOM20 area, indicating reduced mitophagy by lysosomes. Furthermore, upstream autophagic signaling and activation were increased without continuation to autophagosome formation, which was caused by a deficiency of LC3BII, a marker for autophagosomes (Walter et al., 2019). Autophagosome transport was impaired and lower in LRRK2 G2019S iPSCs compared to healthy cells. This might indicate a reduced autophagosome-lysosome fusion that affects mitochondrial clearance (Boecker et al., 2021). Thus, the autophagosomal-lysosomal pathway functionality is reduced in iPSC-derived LRRK2 G2019S NESCs (Walter et al., 2019).

3.5. LRRK2 and mitochondrial calcium homeostasis

Mitochondria play an essential role in calcium (Ca^{2+}) homeostasis, and dysregulation of Ca^{2+} homeostasis has been linked to several brain conditions, including cerebral ischemia (Schäfer et al., 2014) and neurodegeneration, (PD and AD pathogenesis) (Marmolejo-Garza et al., 2022; Trombetta-Lima et al., 2021). The rise in the intracellular Ca^{2+} increases the demand for cytosolic Ca^{2+} clearance; this subsequently results in an elevation of mitochondrial metabolism and increased mitochondrial reactive oxygen species (mitoROS) production, which affects mitochondrial health (Scorziello et al., 2020). A recent study linked *LRRK2* G2019S mutation to an overall increase in intracellular Ca^{2+} level and signaling in hiPSC-derived dopaminergic neurons (Kim et al., 2020b). The aberrant elevation of intracellular Ca^{2+} was attributed to the enhanced translation of Ca^{2+} regulating genes, including voltage-gated Ca^{2+} channel subunits. In addition, the transcription level of respiratory complex proteins was significantly higher in LRRK2 G2019S dopaminergic neurons, indicating an increased metabolic demand (Kim et al., 2020b). The dysfunction in Ca^{2+} dynamics in *LRRK2* G2019S mutation has also been demonstrated in iPSC-derived sensory neurons (Schwab and Ebert, 2015). *LRRK2* G2019S mutant sensory neurons displayed a decrease in KCl-mediated Ca^{2+} response, which in turn affected autophagy by increasing p62 and LC3-II protein levels in mutant sensory neurons compared to control. Inhibition of the kinase activity in *LRRK2* G2019S mutant sensory neurons by treatment with LRRK2 kinase inhibitors increased KCl-induced Ca^{2+} response. Yet, the Ca^{2+} level was still lower than the untreated controls indicating partial rescue in calcium signaling deficits (Schwab and Ebert, 2015).

Contact between mitochondria and endoplasmic reticulum (ER) generates an adjacent network for intracellular Ca^{2+} coordination and regulation (Honrath et al., 2017, 2018). The effect of LRRK2 G2019S on ER intracellular Ca^{2+} was recently reported in iPSC-derived neurons. Neurons carrying *LRRK2* G2019S mutations have disrupted ER Ca^{2+} homeostasis, with decreased basal level of ER Ca^{2+} and a reduced intracellular Ca^{2+} buffer capacity (Korecka et al., 2019).

From the studies with hiPSC-derived dopaminergic neurons, we learned that *LRRK2* mutations contribute to detrimental effects on mitochondrial morphology and function (see overview in Fig. 2). Collectively, the effects of *LRRK2* mutations on mitochondria in hiPSC-derived dopaminergic neurons may elucidate how LRRK2 contributes to the dysfunction of mitochondria in PD pathology. However, more

research is needed to identify the exact molecular pathways underlying these effects.

4. LRRK2 in iPSC-derived peripheral immune cells

LRRK2 proteins are highly expressed in immune cells. The link between *LRRK2* polymorphism and the peripheral immune function also has been found in inflammatory disorders, including autoimmune inflammatory bowel disease (Crohn's disease) and leprosy disease (Ahmadi Rastegar and Dzamko, 2020). Furthermore, peripheral inflammation exacerbates neuroinflammation in the CNS and thereby plays a major role in the progression of PD (Fuzzati-Armentero et al., 2019). The overlap between the functions of LRRK2 in peripheral inflammation and neuroinflammation urges us to understand how LRRK2 proteins affect immune cells. In this part of the review, we will discuss the function of LRRK2 in hiPSC-derived macrophages and monocytes. The effects of *LRRK2* G2019S mutation in peripheral immune cells are summarized in Table 2.

4.1. LRRK2 in hiPSC-derived macrophages

Macrophages are the first line of defense against pathogens and populate most tissues of the body. Macrophages readily phagocytize and kill pathogens. Phagosome maturation is an essential pathway for the degradation of phagocytized pathogens. LRRK2 has been shown to negatively regulate this process in hiPSC-derived macrophages contributing to mycobacterial replication and impairments in the innate immune response (Härtlova et al., 2018). LRRK2 KO iPSC-derived macrophages have limited mycobacterial replication and increased mycobacterial targeting to phagolysosomes for destruction compared to healthy macrophages (Härtlova et al., 2018). The same trend was observed for LRRK2 macrophages in the presence of kinase inhibitors, while the opposite was observed in LRRK2 G2019S iPSC-derived macrophages (Härtlova et al., 2018). This indicates that increased LRRK2 kinase activity reduces macrophage control over mycobacteria. In addition, mycobacterial phagosome maturation is negatively regulated by LRRK2 kinase activity, owing to its active role in the recruitment of the negative regulator of PI3K, Rubicon, to phagosomes (Härtlova et al., 2018). PI3K is a family of kinases that is critical for phagosome maturation.

LRRK2 was shown to be recruited during the late stages of phagosomal maturation in iPSC-derived macrophages, indicating its involvement in later stages rather than the initial phagocytic uptake of bioparticles (Lee et al., 2020). The inhibition of the kinase function of LRRK2 enhances its residency in the phagosome. Remarkably, LRRK2 was essential for the recruitment of RAB8a and RAB10 to the phagosomes (Lee et al., 2020). Rab GTPases are identified as a direct substrate of LRRK2 and have an important function in vesicular trafficking (Steger et al., 2016). Furthermore, it was also discovered that the treatment with LRRK2 kinase inhibitors enhanced LRRK2 "residency" at the phagosomes, demonstrating that recruitment of RAB8a and RAB10 to phagosome depends on LRRK2 kinase activity (Lee et al., 2020). α-syn was found to influence LRRK2 expression and phosphorylation in macrophages, in both human and mouse macrophages and dendritic-like cells. The application of α-syn fibrils induced RAB10 phosphorylation and increased LRRK2 expression. These processes were dependent on JAK-STAT activation (Xu et al., 2022).

4.2. LRRK2 in hiPSC-derived monocytes

Monocytes play an essential role in the innate immune response. Monocytes circulate into the bloodstream and release cytokines and chemokines upon activation by pathogenic insults. Monocytes differentiate into different subtypes, which are distinguished based on the relative expression of CD14 and CD16 surface receptors (Sampath et al., 2018). A study on human peripheral blood mononuclear cells revealed

Table 2
Effects of *LRRK2* G2019S mutation on different hiPSC-derived models.

Models	G2019S mutation effect	References
iPSC-derived dopaminergic neurons	Shortened neurite length	(Borgs et al., 2016; Chang et al., 2021; Korecka et al., 2019; Lin et al., 2016; Qing et al., 2017; Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schwab and Ebert, 2015; Tran et al., 2020)
	Increased neuritic arborization at early developmental stage	(Borgs et al., 2016)
	Increase α-syn accumulation	(Bieri et al., 2019; Chang et al., 2021; Nguyen et al., 2011; Reinhardt et al., 2013; Sánchez-Danés et al., 2012; Schapansky et al., 2018; Skibinski et al., 2014)
	Tau hyperphosphorylation	(Schapansky et al., 2018)
	Increased oxidized dopamine levels	(Nguyen and Krainc, 2018)
iPSC-derived macrophages	Increased mycobacterial replication	(Härtlova et al., 2018)
	Reduced mycobacterial targeting to phagolysosomes	(Härtlova et al., 2018)
iPSC-derived monocytes	Faster differentiation during early maturation phase	(Speidel et al., 2016)
	Reduced maturing capacity	(Speidel et al., 2016)
	Reduced migration activity	(Speidel et al., 2016)
iPSC-derived microglia	Negative regulation of NFAT activity	(Panagiotakopoulou et al., 2020)
	Enhanced immune response to IFN-γ	(Panagiotakopoulou et al., 2020)
	Reduced microglial motility	(Panagiotakopoulou et al., 2020)
	Altered cytokine production (IL-6, TNF-α, IL-8 and MCP-1)	(Panagiotakopoulou et al., 2020)
iPSC-derived astrocytes	Elevated expression level of α-synuclein and reduced ability to degrade it	(Sonninen et al., 2020)
	Decreased expression of MPP2, involved in α-synuclein degradation	(Booth et al., 2017)
	Increased responsivity to inflammatory stimuli (TNFα, IL-1β) and disturbed calcium signaling	(Sonninen et al., 2020)
	Altered metabolism and mitochondrial DNA damage	(Sonninen et al., 2020)
	Disturbed morphology (reduced surface area, low complexity, reduced number) and function (reduced amplitude and integral of calcium transients, altered mitochondrial membrane potentiation)	(Ramos-Gonzalez et al., 2021)
	Mitochondrial impairment (reduced mitochondrial oxygen consumption rate, increased glycolytic capacity, increased basal proton efflux rate, fewer, more fragmented and more rounded mitochondria concentrated perinuclearly, higher basal oxidative status)	(Ramos-Gonzalez et al., 2021)
Co-culture of iPSC-derived dopaminergic neurons and	Decreased number of dopaminergic neurons	(de Rus Jacquet et al., 2021; di Domenico et al., 2019
		(di Domenico et al., 2019)

