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Using patient-derived induced pluripotent stem cells to identify Parkinson's disease-relevant phenotypes

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Abstract

Purpose of Review: Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting older individuals. The specific cause underlying dopaminergic (DA) neuron loss in the substantia nigra, a pathological hallmark of PD, remains elusive. Here we highlight peer-reviewed reports using induced pluripotent stem cells (iPSCs) to model PD *in vitro* and discuss the potential disease-relevant phenotypes that may lead to a better understanding of PD etiology. Benefits of iPSCs are that they retain the genetic background of the donor individual and can be differentiated into specialized neurons to facilitate disease modeling.

Recent Findings: Mitochondrial dysfunction, oxidative stress, ER stress, and alpha-synuclein accumulation are common phenotypes observed in PD iPSC-derived neurons. New culturing technologies, such as directed reprogramming and midbrain organoids, offer innovative ways of investigating intraneuronal mechanisms of PD pathology.

Summary: PD patient-derived iPSCs are an evolving resource to understand PD pathology and identify therapeutic targets.

Keywords

Mitochondria; Oxidative stress; Dopaminergic neurons; Alpha synuclein; LRRK2; Gene editing

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Conflict of Interest

The authors declare no conflict of interest.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a recent study estimating that 1.2 million people in the United States will be living with PD by the year 2030 [1]. PD typically affects older individuals and leads to a host of motor and non-motor symptoms due to the loss of dopaminergic (DA) neurons in the substantia nigra and other areas of the central and peripheral nervous system. Over the last few decades, mutations in a number of genes have been directly linked to PD or linked to an increased risk for developing PD [reviewed in 2]. Environmental factors such as toxins, pesticides, and head trauma have also been linked to PD [reviewed in 3]. However, the vast majority of PD cases do not have a known genetic component or identifiable environmental exposure, and the direct cause of PD still remains elusive. The prevailing hypotheses for neuronal loss in PD combines mitochondrial malfunction, increased oxidative stress, inflammation, and accumulation of misfolded alpha-synuclein protein.

There are a variety of ways to study PD and its mechanisms of neurodegeneration. Models range across the spectrum from in vitro cell lines, to flies and worms, to non-human primates and human patient samples with each system presenting advantages and disadvantages. This review focuses on the human-based model system of induced pluripotent stem cells (iPSCs) generated from PD patients. Shortly after the groundbreaking discovery that human adult somatic cells could be reprogrammed back to an embryonic like state through the forced expression of a subset of pluripotency transcription factors [4, 5], human disease modeling entered a new frontier. Scientists now had the capability of using patient-derived skin cells and other somatic cells (e.g. peripheral blood derived mononuclear cells and excreted kidney epithelial cells in urine) to generate iPSCs that offer a virtually endless source of human cells, retain the unique genetic background of each donor, can be chemically and/or transcriptionally coaxed to become the specific cell type(s) affected in disease, and can be used to specifically interrogate mechanistic processes involved in disease. In that regard, PD studies have preferentially used iPSCs differentiated towards a nigral DA neuron fate, as their loss triggers the typical motor signs of PD. iPSCs serve a unique role in biomedical research, and the goal of this review is to discuss how iPSCs are being used to study PD, with an emphasis on recent phenotypes identified in PD-iPSC derived DA neurons.

PD Relevant Phenotypes Identified from iPSC Models

A major advantage of the iPSC model system for PD is the ability to generate midbrain-patterned DA neurons from human PD patients with diverse genetic backgrounds to characterize morphological and functional deficiencies. As discussed in detail below and shown in the Table, some of the observed phenotypes, such as mitochondrial dysfunction, are shared across different genetic backgrounds and have a clear relation to human PD pathology. However, other phenotypes, such as neurite outgrowth defects, may be more limited to a particular genetic background and may not be directly related to the developmental pathogenesis of PD. Although PD iPSCs have been used to assess a number of disease-relevant characteristics, including calcium signaling, DA release and uptake, and synaptic activity [6–10], below we will specifically discuss the recent studies examining

mitochondrial dysfunction, oxidative stress, endoplasmic reticulum (ER) stress, and alpha-synuclein accumulation in PD iPSC-derived neurons.

Mitochondrial Dysfunction

Mitochondria are dynamic organelles that play important roles for ATP production and cellular metabolism. Several molecular pathologies observed in PD patient samples converge on mitochondrial dysfunction, including decreased mitochondrial Complex I activity [11–14], deregulation of mitochondrial protein expression [15–17], and dysregulation of glucose metabolism [18]. Furthermore, mutations in the PD-associated genes *PARKIN*, *PINK1*, *DJ1*, and *LRRK2* either directly or indirectly disrupt mitochondrial function [reviewed in 19]). Currently, a major focus of the iPSC field has been characterizing mitochondrial defects in PD derived neurons and identifying possible underlying mechanisms that may explain the observed phenotypes.

Mitochondria in PD patient iPSC-derived neurons have morphological and functional alterations (Table 1). Mitochondria from *PARKIN*, *PINK1*, and *GBA* iPSC-derived DA neurons are swollen and disorganized [20–23]. Although these morphological changes have not been thoroughly characterized in postmortem PD brain samples, an increase in swollen and disorganized mitochondria is abnormal and possibly reflects mitochondria malfunction. An increase in fragmented mitochondria [24–29] and a decrease in mitochondrial content [23, 26, 30] were also present in iPSC-derived DA neurons from *PINK1*, *PARKIN*, *SNCA A53T*, *LRRK2 G2019S*, and *OPA1* patients, overall showing a decrease in the number of functional mitochondria available inside neurons. Clearance of unhealthy mitochondria via mitophagy is also impaired in *LRRK2 G2019S* and *PARKIN* iPSC-derived DA neurons, suggesting that other mitochondrial-related cellular mechanisms may be affected and are unable to detect mitochondrial stress [31–33]. Interestingly, mitochondrial content in *LRRK2 G2019S* iPSC-derived glutamatergic and sensory neurons was not found to be significantly different from their control counterparts, suggesting an intrinsic defect to DA neurons [30].

Having fragmented mitochondria and lowered mitochondrial content in DA neurons implies that the cells' source of energy is compromised, and therefore many cellular processes may be affected. For example, two recent studies reported reduced levels of nicotinamide adenine dinucleotide (NAD⁺), a key metabolic substrate involved in cellular energy production, in PD iPSC-derived DA neurons [22, 30]. In the first study, *LRRK2 G2019S* iPSC-derived DA neurons, but not glutamatergic or sensory neurons, had diminished levels of NAD⁺, perhaps making DA neurons susceptible to bioenergetic issues [30]. Furthermore, sirtuins, NAD⁺-dependent deacetylases that have important roles in mitochondrial health, metabolism, and aging, had decreased activity, suggesting a possible mechanism by which mitochondrial dysfunction is manifesting. In the second study, *GBA* patient iPSC-derived DA neurons had a decreased ratio of NAD⁺/NADH and alterations in expression of proteins that regulate NAD⁺ levels [22]. Treatment with the NAD⁺ precursor nicotinamide riboside (NR) increased NAD⁺ levels in *GBA* DA neurons and rescued mitochondrial respiration and dynamics, identifying a nexus between decreased NAD⁺ levels and mitochondrial dysfunction in PD DA neurons.

Mitochondrial respiration and bioenergetics are altered in *OPA1*, *GBA*, *LRRK2 G2019S*, *PINK1*, and *PARKIN* patient iPSC-derived neurons. By measuring oxygen consumption rate (OCR), it was found that basal respiration, spare respiration, and ATP-linked respiration are decreased along with ATP levels in PD patient iPSC-derived neural progenitor cells and DA neurons [22, 26, 30]. These results match findings in the substantia nigra of PD patients where ATP synthase levels were also decreased [15]. Damage to mitochondrial DNA (mtDNA) can be found in nigral samples of PD patients and correlates to defects in respiration [34]. Interestingly, *LRRK2 G2019S* PD iPSC-derived DA neurons show increased mtDNA damage [35], potentially explaining respiratory defects in these cells. Of note, the mtDNA damage was only observed in the differentiated *LRRK2 G2019S* DA neurons and not in the parental fibroblasts or in the undifferentiated iPSCs [35], which points to the idea that certain pathological processes may develop in a cell-type specific way. Since the genes that mtDNA encode make up subunits of the electron transport chain [36], it is highly possible that mtDNA damage could underlie the complex I deficiency observed in PD patients [14, 37–39] and in *PARKIN* mutant iPSC-derived neurons [29]. As the electron transport chain is the main source of reactive oxygen species (ROS) in the cell, these mitochondrial defects could lead to an increase in oxidative stress within PD DA neurons.