(*continued on next page*)

Table 2 (*continued*)

Models	G2019S mutation effect	References
astrocytes (Patient astrocytes and healthy neurons)	Fewer and shorter neurites, abundance of beaded-necklace neurites, compromised cell survival of neurons	
	Accumulation of α-synuclein in neurons caused by direct transfer from astrocytes, cleared by treatment with chaperone-mediated autophagy activator	(di Domenico et al., 2019)
	Dysregulated homeostasis of astrocyte-to-neuron via EV, leading to neuronal atrophy	(de Rus Jacquet et al., 2021)
	Altered secretion of neurotrophic and astrocyte-secreted exosome uptake by neurons	(de Rus Jacquet et al., 2021)
Co-culture of iPSC-derived dopaminergic neurons and astrocytes (Patient neurons and healthy astrocytes)	Partially recovered neurite number and complex neurite arborization	(di Domenico et al., 2019)
	Prevention of α-synuclein accumulation in neurons	(di Domenico et al., 2019)
Brain organoids	Decreased dopaminergic neuronal marker expression and mature neuronal markers	(Kim et al., 2019)
	Decreased neurite length of dopaminergic neurons in the organoids	(Kim et al., 2019)
	Increased cell death of the organoid cells	(Kim et al., 2019)
	Increased α-synuclein positive vesicles	(Kim et al., 2019)
	Decreased dopaminergic neurons, relieved by LRRK2 inhibitor	(Ha et al., 2020; Smits et al., 2019)
	Increased autophagy, relieved by LRRK2 inhibitor	(Ha et al., 2020)
Intestinal organoids	Altered intestinal cell gene expression for genes involved in synaptic vesicle trafficking	(Son et al., 2017)
	Altered gene expression, including upregulation of IRX2 and LMO3 and downregulation of ANXA10 and TFF2	(Sim et al., 2020b)

ANXA10: Annexin A10, EV: Extracellular vesicles, IFN-γ: Interferon-gamma, IL-1β: Interleukin-1 beta, IL-6: Interleukin-6, IL-8: Interleukin-8, IRX2: Iroquois homeobox 2, MCP-1: Monocyte chemoattractant protein-1, NFAT: Nuclear factor of activated T-cells, TFF2: Nuclear factor of activated T-cells, TNF-α: Tumor necrosis factor-alpha.

that LRRK2 is present in $CD14^{+}$ monocytes and, to a higher extent, in the $CD14^{+}$ $CD16^{+}$ subpopulation of monocytes, which is a more mature subset of monocytes. In addition, stimulation of monocytes with IFN-γ intensively elevates LRRK2 levels, thereby shifting the cells from $CD14^{+}CD16^{-}$ towards $CD14^{+}CD16^{+}$, which was diminished with LRRK2 kinase inhibitor, IN-1 (Thévenet et al., 2011). These findings thus show a possible role of LRRK2 in monocyte maturation. The effect of *LRRK2* G2019S mutation on monocyte maturation was studied in monocytes differentiated from hiPSCs using patient-derived LRRK2 G2019S iPSCs and corresponding isogenic control line as well as control iPSCs and the LRRK2 G2019S knock-in lines (Speidel et al., 2016). *LRRK2* G2019S mutant cells were found to differentiate faster to monocytes during the early maturation phase compared to the isogenic controls. However, the expression level of CD16 (patrolling subpopulation of monocytes) was significantly lower in G2019S monocytes compared to their corresponding isogenic controls, indicating an alteration in the differentiation pattern of monocytes and deficiency of immune surveillance in the mutant cells (Speidel et al., 2016). Furthermore, LRRK2 G2019S iPSC-derived monocytes showed a slightly lower migration capacity compared to their corresponding isogenic controls upon administration of ATP (stimulates monocyte migration), although LPS-induced inflammatory cytokine production was similar in both mutant and control cells (Speidel et al., 2016). These results direct to the possible role of LRRK2 in immune cell maturation and consequently in PD progression.

5. LRRK2 in iPSC-derived glial cells

Glial cells are the immune guardians in keeping the brain at homeostasis conditions. LRRK2 is also expressed in glial cells, including microglia and astrocytes (Miklossy et al., 2006). In response to stress stimulus, glial cells are activated by releasing proinflammatory signals to mediate neuroinflammation in order to omit the toxic substances (Gao and Hong, 2008). However, prolonged microglial activation leads to tissue damage, which is also considered as a neuropathological hallmark in PD. Early observation supported that reactive microglia are increased in the SNPc post-mortem PD brain (Joers et al., 2017). Understanding the functional alterations in human LRRK2 microglia is central to elucidating the different mechanisms of PD asmicroglial activation has been described in PD pathology (Sabogal-Guáqueta et al., 2020). This part of the review will discuss the effect of *LRRK2* mutant in the human PD glial cells (Table 2).

5.1. LRRK2 in hiPSC-derived microglia

LRRK2 has been described as a positive regulator of inflammation in murine microglia, where its mutations could increase neuroinflammation (Russo et al., 2014). However, so far, few data are available from iPSCs - derived microglia from PD patients. A recent study showed that IFN-γ increased LRRK2 expression in iPSC-derived microglia and neurons (Panagiotakopoulou et al., 2020). Besides, LRRK2 expression negatively regulated the Nuclear factor of activated T-cells (NFAT) activity, specially, in microglia with *LRRK2* G2019S mutation. LRRK2 G2019S microglia showed a reduction in microglial mobility in response to IFN-γ. LPS stimulation resulted in a reduction in IL-6, TNF-α, IL-8, and MCP-1 production in LRRK2 G2019S microglia compared to control microglia, while the opposite was observed in LRRK2 KO microglia. These findings indicate a defective immune response in LRRK2 G2019S microglia (Panagiotakopoulou et al., 2020).

Interestingly, it has been shown that LRRK2-driven immunological changes in G2019S microglia activation lead to neurite shortening, indicating a possible neurotoxic effect (Panagiotakopoulou et al., 2020). Another study found that increased LRRK2 activity and Rab10-phosphorylation was observed following lysosomal stress and inflammatory stimuli indicating that perturbed immune responses in PD patient brains could upregulate the LRRK2 signaling pathway (Wang et al., 2021). Rab8a is a LRRK2 kinase substrate that regulates receptor-mediated recycling and endocytic trafficking of transferrin. iPSC-derived microglia from LRRK2-G2019S patients showed increased Rab8 phosphorylation and retained transferrin in the perinuclear recycling compartment area associated with lysosomes under proinflammatory conditions, resulting in a clear dysfunction of iron homeostasis and misbalance in the recycling mechanism. These data were corroborated *in vivo* where G2019S *Lrrk2* knock-in mice displayed a marked increase in iron and ferritin accumulation in microglia cells from the striatum compared to wildtype and *Lrrk2* KO (Mamais et al., 2021). More studies are needed to understand the emerging function of LRRK2 in microglia and to get insight into the molecular pathways underlying neuroinflammation, and consequently, identify novel targets for PD therapeutics.

5.2. LRRK2 in hiPSC-derived astrocytes and hiPSC-derived oligodendrocytes

Neurons interact with non-neuronal cells, particularly astrocytes, in the highly dynamic brain microenvironment. Astrocytes are the most enriched glial cells in the central nervous system; they regulate neural firing activity, neurotransmitter uptake, synapse formation, and supply energy for neurons by secreting neurotrophic factors (Sofroniew, 2020). There is mounting evidence suggesting a link between disrupted astrocytes and PD pathogenesis. Eight causative PD genes, including *LRRK2*, overlapped with astrocyte biology (Booth et al., 2017). LRRK2 is expressed in human astrocytes, albeit at a lower expression level than neurons (Miklossy et al., 2006) and the low expression levels of LRRK2 were shown in iPSC-derived midbrain patterned astrocytes (Booth et al., 2019).