Oxidative Stress

Oxidative stress is recognized as a main factor in the pathogenesis of PD [reviewed in 40]). The total levels of oxidized proteins are highly increased in the brains of PD patients [41–43], including the levels of oxidized mitochondrial proteins [12, 44], as well as DNA and lipids [45]. In addition, several antioxidant proteins are also dysregulated in PD patients [12, 46, 47]. These oxidative stress phenotypes are most likely due to a culmination of mitochondrial dysfunction as well as intrinsic dopamine oxidation occurring within DA neurons [48].

PD iPSC-derived DA neurons present oxidative stress phenotypes similar to the ones found in nigral samples of PD patients (Table 1). An increase in carbonylated proteins, which reflects protein oxidation, has been observed in *PARKIN*, *LRRK2 I2020T*, and *OPA1* patient iPSC-derived DA neurons [6, 8, 26]. Monoamine oxidase (MAO)-A and MAO-B, which are proteins involved in the oxidation of dopamine, have also been found to be upregulated in *PARKIN*, *SNCA triplication*, and *LRRK2 G2019S* iPSC-derived DA neurons [6, 49, 50]. Interestingly, decreases in MAO-A and MAO-B expression and a reduction in carbonylated proteins were found when wild type parkin was overexpressed, suggesting that there is a direct correlation between a PD-related gene and oxidative stress [6]. Antioxidant genes, including Nrf2 [21, 26], NQO1 [21], and SOD1 and SOD2 [26] are also upregulated in *PARKIN* and *OPA1* iPSC-derived DA neurons, hinting that endogenous upregulation of antioxidant pathways may be a compensatory mechanism to prolong DA neuron health and function.

Endoplasmic Reticulum (ER) Stress

The main functions of the ER are protein synthesis and protein folding [51]. During ER dysfunction and stress, proteins become unfolded and can aggregate in the cell. In response to ER stress, the unfolded protein response (UPR) becomes activated to mitigate cellular

damage that can occur from the accumulation of misfolded proteins. Consistent with a relationship between protein aggregation and cellular stress [reviewed in 40]), current evidence demonstrates an upregulation of ER stress and UPR related genes in the substantia nigra of PD patients [52–54]. Although still underexplored compared to mitochondrial dysfunction, ER stress has been gaining attention in the iPSC field (Table 1). Increased levels of ER stress and UPR related genes, including *BiP*, *PDI*, *phospho-eIF2 α* , *IRE1 α* , and *nicotrin*, were identified in *SNCA A53T*, *LRRK2 G2019S*, and *GBA* iPSC-derived DA and cortical neurons [22, 55–58]. Since protein aggregation and the accumulation of misfolded alpha-synuclein is a common phenotype found in PD patients, using iPSC models may help us better understand the molecular mechanisms underlying ER stress that could be contributing to protein aggregation and DA neuron loss in PD.

Alpha-Synuclein Accumulation

The alpha-synuclein locus (*SNCA*) was the first gene to be linked to PD [59]. *SNCA* mutations include A53T, A30P, E46K, G51D, and multiplications, all causing dominant early-onset PD [60, 61]. Interestingly, the T53 sequence variant of alpha-synuclein is only pathological to humans as most other vertebrate species naturally express T53 with no known neuronal deficit [62]. Alpha-synuclein is a presynaptic protein that can be found free and soluble in the cytoplasm or bound to the intracellular aspect of the lipid bilayer [63]. It is involved in cell survival, antioxidation, neuronal differentiation, and regulation of dopamine biosynthesis [64]. Abnormal alpha-synuclein aggregation has long been implicated in PD pathogenesis as it is the main protein found in Lewy bodies (LBs; intracytoplasmic eosinophilic aggregates) and Lewy neurites (LNs; abnormal filament-containing neurites), hallmark features of both familial and sporadic PD [65]. LB formation is proposed to start with abnormal accumulation and phosphorylation of alpha-synuclein in the neuronal cytoplasm, which then forms pale bodies (irregularly dense aggregates), that then become the stereotypical dense core and translucent peripheral halo structure that characterize LBs [65]. Thus, a focus in the iPSC disease modeling field is to recapitulate alpha-synuclein accumulation in iPSC-derived neurons to understand the potential toxic properties of alpha-synuclein aggregation.