LRRK2 G2019S iPSC-derived astrocytes showed elevated levels of α-syn, increased responsivity to inflammatory stimuli, impaired Ca^{2+} signaling, altered metabolism, and increased mtDNA (Sonninen et al., 2020). Recently, it was shown that LRRK2 G2019S iPSC-derived astrocytes have an altered morphology and impaired mitochondria (Ramos-Gonzalez et al., 2021). LRRK2 G2019S midbrain astrocytes show reduced expression of transforming growth factor-beta 1 (TGFβ1) and matrix metallopeptidase 2 (MMP2) (Booth et al., 2019). MMP2 regulates the degradation of α-syn (Oh et al., 2017). Consequently, the midbrain astrocytes carrying the *LRRK2* G2019S mutation have decreased capacity to degrade α-syn, and a lower neuroprotective capacity (Booth et al., 2019). Co-culture neuronal-astrocyte studies revealed that the number of dopaminergic neurons is much lower in the presence of iPSC-derived astrocytes carrying *LRRK2* G2019S mutations (di Domenico et al., 2019; de Rus Jacquet et al., 2021). Interestingly, LRRK2 G2019S could dysregulate the homeostasis of astrocyte-to-neuron communication via secreted extracellular vesicles (EV), leading to atrophy of the hiPSC-derived neurons (de Rus Jacquet et al., 2021). LRRK2 G2019S induced a reduction of the secretion of neurotrophic factors, and therefore the uptake of astrocyte-secreted exosome by iPSC-derived dopaminergic neurons was reduced (de Rus Jacquet et al., 2021). hiPSC astrocytes carrying *LRRK2* G2019S mutation showed a dysregulation of macroautophagy and chaperone-mediated autophagy, which resulted in an increase of α-syn inside the neurons and led to neuronal degeneration (di Domenico et al., 2019). Although only limited studies evaluated *LRRK2* mutation in iPSC-derived astrocytes, it seems that LRRK2 G2019S disrupts the communication between astrocytes and dopaminergic neurons and thereby indirectly contributes to the α-syn accumulation and neuronal loss.

Taken together, these results from various studies on astroglia in iPSC-derived cells demonstrate an essential role for LRRK2 signaling but also a complex interplay between the CNS and the innate immune system. Furthermore, it becomes apparent that there are several gaps in knowledge regarding iPSC-based modeling as many of the immune system's cells are not yet represented in the body of literature concerning PD and *LRRK2* mutations. In the future, it might also interesting to study the function of LRRK2 in hiPSC-derived oligodendrocytes. Although oligodendrocytes express endogenous LRRK2 (Miklossy et al., 2006), so far, there is no data available on the role of LRRK2 in iPSC-derived oligodendrocytes carrying *LRRK2* mutations. Other studies have used iPSC-derived oligodendrocytes to investigate myelin diseases, e.g., multiple sclerosis, schizophrenia, and Huntington's disease (Chanoumidou et al., 2020). In addition, many studies focused on optimizing the generation and differentiation of hiPSC-derived oligodendrocytes and oligodendrocyte progenitor cells (Ehrlich et al., 2017). Oligodendrocytes are responsible for maintaining the myelin sheaths of neurons. In the typical healthy dynamic environment, the firing activity of neurons is sustained by the myelination process mediated by oligodendrocytes (Bradl and Lassmann, 2010). Oligodendrocytes are highly vulnerable to oxidative stress due to the high demand for oxygen, ATP, and co-enzyme iron for the myelination process. An abundant iron level is stored in the form of ferritin in oligodendrocytes. Failure in iron storage or transport may contribute to neurodegenerative disease pathogenesis, which overlapped with the theory of a newly regulated cell death, ferroptosis, in PD (Xu et al., 2018). There are still no straightforward findings to define the role of oligodendrocytes in PD pathogenesis, although PD heritability in the lysosomal gene was also found in glial cells, including oligodendrocytes (Reynolds et al., 2019).

6. LRRK2 in 3D models

Although the two-dimensional (2D) models of iPSC described above are a powerful and reproducible tool in PD research, it has some limitations. First, a typical 2D model cannot display the complexity of the human brain. Moreover, the 2D cell model cannot represent a complex cell-cell interaction because it is only limited to horizontal contact. Importantly, the 2D model shows a lack of representation for brain organization. This 2D model limitation urges the generation and development of a three-dimensional (3D) brain organoid model that can recapitulate the complex organization of the human brain and the disease phenotype closely.

The 3D model exhibits critical differences compared to the 2D model. For example, the 3D culture of dopaminergic neurons with *LRRK2* G2019S mutation showed a significant reduction of tyrosine hydroxylase positive dopaminergic neurons after 6 weeks of differentiation compared to the isogenic control cell lines without the application of stressor. At that time point, the 2D cultures under the same conditions did not show alteration in the amount of tyrosine hydroxylase positive dopaminergic neurons indicating that 3D cultures can mimic better PD-specific phenotypes than the 2D cultures (Bolognin et al., 2019). In addition, gene expression patterns and cellular phenotypes in 3D cultures were more similar to physiological conditions compared to 2D cultures, and mitochondrial defects were observed even without a chemical stressor (Bolognin et al., 2019). Another recent study comparing the 2D and 3D cultures of dopaminergic neurons with and without *LRRK2* G20129S mutation revealed comparable dopaminergic neurons content in both cultures. However, the accumulation of α-syn was significantly higher in 3D cultures of LRRK2 G20129S dopaminergic neurons compared to the 2D cultures (Fiore et al., 2022). Moreover, differentially expressed genes and the transcriptomic profile of 3D cultures are associated with different cellular pathways compared to 2D cultures (Fiore et al., 2022). Changes in cell behavior cultivated as 2D or 3D cultures were reported in various cell types. Healthy dopaminergic neurons cultivated as 2D and 3D cultures displayed many differences in a variety of biological processes. For instance, 3D cultures showed upregulation of glycolysis, synaptic transmission and action potential compared to 2D cultures indicating more physiologically relevant conditions (Fiore et al., 2022). These data entail the potential of 3D models to mimic physiological conditions. The main effects of *LRRK2* G2019S mutation in brain organoid models are summarized in Table 2.

6.1. LRRK2 in human-derived midbrain organoids (hMOs)

The intrinsic capacity of self-organization of iPSCs and embryonic stem cells (ESCs) with a controlled growth condition becomes an essential factor in the generation of 3D human brain organoids (Amin and Paşca, 2018). The optimization of cocktail signaling molecules via different protocols can generate diverse brain regions, e.g., cerebral cortex, midbrain, and forebrain organoids (Lancaster et al., 2013; Monzel et al., 2017; Smits et al., 2019). Human brain organoids consist of heterogenous neural progenitors, which develop into different neuronal subtypes and active neuronal networks. The recapitulated tissue complexity given by the brain organoid allows the investigation of neuronal connectivity, neurodevelopment, neuro-immune interaction, and transcriptional diversity (Amin and Paşca, 2018).

Human midbrain organoids (hMOs) derived from hiPSCs established an advanced 3D *in vitro* model for PD research. The current hMOs

approaches are based on the differentiation protocols used first to generate 2D cultures. The comparison of the hMOs protocols has been well documented in a recent review (Smits and Schwamborn, 2020). hMOs exhibited shared features of the human midbrain, including mature dopaminergic neurons, dopamine secretion, non-neuronal cell types (e.g., astrocytes and oligodendrocytes), and even neuromelanin-like granules that are similar to those found in the human substantia nigra tissue (Jo et al., 2016; Monzel et al., 2017). Differentiated hMOs were shown to be functionally active, with synaptic connections and active neuronal network (Monzel et al., 2017). The gene expression profile of hMOs was similar to prenatal midbrain compared to 2D cultured mature dopaminergic neurons (Jo et al., 2016). These findings illustrated that 3D midbrain organoids resemble better the human midbrain, providing a better disease modeling tool for PD research.

LRRK2 G2019S mutant has been studied in 3D organoid models, as well. In 2017, 3D human neuroectodermal spheres (hNESs) and human intestinal organoids (hIOs) were generated from the same iPSC line derived from PD patients carrying *LRRK2* G2019S to study the link between gastrointestinal dysfunction and PD pathophysiology (Son et al., 2017). The gene expression profile of both hNESs and hIOs was altered in cells carrying *LRRK2* mutation. Interestingly, the gene alterations were more evident in the hIOs compared to hNESs. Based on their gene ontology and pathway analysis, synaptic vesicle trafficking was found to be the most impaired pathway in both types of organoids (Son et al., 2017). Recently, a comprehensive study successfully modeled LRRK2 G2019S in 3D human midbrain organoids, recapitulating PD pathological hallmarks observed in monolayer 2D cell cultures (Kim et al., 2019). For example, the neurite length and branching number of dopaminergic neurons in the hMOs were found to be decreased, similarly to the 2D cell cultures (Kim et al., 2019; Smits et al., 2019). However, LRRK2 G2019S hMOs were more susceptible to neurotoxicity compared to wildtype hMOs (Kim et al., 2019) and the gene expression profile of LRRK2 G2019S hMOs showed similarity with patients having mutant *LRRK2*-associated sporadic PD. Interestingly, they found that TXNIP (a thiol oxidoreductase) had an important role in the development of organoids and might contribute to *LRRK2* mutant-associated PD phenotypes (Kim et al., 2019). Another study reported a lower number of dopaminergic neurons in LRRK2 G2019S-simplified brain organoids (Smits et al., 2019). The reduced dopaminergic neuronal network was confirmed in a different study that also reported increased autophagy. Both effects were prevented by treatment with LRRK2 kinase inhibitor (Ha et al., 2020). Although there are fewer studies of *LRRK2* mutations in 3D midbrain organoids, the findings above reassured that 3D organoids could represent a supportive model to investigate the role of LRRK2 in association with PD, besides the 2D cultures.

6.2. LRRK2 in human-derived intestinal organoids (hIOs)

Gastrointestinal dysfunction is one of the most common non-motor symptoms in PD and is multifactorial. In fact, disturbed gastrointestinal motility is a PD symptom that precedes the clinical diagnosis of the disease for years and could potentially serve as an early indicator of the premotor phase (Jost, 2010). Furthermore, the deposition of α-syn in the enteric nervous system and dysfunction of enteric and central innervation of the gut has been previously reported (Chukwurah et al., 2019; Su et al., 2017). There is compelling evidence suggesting that altered gastrointestinal microbiome composition is the cause of the aforementioned features (Lubomski et al., 2020; Sharma et al., 2019). Son & collaborators generated human intestinal organoids (hIOs) from PD patient-specific iPSCs carrying a *LRRK2* G2019S mutation as a new model to study the contribution of other organs to PD pathology. Expression profiles were distinctive from wildtype controls, and gene ontology data showed divergence in metabolic processes, cellular process, biological regulation, and synaptic vesicle trafficking that involves proteins such as G-proteins, cadherins, and B cell activation. Surprisingly, gene expression changes associated with *LRRK2* G2019S mutation were higher in intestinal tissue than in neural tissue, suggesting an important role of the gastrointestinal system in PD (Son et al., 2017). Herewith, Son et al. (2017) provided the first evidence of LRRK2 G2019S also causing significant changes in the intestinal cell gene expression, aside from its effects on brain cells. More recently, the generation of *in vitro* hIOs and *ex vivo* mouse small intestine from old transgenic mouse harboring *Lrrk2* G2019S mutation allow the identification of upregulated genes, i.e., iroquois-class homeodomain protein 2 (*IRX2* gene) and LIM domain only protein 3 (*LMO3* gene), and down-regulated genes, i.e. Annexin A10 (*ANXA10* gene) and Trefoil factor 2 (*TFF2* gene). To corroborate these data, the authors included human neuroectodermal spheres, and they found *IRX2* exhibiting a similar expression pattern to human and murine organoids, proposing this particular gene as a potential and functional biomarker in PD (Sim et al., 2020b).

7. Modeling LRRK2 expression and activity in microfluidic models

Microfluidic technology enables scientists to study cellular mechanisms and differentiation under certain experimental conditions. Microfluidic chips offer numerous advantages over conventional cell culturing. Experienced labs capable of fabricating microfluidic chambers from Polydimethylsiloxane (PDMS) could generate microfluidic devices with a relatively low cost of fabrication. Some of the limitations of these devices are represented by i) generation of the microfluidic devices is time-consuming, ii) the viability and iii) long-term culturing of neuronal cells, and iv) reproducibility of the results. Small volumes of cell culture reagents and lower cell counts make the chips highly cost-effective. Microfluidics also offers high reproducibility and precise control of experimental variables at a faster pace. Moreover, the creation of chemical gradient and barrier conditions resembling biological systems makes the use of microfluidic chips highly attractive for studying biological functions in different neurodegenerative diseases, particularly PD (Mofazzal Jahromi et al., 2019; Neto et al., 2016).

In a recent study (Devold Valderhaug et al., 2020), custom-designed multimodal microfluidic chips coupled to a microelectrode array have been implemented to study the impact of *LRRK2* G2019S mutation on neuronal networks. The authors used control hiPSC-derived H9N neuronal stem cells along with the homozygous CRISPR-Cas9 inserted G2019S mutation. They showed a striking increase in the neuritic density in the LRRK2 G2019S neuronal network compared to the control neuronal network (Devold Valderhaug et al., 2020). This finding contradicts other studies that reported a reduction in the neuritic length and branching in neurons with *LRRK2* G2019S mutation (Nguyen et al., 2011; Qing et al., 2017; Reinhardt et al., 2013; Sánchez-Danés et al., 2012). In addition, the average mean firing rate of the G2019S LRRK2 neuronal network was consistently higher than the control neurons under basal conditions. However, the G2019S LRRK2 neuronal network displayed a lower total network correlation compared to the control neuronal network and aberrant outgrowth in the synaptic contacts. This, thus, demonstrates that the G2019S mutant induces an overall impairment in network development and less efficient signal propagation (Devold Valderhaug et al., 2020). Following kainic acid stimulation, which induces increased neuronal excitability, the neuronal networks of the healthy neurons displayed greater responses compared to the G2019S LRRK2 neuronal networks. These responses included i) marked reduction in active mitochondria and mean firing rates, ii) increased size of synaptic contacts, and iii) higher total network correlation (unlike the LRRK2 G2019S that did not show further increase in total network correlation following kainic acid) indicating a potential impairment in synaptic remodeling in the LRRK2 G2019S neuronal network (Devold Valderhaug et al., 2020). This study illustrates that using microfluidic chips together with the microelectrode array can capture alterations in the neuronal network activity and allows analyses of the neuronal network stress responses upon confined neuronal overexcitation.

8. Conclusion and prospective

hiPSCs present a powerful tool to study neurodegenerative diseases. hiPSC models allow the production of the desired cell type and present disease-related phenotypes (Bose et al., 2022). Nevertheless, the use of iPSC-based models suffers from some limitations reviewed in (Doss and Sachinidis, 2019; Kim et al., 2020a). For instance, intrinsic variability between donors results in huge discrepancies. iPSCs' characteristics might be altered during the reprogramming process and might not reflect the actual disease phenotype (Kim et al., 2011; Nishino et al., 2011). The lack of standardized protocol to assess the validity and reduce the variability of the produced models is another challenge. In addition, the higher complexity of 3D and organoid models makes it more challenging to control the experimental conditions (Kim et al., 2020a). Furthermore, organoid cultures are relatively more expensive and time-consuming (Huang et al., 2021).

Despite the mentioned limitations, hiPSC-derived models allow *in vitro* comprehensive study of molecular pathways underlying neurodegenerative diseases since they might recapitulate some of the disease phenotypes similar to human samples than cell lines or animal models (Avazzadeh et al., 2021). In addition, the introduction of genome-editing techniques provides better comparison based on a single mutation, through mutation correction or insertion, eliminating variability between donors and allowing accurate comparisons in a specific cell population (De Masi et al., 2020). hiPSC-derived models offer appropriate models to disclose unique information under physiological and pathological conditions that were previously possible only from post-mortem biopsies. The availability of differentiation protocols for hiPSC-models led to extensive studies focusing on the characterization of 2D and 3D models derived from PD patient hiPSCs with monogenic mutations such as *LRRK2* G2019S.

Studying 2D hiPSC-derived neuronal models does not completely reflect the *in vivo* conditions concerning the synaptic connection with other cell types and fully mature neuronal phenotypes (Centeno et al., 2018). That is why organoid models could represent a superior model to 2D culture, particularly when studying cell-cell interaction. The adaptability of brain organoid models facilitates studying the interaction between different brain regions, which is an important feature during disease development. However, the use of hiPSC-derived organoids suffers from limitations including; 1) the transcriptional profile is similar to prenatal brains than to aging ones making it difficult to mimic aging phenotypes, 2) the lack of vascularization and blood-brain barrier, 3) the lack of microglia, and 4) the variability in the produced organoids. The limitations and future potentials of hiPSC-derived models to mimic neurodegenerative diseases are thoroughly explained in (Baena-Montes et al., 2021; Bubnys and Tsai, 2022; Papaspyropoulos et al., 2020).

The combination of microfluidic models with microelectrode array is a recent *in vitro* advancement permitting precise control of studying neuronal activity and stressor responses in healthy and diseased conditions giving an accurate image of perturbations happening in neurodegenerative diseases (Devold Valderhaug et al., 2020).

In a nutshell, LRRK2-iPSCs models provide powerful *in vitro* systems that can mimic pathological conditions related to PD, as described in this review. These models can elucidate possible molecular interactions of LRRK2 that help identify the role of LRRK2 in PD pathogenesis which in turn will allow for identifying PD biomarkers and improving drug development approaches.

Data availability

No data was used for the research described in the article.

Acknowledgements

AK is a recipient of a TÜBITAK 2232 fellowship. AMD and AK are recipients of a ParkinsonFonds grant and AD was supported by a Rosalind Franklin Fellowship co-funded by the European Union and the University of Groningen.