Normal levels of alpha-synuclein have been observed in *SNCA A53T* and sporadic patient iPSC-derived DA neurons [27, 66], whereas other studies examining *SNCA triplication*, *LRRK2 G2019S*, *PARKIN*, *PINK1*, *GBA*, and sporadic patient derived DA, cortical, and sensory neurons have noted increased protein levels of alpha-synuclein (Table 1). Only a handful of studies have characterized the accumulation of abnormal phosphorylated, aggregated, and insoluble forms of alpha-synuclein [7, 20, 23, 67]. Phosphorylated alpha-synuclein at serine 129 (pS129) is the most abundant form found in LBs [68], making this post-translationally modified protein important to identify in patient iPSC-derived neurons in vitro. Increased levels of phosphorylated alpha-synuclein were identified in *LRRK2 G2019S*, *SNCA triplication*, *PARKIN*, and sporadic patient iPSC-derived DA neurons indicating that this abnormal protein expression is common across different PD genotypes [69]. Elevated levels of phosphorylated alpha-synuclein were also present in iPSC-derived cortical neurons from *SNCA A53T* mutation carriers [7], while aggregated alpha-synuclein (observed as increased immunolabeled puncta) was found in iPSC-derived cortical neurons

from a patient with the *SNCA* triplication [67]. Accumulation of insoluble alpha-synuclein (assessed by western blot using insoluble extracts) in *PARKIN* and *PINK1* iPSC-derived DA neurons was also reported [20]. Together, these data indicate that PD patient-derived DA neurons can exhibit abnormally altered alpha-synuclein protein profiles, but it is important to note that alpha-synuclein accumulation associated with LB formation has not yet been observed. Additionally, the processes by which accumulated alpha-synuclein could induce DA neuron toxicity remain to be elucidated.

Reversal of phenotypes using gene editing

From the observation that iPSC-derived DA neurons generated from PD patients exhibit potentially disease-relevant phenotypes, critical questions have emerged regarding whether specific mutations are responsible for the deficits. Genomic editing techniques, including clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 and zinc finger nuclease (ZFN) technology allow for site-directed mutagenesis to manipulate the cell and help address these questions.

The role of genomic editing in the *in vitro* modeling of PD is expanding ([reviewed in 70]). As described above, patient-derived cells expressing mutant *LRRK2 G2019S*, *SNCA triplication*, and *SNCA A53T* have been characterized to present PD-related pathology including mitochondrial dysfunction, oxidative stress, ER stress, and alpha-synuclein accumulation (Table 1). ZFN or CRISPR mediated correction of these mutations by way of site-directed mutagenesis has provided further evidence that PD-associated mutations are responsible for many of the observed cellular dysfunctions in these cells [35, 57, 67, 71–73]. For example, isogenic gene corrected lines using ZFN from two female heterozygous G2019S *LRRK2* PD patients were found to normalize DA neuron neurite outgrowth, defects in autophagy, altered alpha-synuclein and tau levels, and susceptibility to oxidative stress when compared to unedited G2019S lines [73]. A similar restoration of DA neurite outgrowth was found using CRISPR correction of G2019S *LRRK2* iPSCs [72]. In a different study, ZFN gene correction of the G2019S *LRRK2* mutation significantly reduced the levels of damaged mtDNA in the gene-corrected DA neurons compared to the isogenic unmodified DA neurons, despite similar levels of mtDNA genomes [35].