References

Ahmadi Rastegar, D., Dzamko, N., 2020. Leucine rich repeat kinase 2 and innate immunity. Front. Neurosci. 14, 1–11. https://doi.org/10.3389/fnins.2020.00193.
Amin, N.D., Paşca, S.P., 2018. Building models of brain disorders with three-dimensional organoids. Neuron 100, 389–405. https://doi.org/10.1016/j.neuron.2018.10.007.
Avazzadeh, S., Baena, J.M., Keighron, C., Feller-Sanchez, Y., Quinlan, L.R., 2021. Modelling parkinson's disease: Ipscs towards better understanding of human pathology. Brain Sci. 11 https://doi.org/10.3390/brainsci11030373.
Baena-Montes, J.M., Avazzadeh, S., Quinlan, L.R., 2021. α-Synuclein pathogenesis in hiPSC models of Parkinson's disease. Neuronal Signal. 5, 20210021. https://doi.org/10.1042/ns20210021.
Bahnassawy, L., Nicklas, S., Palm, T., Menzl, I., Birzele, F., Gillardon, F., Schwamborn, J. C., 2013. The Parkinson's disease-associated LRRK2 mutation R1441G inhibits neuronal differentiation of neural stem cells. Stem Cells Dev. 22, 2487–2496. https://doi.org/10.1089/scd.2013.0163.
Bieri, G., Brahic, M., Bousset, L., Couthouis, J., Kramer, N.J., Ma, R., Nakayama, L., Monbureau, M., Defensor, E., Schüle, B., et al., 2019. LRRK2 modifies α-syn pathology and spread in mouse models and human neurons. Acta Neuropathol. 137, 961–980. https://doi.org/10.1007/s00401-019-01995-0.
Boecker, C.A., Goldsmith, J., Dou, D., Cajka, G.G., Holzbaur, E.L.F., 2021. Increased LRRK2 kinase activity alters neuronal autophagy by disrupting the axonal transport of autophagosomes. Curr. Biol. 31, 2140–2154.e6. https://doi.org/10.1016/j.cub.2021.02.061.
Bolognin, S., Fossépré, M., Qing, X., Jarazo, J., Ščančar, J., Moreno, E.L., Nickels, S.L., Wasner, K., Ouzren, N., Walter, J., et al., 2019. 3D cultures of Parkinson's disease-specific dopaminergic neurons for high content phenotyping and drug testing. Adv. Sci. 6, 1800927. https://doi.org/10.1002/advs.201800927.
Booth, H.D.E., Hirst, W.D., Wade-Martins, R., 2017. The role of astrocyte dysfunction in Parkinson's disease pathogenesis. Trends Neurosci. 40, 358–370. https://doi.org/10.1016/j.tins.2017.04.001.
Booth, H.D.E., Wessely, F., Connor-Robson, N., Rinaldi, F., Vowles, J., Browne, C., Evetts, S.G., Hu, M.T., Cowley, S.A., Webber, C., et al., 2019. RNA sequencing reveals MMP2 and TGFB1 downregulation in LRRK2 G2019S Parkinson's iPSC-derived astrocytes. Neurobiol. Dis. 129, 56–66. https://doi.org/10.1016/j.nbd.2019.05.006.
Borgs, L., Peyre, E., Alix, P., Hanon, K., Grobarczyk, B., Godin, J.D., Purnelle, A., Krusy, N., Maquet, P., Lefebvre, P., et al., 2016. Dopaminergic neurons differentiating from LRRK2 G2019S induced pluripotent stem cells show early neuritic branching defects. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep33377.
Bose, A., Petsko, G.A., Studer, L., 2022. Induced pluripotent stem cells: a tool for modeling Parkinson's disease. Trends Neurosci. https://doi.org/10.1016/j.tins.2022.05.001.
Bradl, M., Lassmann, H., 2010. Oligodendrocytes: biology and pathology. Acta Neuropathol. 119, 37–53. https://doi.org/10.1007/s00401-009-0601-5.
Bubnys, A., Tsai, L.H., 2022. Harnessing cerebral organoids for Alzheimer's disease research. Curr. Opin. Neurobiol. 72, 120–130. https://doi.org/10.1016/j.conb.2021.10.003.
Centeno, E.G.Z.Z., Cimarosti, H., Bithell, A., 2018. 2D Versus 3D Human Induced Pluripotent Stem Cell-Derived Cultures for Neurodegenerative Disease Modelling. BioMed Central Ltd. https://doi.org/10.1186/s13024-018-0258-4.
Chang, K.H., Huang, C.Y., Ou-Yang, C.H., Ho, C.H., Lin, H.Y., Hsu, C.L., Chen, Y.T., Chou, Y.C., Chen, Y.J., Chen, Y., et al., 2021. In vitro genome editing rescues parkinsonism phenotypes in induced pluripotent stem cells-derived dopaminergic neurons carrying LRRK2 p.G2019S mutation. Stem Cell Res Ther 12, 508. https://doi.org/10.1186/s13287-021-02585-2.
Chanoumidou, K., Mozafari, S., Baron-Van Evercooren, A., Kuhlmann, T., 2020. Stem cell derived oligodendrocytes to study myelin diseases. Glia 68, 705–720. https://doi.org/10.1002/glia.23733.
Chen, C., Turnbull, D.M., Reeve, A.K., 2019. Mitochondrial dysfunction in Parkinson's disease—cause or consequence? Biology (Basel). 8, 1–26. https://doi.org/10.3390/biology8020038.
Chukwurah, E., Osmundsen, A., Davis, S.W., Lizarraga, S.B., 2019. All together now: modeling the interaction of neural with non-neural systems using organoid models. Front. Neurosci. 13, 1–16. https://doi.org/10.3389/fnins.2019.00582.
Connor-Robson, N., Booth, H., Martin, J.G., Gao, B., Li, K., Doig, N., Vowles, J., Browne, C., Klinger, L., Juhasz, P., et al., 2019. An integrated transcriptomics and proteomics analysis reveals functional endocytic dysregulation caused by mutations in LRRK2. Neurobiol. Dis. 127, 512–526. https://doi.org/10.1016/j.nbd.2019.04.005.
Cooper, O., Seo, H., Andrabi, S., Guardia-Laguarta, C., Graziotto, J., Sundberg, M., McLean, J.R., Carrillo-Reid, L., Xie, Z., Osborn, T., et al., 2012. Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. Sci. Transl. Med. 4, 141ra90. https://doi.org/10.1126/scitranslmed.3003985.
De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G., Sangiuolo, F., 2020. Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery. Hum. Genomics 14, 1–12. https://doi.org/10.1186/s40246-020-00276-2.
de Rus Jacquet, A., Tancredi, J.L., Lemire, A.L., Desantis, M.C., Li, W.P., O'shea, E.K., 2021. The lrrk2 g2019s mutation alters astrocyte-to-neuron communication via extracellular vesicles and induces neuron atrophy in a human ipsc-derived model of

parkinson's disease. Elife 10. https://doi.org/10.7554/eLife.73062, 2020.07.02.178574.
Devold Valderhaug, V., Huse Ramstad, O., van de Wijdeven, R., Heiney, K., Nichele, S., Sandvig, A., Sandvig, I., 2020. Structural and functional alterations associated with the LRRK2 G2019S mutation revealed in structured human neural networks. BioRxiv 2020 (05), 02.073726. https://doi.org/10.1101/2020.05.02.073726.
di Domenico, A., Carola, G., Calatayud, C., Pons-Espinal, M., Muñoz, J.P., Richaud-Patin, Y., Fernandez-Carasa, I., Gut, M., Faella, A., Parameswaran, J., et al., 2019. Patient-specific iPSC-derived astrocytes contribute to non-cell-autonomous neurodegeneration in Parkinson's disease. Stem Cell Rep. 12, 213–229. https://doi.org/10.1016/j.stemcr.2018.12.011.
Di Maio, R., Hoffman, E.K., Rocha, E.M., Keeney, M.T., Sanders, L.H., De Miranda, B.R., Zharikov, A., Van Laar, A., Stepan, A.F., Lanz, T.A., et al., 2018. LRRK2 activation in idiopathic Parkinson's disease. Sci. Transl. Med. 10, 1–13. https://doi.org/10.1126/scitranslmed.aar5429.
Doss, M.X., Sachinidis, A., 2019. Current challenges of iPSC-based disease modeling and therapeutic implications. Cells 8. https://doi.org/10.3390/cells8050403.
Ehrlich, M., Mozafari, S., Glatza, M., Starost, L., Velychko, S., Hallmann, A.L., Cui, Q.L., Schambach, A., Kim, K.P., Bachelin, C., et al., 2017. Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. Proc. Natl. Acad. Sci. U. S. A. 114, E2243–E2252. https://doi.org/10.1073/pnas.1614412114.
Eldeeb, M.A., Thomas, R.A., Ragheb, M.A., Fallahi, A., Fon, E.A., 2022. Mitochondrial quality control in health and in Parkinson's disease. Physiol. Rev. https://doi.org/10.1152/physrev.00041.2021.
Fiore, N.J., Ganat, Y.M., Devkota, K., Batorsky, R., Lei, M., Lee, K., Cowen, L.J., Croft, G., Noggle, S.A., Nieland, T.J.F., et al., 2022. Bioengineered models of Parkinson's disease using patient-derived dopaminergic neurons exhibit distinct biological profiles in a 3D microenvironment. Cell. Mol. Life Sci. 79, 3. https://doi.org/10.1007/s00018-021-04047-7.
Fuzzati-Armentero, M.T., Cerri, S., Blandini, F., 2019. Peripheral-central neuroimmune crosstalk in parkinson's disease: what do patients and animal models tell us? Front. Neurol. 10 https://doi.org/10.3389/fneur.2019.00232.
Gao, H.M., Hong, J.S., 2008. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol. 29, 357–365. https://doi.org/10.1016/j.it.2008.05.002.
Ha, J., Kang, J.S., Lee, M., Baek, A., Kim, S., Chung, S.K., Lee, M.O., Kim, J., 2020. Simplified brain organoids for rapid and robust modeling of brain disease. Front. Cell Dev. Biol. 8 https://doi.org/10.3389/fcell.2020.594090.
Han, J.M., Jeong, S.J., Park, M.C., Kim, G., Kwon, N.H., Kim, H.K., Ha, S.H., Ryu, S.H., Kim, S., 2012. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. Cell 149, 410–424. https://doi.org/10.1016/j.cell.2012.02.044.
Härtlova, A., Herbst, S., Peltier, J., Rodgers, A., Bilkei-Gorzo, O., Fearns, A., Dill, B.D., Lee, H., Flynn, R., Cowley, S.A., et al., 2018. LRRK2 is a negative regulator of *Mycobacterium tuberculosis* phagosome maturation in macrophages. EMBO J. 37, 1–17. https://doi.org/10.15252/embj.201798694.
Hirsch, L., Jette, N., Frolkis, A., Steeves, T., Pringsheim, T., 2016. The incidence of Parkinson's disease: a systematic review and meta-analysis. Neuroepidemiology 46, 292–300. https://doi.org/10.1159/000445751.
Ho, D.H., Kim, H.G.H., Nam, D., Sim, H., Kim, J., Kim, H.G.H., Son, I., Seol, W., 2018. LRRK2 impairs autophagy by mediating phosphorylation of leucyl-tRNA synthetase. Cell Biochem. Funct. 36, 431–442. https://doi.org/10.1002/cbf.3364.
Honrath, B., Metz, I., Bendridi, N., Rieusset, J., Culmsee, C., Dolga, A.M., 2017. Glucose-regulated protein 75 determines ER–mitochondrial coupling and sensitivity to oxidative stress in neuronal cells. Cell Death Dis. 3, 17076. https://doi.org/10.1038/cddiscovery.2017.76.
Honrath, B., Culmsee, C., Dolga, A.M., 2018. One protein, different cell fate: the differential outcome of depleting GRP75 during oxidative stress in neurons. Cell Death Dis. 9, 1–3. https://doi.org/10.1038/s41419-017-0148-7.
Hsieh, C.H., Shaltouki, A., Gonzalez, A.E., Bettencourt da Cruz, A., Burbulla, L.F., St. Lawrence, E., Schüle, B., Krainc, D., Palmer, T.D., Wang, X., 2016. Functional impairment in Miro degradation and Mitophagy is a shared feature in familial and sporadic Parkinson's disease. Cell Stem Cell 19, 709–724. https://doi.org/10.1016/j.stem.2016.08.002.
Huang, Y., Huang, Z., Tang, Z., Chen, Y., Huang, M., Liu, H., Huang, W., Ye, Q., Jia, B., 2021. Research progress, challenges, and breakthroughs of organoids as disease models. Front. Cell Dev. Biol. 9, 3259. https://doi.org/10.3389/fcell.2021.740574.
Jo, J., Xiao, Y., Sun, A.X., Cukuroglu, E., Tran, H.D., Göke, J., Tan, Z.Y., Saw, T.Y., Tan, C.P., Lokman, H., et al., 2016. Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. Cell Stem Cell 19, 248–257. https://doi.org/10.1016/j.stem.2016.07.005.
Joers, V., Tansey, M.G., Mulas, G., Carta, A.R., 2017. Microglial phenotypes in Parkinson's disease and animal models of the disease. Prog. Neurobiol. 155, 57–75. https://doi.org/10.1016/j.pneurobio.2016.04.006.
Jost, W.H., 2010. Gastrointestinal dysfunction in Parkinson's disease. J. Neurol. Sci. 289, 69–73. https://doi.org/10.1016/j.jns.2009.08.020.
Kim, K., Zhao, R., Doi, A., Ng, K., Unternaehrer, J., Cahan, P., Hongguang, H., Loh, Y.H., Aryee, M.J., Lensch, M.W., et al., 2011. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat. Biotechnol. 29, 1117–1119. https://doi.org/10.1038/nbt.2052.
Kim, H., Park, H.J., Choi, H., Chang, Y., Park, H., Shin, J., Kim, J., Lengner, C.J., Lee, Y. K., Kim, J., 2019. Modeling G2019S-LRRK2 sporadic Parkinson's disease in 3D midbrain organoids. Stem Cell Rep. 12, 518–531. https://doi.org/10.1016/j.stemcr.2019.01.020.
Kim, J., Koo, B.K., Knoblich, J.A., 2020a. Human organoids: model systems for human biology and medicine. Nat. Rev. Mol. Cell Biol. 21, 571–584. https://doi.org/10.1038/s41580-020-0259-3.
Kim, J.W., Yin, X., Jhaldiyal, A., Khan, M.R., Martin, I., Xie, Z., Perez-Rosello, T., Kumar, M., Abalde-Atristain, L., Xu, J., et al., 2020b. Defects in mRNA translation in LRRK2-mutant hiPSC-derived dopaminergic neurons Lead to dysregulated calcium homeostasis. Cell Stem Cell 27, 633–645.e7. https://doi.org/10.1016/j.stem.2020.08.002.
Korecka, J.A., Talbot, S., Osborn, T.M., De Leeuw, S.M., Levy, S.A., Ferrari, E.J., Moskites, A., Atkinson, E., Jodelka, F.M., Hinrich, A.J., et al., 2019. Neurite collapse and altered ER Ca 2+ control in human Parkinson disease patient iPSC-derived neurons with LRRK2 G2019S mutation. Stem Cell Reports 12, 29–41. https://doi.org/10.1016/j.stemcr.2018.11.021.
Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., Knoblich, J.A., 2013. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379. https://doi.org/10.1038/nature12517.
Lee, H., Flynn, R., Sharma, I., Haberman, E., Carling, P.J., Nicholls, F.J., Stegmann, M., Vowles, J., Haenseler, W., Wade-Martins, R., et al., 2020. LRRK2 is recruited to phagosomes and co-recruits RAB8 and RAB10 in human pluripotent stem cell-derived macrophages. Stem Cell Rep. 14, 940–955. https://doi.org/10.1016/j.stemcr.2020.04.001.
Lees, A.J., Hardy, J., Revesz, T., 2009. Parkinson's disease. Lancet 373, 2055–2066. https://doi.org/10.1016/S0140-6736(09)60492-X.
Lin, L., Göke, J., Cukuroglu, E., Dranias, M.R., VanDongen, A.M.J., Stanton, L.W., 2016. Molecular features underlying neurodegeneration identified through in vitro modeling of genetically diverse Parkinson's disease patients. Cell Rep. 15, 2411–2426. https://doi.org/10.1016/j.celrep.2016.05.022.
Lis, P., Burel, S., Steger, M., Mann, M., Brown, F., Diez, F., Tonelli, F., Holton, J.L., Ho, P. W., Ho, S.L., et al., 2018. Development of phospho-specific Rab protein antibodies to monitor in vivo activity of the LRRK2 Parkinson's disease kinase. Biochem. J. 475, 1–22. https://doi.org/10.1042/BCJ20170802.
Liu, G.-H., Qu, J., Suzuki, K., Nivet, E., Li, M., Montserrat, N., Yi, F., Xu, X., Ruiz, S., Zhang, W., et al., 2012. Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. Nature. https://doi.org/10.1038/nature11557.
López de Maturana, R., Lang, V., Zubiarrain, A., Sousa, A., Vázquez, N., Gorostidi, A., Águila, J., López de Munain, A., Rodríguez, M., Sánchez-Pernaute, R., 2016. Mutations in LRRK2 impair NF-KB pathway in iPSC-derived neurons. J. Neuroinflammation 13. https://doi.org/10.1186/s12974-016-0761-x.
Lubomski, M., Tan, A.H., Lim, S.Y., Holmes, A.J., Davis, R.L., Sue, C.M., 2020. Parkinson's disease and the gastrointestinal microbiome. J. Neurol. 267, 2507–2523. https://doi.org/10.1007/s00415-019-09320-1.
Mamais, A., Kluss, J.H., Bonet-Ponce, L., Landeck, N., Langston, R.G., Smith, N., Beilina, A., Kaganovich, A., Ghosh, M.C., Pellegrini, L., et al., 2021. Mutations in LRRK2 linked to Parkinson disease sequester Rab8a to damaged lysosomes and regulate transferrin-mediated iron uptake in microglia. PLoS Biol. 19, e3001480 https://doi.org/10.1371/JOURNAL.PBIO.3001480.
Marmolejo-Garza, A., Medeiros-Furquim, T., Rao, R., Eggen, B.J.L., Boddeke, E., Dolga, A.M., 2022. Transcriptomic and epigenomic landscapes of Alzheimer's disease evidence mitochondrial-related pathways. Biochim. Biophys. Acta, Mol. Cell Res. 1869, 119326 https://doi.org/10.1016/J.BBAMCR.2022.119326.
Marrone, L., Bus, C., Schöndorf, D., Fitzgerald, J.C., Kübler, M., Schmid, B., Reinhardt, P., Reinhardt, L., Deleidi, M., Levin, T., et al., 2018. Generation of iPSCs carrying a common LRRK2 risk allele for in vitro modeling of idiopathic Parkinson's disease. PLoS One 13, e0192497. https://doi.org/10.1371/journal.pone.0192497.
Mata, I.F., Wedemeyer, W.J., Farrer, M.J., Taylor, J.P., Gallo, K.A., 2006. LRRK2 in Parkinson's disease: protein domains and functional insights. Trends Neurosci. 29, 286–293. https://doi.org/10.1016/j.tins.2006.03.006.
Mauro, C., Leow, S.C., Anso, E., Rocha, S., Thotakura, A.K., Tornatore, L., Moretti, M., De Smaele, E., Beg, A.A., Tergaonkar, V., et al., 2012. NF-κB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration Europe PMC Funders Group. Nat. Cell Biol. 13, 1272–1279. https://doi.org/10.1038/ncb2324.
Miklossy, J., Arai, T., Guo, J.P., Klegeris, A., Yu, S., McGeer, E.G., McGeer, P.L., 2006. LRRK2 expression in normal and pathologic human brain and in human cell lines. J. Neuropathol. Exp. Neurol. 65, 953–963. https://doi.org/10.1097/01.jnen.0000235121.98052.54.
Mofazzal Jahromi, M.A., Abdoli, A., Rahmanian, M., Bardania, H., Bayandori, M., Moosavi Basri, S.M., Kalbasi, A., Aref, A.R., Karimi, M., Hamblin, M.R., 2019. Microfluidic brain-on-a-Chip: perspectives for mimicking neural system disorders. Mol. Neurobiol. 56, 8489–8512. https://doi.org/10.1007/s12035-019-01653-2.
Monzel, A.S., Smits, L.M., Hemmer, K., Hachi, S., Moreno, E.L., van Wuellen, T., Jarazo, J., Walter, J., Brüggemann, I., Boussaad, I., et al., 2017. Derivation of human midbrain-specific organoids from neuroepithelial stem cells. Stem Cell Rep. 8, 1144–1154. https://doi.org/10.1016/j.stemcr.2017.03.010.
Neto, E., Leitão, L., Sousa, D.M., Alves, C.J., Alencastre, I.S., Aguiar, P., Lamghari, X.M., Lamghari, M., 2016. Compartmentalized microfluidic platforms: the unrivaled breakthrough of in vitro tools for neurobiological research. J. Neurosci. 36, 11573–11584. https://doi.org/10.1523/JNEUROSCI.1748-16.2016.
Nguyen, M., Krainc, D., 2018. LRRK2 phosphorylation of auxilin mediates synaptic defects in dopaminergic neurons from patients with Parkinson's disease. Proc. Natl. Acad. Sci. 115, 5576–5581.
Nguyen, H.N., Byers, B., Cord, B., Shcheglovitov, A., Byrne, J., Gujar, P., Kee, K., Schüle, B., Dolmetsch, R.E., Langston, W., et al., 2011. LRRK2 mutant iPSC-derived da neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 8, 267–280. https://doi.org/10.1016/j.stem.2011.01.013.

Nishino, K., Toyoda, M., Yamazaki-Inoue, M., Fukawatase, Y., Chikazawa, E., Sakaguchi, H., Akutsu, H., Umezawa, A., 2011. DNA methylation dynamics in human induced pluripotent stem cells over time. PLoS Genet. 7, 1002085. https://doi.org/10.1371/journal.pgen.1002085.

Oh, S.H., Kim, H.N., Park, H.J., Shin, J.Y., Kim, D.Y., Lee, P.H., 2017. The cleavage effect of mesenchymal stem cell and its derived matrix metalloproteinase-2 on extracellular α-Synuclein aggregates in parkinsonian models. Stem Cells Transl. Med. 6, 949–961. https://doi.org/10.5966/sctm.2016-0111.

Ohta, E., Nihira, T., Uchino, A., Imaizumi, Y., Okada, Y., Akamatsu, W., Takahashi, K., Hayakawa, H., Nagai, M., Ohyama, M., et al., 2015. I2020T mutant LRRK2 iPSC-derived neurons in the Sagamihara family exhibit increased Tau phosphorylation through the AKT/GSK-3β signaling pathway. Hum. Mol. Genet. 24, 4879–4900. https://doi.org/10.1093/hmg/ddv212.

Pan, L., Meng, L., He, M., Zhang, Z., 2021. Tau in the Pathophysiology of Parkinson's Disease. J. Mol. Neurosci. 71, 2179–2191. https://doi.org/10.1007/s12031-020-01776-5.

Panagiotakopoulou, V., Ivanyuk, D., De Cicco, S., Haq, W., Arsić, A., Yu, C., Messelodi, D., Oldrati, M., Schöndorf, D.C., Perez, M.J., et al., 2020. Interferon-γ signaling synergizes with LRRK2 in neurons and microglia derived from human induced pluripotent stem cells. Nat. Commun. 11, 5163. https://doi.org/10.1038/s41467-020-18755-4.

Papaspyropoulos, A., Tsolaki, M., Foroglou, N., Pantazaki, A.A., 2020. Modeling and targeting Alzheimer's disease with organoids. Front. Pharmacol. 11, 396. https://doi.org/10.3389/fphar.2020.00396.

Potashkin, J.A., Blume, S.R., Runkle, N.K., 2011. Limitations of animal models of Parkinson's disease. Parkinsons. Dis. 2011 https://doi.org/10.4061/2011/658083.

Qing, X., Walter, J., Jarazo, J., Arias-Fuenzalida, J., Hillje, A.L., Schwamborn, J.C., 2017. CRISPR/Cas9 and piggyBac-mediated footprint-free LRRK2-G2019S knock-in reveals neuronal complexity phenotypes and α-Synuclein modulation in dopaminergic neurons. Stem Cell Res. 24, 44–50. https://doi.org/10.1016/j.scr.2017.08.013.

Ramos-Gonzalez, P., Mato, S., Chara, J.C., Verkhratsky, A., Matute, C., Cavaliere, F., 2021. Astrocytic atrophy as a pathological feature of Parkinson's disease with LRRK2 mutation. Npj Park. Dis. 7 https://doi.org/10.1038/s41531-021-00175-w.

Reinhardt, P., Schmid, B., Burbulla, L.F., Schöndorf, D.C., Wagner, L., Glatza, M., Höing, S., Hargus, G., Heck, S.A.S., Dhingra, A., et al., 2013. Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. Cell Stem Cell 12, 354–367. https://doi.org/10.1016/j.stem.2013.01.008.

Reynolds, R.H., Botía, J., Nalls, M.A., Noyce, A.J., Nicolas, A., Cookson, M.R., Bandres-Ciga, S., Gibbs, J.R., Hernandez, D.G., Singleton, A.B., et al., 2019. Moving beyond neurons: the role of cell type-specific gene regulation in Parkinson's disease heritability. Npj Park. Dis. 5, 1–14. https://doi.org/10.1038/s41531-019-0076-6.

Rosenbusch, K.E., Oun, A., Sanislav, O., Lay, S.T., Keizer-Gunnink, I., Annesley, S.J., Fisher, P.R., Dolga, A.M., Kortholt, A., 2021. A conserved role for LRRK2 and Roco proteins in the regulation of mitochondrial activity. Front. Cell Dev. Biol. 9, 1–14. https://doi.org/10.3389/fcell.2021.734554.

Russo, I., Bubacco, L., Greggio, E., 2014. LRRK2 and neuroinflammation: partners in crime in Parkinson's disease? J. Neuroinflammation 11, 1–9. https://doi.org/10.1186/1742-2094-11-52.

Sabogal-Guáqueta, A.M., Marmolejo-Garza, A., de Pádua, V.P., Eggen, B., Boddeke, E., Dolga, A.M., 2020. Microglia alterations in neurodegenerative diseases and their modeling with human induced pluripotent stem cell and other platforms. Prog. Neurobiol. 190 https://doi.org/10.1016/j.pneurobio.2020.101805.

Sampath, P., Moideen, K., Ranganathan, U.D., Bethunaickan, R., 2018. Monocyte subsets: phenotypes and function in tuberculosis infection. Front. Immunol. 9, 1726. https://doi.org/10.3389/fimmu.2018.01726.

Sánchez-Danés, A., Richaud-Patin, Y., Carballo-Carbajal, I., Jiménez-Delgado, S., Caig, C., Mora, S., Di Guglielmo, C., Ezquerra, M., Patel, B., Giralt, A., et al., 2012. Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. EMBO Mol. Med. 4, 380–395. https://doi.org/10.1002/emmm.201200215.

Sanders, L.H., Laganière, J., Cooper, O., Mak, S.K., Vu, B.J., Huang, Y.A., Paschon, D.E., Vangipuram, M., Sundararajan, R., Urnov, F.D., et al., 2014. LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. Neurobiol. Dis. 62, 381–386. https://doi.org/10.1016/j.nbd.2013.10.013.

Schäfer, M.K.E., Pfeiffer, A., Jaeckel, M., Pouya, A., Dolga, A.M., Methner, A., 2014. Regulators of mitochondrial Ca2+ homeostasis in cerebral ischemia. Cell Tissue Res. 357, 395–405. https://doi.org/10.1007/s00441-014-1807-y.

Schapansky, J., Khasnavis, S., DeAndrade, M.P., Nardozzi, J.D., Falkson, S.R., Boyd, J.D., Sanderson, J.B., Bartels, T., Melrose, H.L., LaVoie, M.J., 2018. Familial knockin mutation of LRRK2 causes lysosomal dysfunction and accumulation of endogenous insoluble α-synuclein in neurons. Neurobiol. Dis. 111, 26–35. https://doi.org/10.1016/j.nbd.2017.12.005.

Schwab, A.J., Ebert, A.D., 2015. Neurite aggregation and calcium dysfunction in iPSC-derived sensory neurons with Parkinson's disease-related LRRK2 G2019S mutation. Stem Cell Rep. 5, 1039–1052. https://doi.org/10.1016/j.stemcr.2015.11.004.

Schwab, A.J., Sison, S.L., Meade, M.R., Broniowska, K.A., Corbett, J.A., Ebert, A.D., 2017. Decreased Sirtuin deacetylase activity in LRRK2 G2019S iPSC-derived dopaminergic neurons. Stem Cell Rep. 9, 1839–1852. https://doi.org/10.1016/j.stemcr.2017.10.010.

Scorziello, A., Borzacchiello, D., Sisalli, M.J., Di Martino, R., Morelli, M., Feliciello, A., 2020. Mitochondrial homeostasis and signaling in Parkinson's disease. Front. Aging Neurosci. 12, 100. https://doi.org/10.3389/fnagi.2020.00100.

Sharma, S., Awasthi, A., Singh, S., 2019. Altered gut microbiota and intestinal permeability in Parkinson's disease: pathological highlight to management. Neurosci. Lett. 712 https://doi.org/10.1016/j.neulet.2019.134516.

Sheng, Z.H., 2017. The interplay of axonal energy homeostasis and mitochondrial trafficking and anchoring. Trends Cell Biol. 27, 403–416. https://doi.org/10.1016/j.tcb.2017.01.005.

Shu, Y., Ming, J., Zhang, P., Wang, Q., Jiao, F., Tian, B., 2016. Parkinson-related LRRK2 mutation R1628P enables Cdk5 phosphorylation of LRRK2 and upregulates its kinase activity. PLoS One 11, e0149739. https://doi.org/10.1371/journal.pone.0149739.

Sim, H., Seo, J.H., Kim, J., Oh, M., Lee, J.E., Baek, A., Lee, S.Y., Chung, S.K., Son, M.Y., Chae, J. Il, et al., 2020a. Quantitative proteomic analysis of primitive neural stem cells from lrrk2 g2019s-associated parkinson's disease patient-derived ipscs. Life 10, 1–19. https://doi.org/10.3390/life10120331.

Sim, H., Lee, J.-E.E., Yoo, H.M., Cho, S., Lee, H., Baek, A., Kim, J.J., Seo, H., Kweon, M.-N.N., Kim, H.G., et al., 2020b. Iroquois homeobox protein 2 identified as a potential biomarker for Parkinson's disease. Int. J. Mol. Sci. 21, 1–16. https://doi.org/10.3390/ijms21103455.

Singh, A., Zhi, L., Zhang, H., 2019. LRRK2 and mitochondria: recent advances and current views. Brain Res. 1702, 96–104. https://doi.org/10.1016/j.brainres.2018.06.010.

Skibinski, G., Nakamura, K., Cookson, M.R., Finkbeiner, S., 2014. Mutant LRRK2 toxicity in neurons depends on LRRK2 levels and synuclein but not kinase activity or inclusion bodies. J. Neurosci. 34, 418–433. https://doi.org/10.1523/JNEUROSCI.2712-13.2014.

Smits, L.M., Schwamborn, J.C., 2020. Midbrain organoids: a new tool to investigate Parkinson's disease. Front. Cell Dev. Biol. 8, 359. https://doi.org/10.3389/fcell.2020.00359.

Smits, L.M., Reinhardt, L., Reinhardt, P., Glatza, M., Monzel, A.S., Stanslowsky, N., Rosato-Siri, M.D., Zanon, A., Antony, P.M., Bellmann, J., et al., 2019. Modeling Parkinson's disease in midbrain-like organoids. Npj Park. Dis. 5, 1–8. https://doi.org/10.1038/s41531-019-0078-4.

Sofroniew, M.V., 2020. Astrocyte reactivity: subtypes, states, and functions in CNS innate immunity. Trends Immunol. 41, 758–770. https://doi.org/10.1016/j.it.2020.07.004.

Son, M.Y., Sim, H., Son, Y.S., Jung, K.B., Lee, M.O., Oh, J.H., Chung, S.K., Jung, C.R., Kim, J., 2017. Distinctive genomic signature of neural and intestinal organoids from familial Parkinson's disease patient-derived induced pluripotent stem cells. Neuropathol. Appl. Neurobiol. 43, 584–603. https://doi.org/10.1111/nan.12396.

Sonninen, T.M., Hämäläinen, R.H., Koskuvi, M., Oksanen, M., Shakirzyanova, A., Wojciechowski, S., Puttonen, K., Naumenko, N., Goldsteins, G., Laham-Karam, N., Lehtonen, M., et al., 2020. Metabolic alterations in parkinson's disease astrocytes. Sci. Rep. 10, 14474. https://doi.org/10.1038/s41598-020-71329-8.

Speidel, A., Felk, S., Reinhardt, P., Sterneckert, J., Gillardon, F., 2016. Leucine-rich repeat kinase 2 influences fate decision of human monocytes differentiated from induced pluripotent stem cells. PLoS One 11, 1–19. https://doi.org/10.1371/journal.pone.0165949.

Steger, M., Tonelli, F., Ito, G., Davies, P., Trost, M., Vetter, M., Wachter, S., Lorentzen, E., Duddy, G., Wilson, S., et al., 2016. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. Elife 5, 1–28. https://doi.org/10.7554/eLife.12813.

Su, Y.-C., Qi, X., 2013. Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by LRRK2 G2019S mutation. Hum. Mol. Genet. 22, 4545–4561. https://doi.org/10.1093/hmg/ddt301.

Su, A., Gandhy, R., Barlow, C., Triadafilopoulos, G., 2017. A practical review of gastrointestinal manifestations in Parkinson's disease. Parkinsonism Relat. Disord. 39, 17–26. https://doi.org/10.1016/j.parkreldis.2017.02.029.

Thévenet, J., Gobert, R., van Huijsduijnen, R.H., Wiessner, C., Sagot, Y.J., Pescini Gobert, R., Hooft van Huijsduijnen, R., Wiessner, C., Sagot, Y.J., Gobert, R., et al., 2011. Regulation of LRRK2 expression points to a functional role in human monocyte maturation. PLoS One 6, e21519. https://doi.org/10.1371/journal.pone.0021519.

Tran, J., Anastacio, H., Bardy, C., 2020. Genetic predispositions of Parkinson's disease revealed in patient-derived brain cells. Npj Park. Dis. 6, 1–18. https://doi.org/10.1038/s41531-020-0110-8.

Trombetta-Lima, M., Sabogal-Guáqueta, A.M., Dolga, A.M., 2021. Mitochondrial dysfunction in neurodegenerative diseases: a focus on iPSC-derived neuronal models. Cell Calcium 94, 102362. https://doi.org/10.1016/j.ceca.2021.102362.

Wallings, R., Manzoni, C., Bandopadhyay, R., 2015. Cellular processes associated with LRRK2 function and dysfunction. FEBS J. 282, 2806–2826. https://doi.org/10.1111/febs.13305.

Walter, J., Bolognin, S., Antony, P.M.A.A., Nickels, S.L., Poovathingal, S.K., Salamanca, L., Magni, S., Perfeito, R., Hoel, F., Qing, X., et al., 2019. Neural stem cells of Parkinson's disease patients exhibit aberrant mitochondrial morphology and functionality. Stem Cell Rep. 12, 878–889. https://doi.org/10.1016/j.stemcr.2019.03.004.

Wang, X., Negrou, E., Maloney, M.T., Bondar, V.V., Andrews, S.V., Montalban, M., Llapashtica, C., Maciuca, R., Nguyen, H., Solanoy, H., et al., 2021. Understanding LRRK2 kinase activity in preclinical models and human subjects through quantitative analysis of LRRK2 and pT73 Rab10. Sci. Rep. 11 https://doi.org/10.1038/s41598-021-91943-4.

Xu, H., Wang, Y., Song, N., Wang, J., Jiang, H., Xie, J., 2018. New progress on the role of glia in iron metabolism and iron-induced degeneration of dopamine neurons in Parkinson's disease. Front. Mol. Neurosci. 10 https://doi.org/10.3389/fnmol.2017.00455.

Xu, E., Boddu, R., Abdelmotilib, H.A., Sokratian, A., Kelly, K., Liu, Z., Bryant, N., Chandra, S., Carlisle, S.M., Lefkowitz, E.J., et al., 2022. Pathological α-synuclein

recruits LRRK2 expressing pro-inflammatory monocytes to the brain. Mol. Neurodegener. 17, 1–19. https://doi.org/10.1186/s13024-021-00509-5.

Zhang, X., Gao, F., Wang, D., Li, C., Fu, Y., He, W., Zhang, J., 2018. Tau pathology in Parkinson's disease. Front. Neurol. 9, 809. https://doi.org/10.3389/fneur.2018.00809.

Zhao, Y., Keshiya, S., Perera, G., Schramko, L., Halliday, G.M., Dzamko, N., 2020. LRRK2 kinase inhibitors reduce alpha-synuclein in human neuronal cell lines with the G2019S mutation. Neurobiol. Dis. 144, 105049 https://doi.org/10.1016/j.nbd.2020.105049.

Zheng, Y.R., Zhang, X.N., Chen, Z., 2019. Mitochondrial transport serves as a mitochondrial quality control strategy in axons: Implications for central nervous system disorders. CNS Neurosci. Ther. 25, 876–886. https://doi.org/10.1111/cns.13122.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R.J., Calne, D.B., et al., 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44, 601–607. https://doi.org/10.1016/j.neuron.2004.11.005.