Aberrant alpha-synuclein levels and ER stress have also been reversed in PD iPSC-derived cortical neurons using gene editing techniques. Using ZFNs to correct *SNCA A53T* in iPSCs resulted in lower alpha-synuclein protein levels in corrected neurons compared to unedited neurons [71]. Similarly, following CRISPR-mediated removal of two *SNCA* alleles from *SNCA triplication* iPSCs, the differentiated neurons exhibited lowered levels of alpha-synuclein aggregation, normalization of NADH levels, and lowered levels of the ER stress response compared to unedited *triplication* neurons [57, 67]. It is important to note that these three studies were performed using cortical neuron cultures rather than DA neuron cultures, which suggests that some PD-related cellular defects may be shared across different neuronal subtypes. Taken together, as gene editing technology moves closer to a therapeutic reality [reviewed in 74]), iPSC-based studies offer valuable insight into what role specific PD-associated gene mutations play in human neuronal pathology.

Limitations of Current iPSC Studies and Potential Alternatives

Among the numerous advantages that patient-derived iPSCs have as an experimental model system for human disease are their virtually limitless proliferative capacity and their ability to differentiate into essentially all cell types in the human body. These are particularly important features, as access to postmortem human tissue samples is rare, the postmortem tissues that are available are not easily expandable, and the cell types impacted by disease may not be accessible at all. However, despite these advantages, iPSCs are not a perfect system and do have important caveats to their use.

DA neuron differentiation protocols are effective, but overall differentiation efficiencies can vary from protocol to protocol, which may cause inconsistent results across laboratories [75]. Additionally, neurons generated from iPSCs are fetal-like with respect to their transcriptional profiles and electrical maturation [76, reviewed in 77], although one study found that purifying the iPSC-derived DA neuron population improves the transcriptional alignment with more mature DA neurons [78]. As PD is a disease associated with aging, their “youthfulness” may limit iPSC-derived neurons’ ability to accurately model phenotypes found in PD patients. In that regard, one of the major features of PD is loss of DA neurons, yet iPSC-derived DA neurons generated from PD patients have no viability problems in culture without the addition of an exogenous stressor [8, 23, 49, 50, 69, 73, 79, 80]. A few options have emerged to overcome the “fetal-age” limitation of iPSC-derived cells. One study utilized the overexpression of progerin, a mutant form of laminin A associated with the premature aging disease Hutchinson-Gilford Progeria Syndrome, to accelerate an aging process in *PINK1* and *PARKIN* PD patient iPSC-derived DA neurons [81]. Progerin overexpression lead to enhanced apoptosis activation and shortened neurites in PD iPSC-derived DA neurons. More interestingly, when the progerin-treated DA neurons were transplanted into the striatum of 6-hydroxydopamine treated mice, the transplanted DA neurons exhibited reduced survival, multilamellar inclusions, fibrillar bodies, and intracellular accumulation of neuromelanin [81]. A caveat to this approach is that since aberrant expression of progerin causes abnormal aging, it is difficult to distinguish between disease relevant age-related phenotypes and progerin-specific phenotypes. Alternatively, another study treated *PINK1* and *PARKIN* PD patient iPSC-derived DA neurons with the telomerase inhibitor 2-[(E)-3-naphthalen-2-yl-but-2-enoylamino]-benzoic acid (BIBR1532) and found some modest reduction in neurite branching in the *PARKIN* neurons; and increased mitochondrial ROS, DNA damage marks, and a reduction in overall tyrosine hydroxylase staining in both *PINK1* and *PARKIN* neurons [82]. Finally, newer directed reprogramming protocols have been developed in which somatic cells are directly induced into the cell type of interest [reviewed in 83]. These induced cells retain epigenetic marks associated with aged tissues, as well as disease-specific phenotypes that are not readily observed in iPSC-derived neurons, including protein aggregation and degeneration [84–86]. For example, a recent study using directed reprogramming of Huntington’s disease (HD) patient fibroblasts to medium spiny neurons showed robust neuronal huntingtin protein aggregation and endogenous neuronal toxicity [84], neither of which were previously demonstrated for HD iPSC-derived medium spiny neurons [87–89]. In the context of PD, only a small number of papers describe the directed reprogramming of fibroblasts to DA

neurons [90–93]; however, direct reprogramming has not been used to specifically model PD, thereby offering new areas of exploration.

Another potential pitfall to the current PD iPSC-based studies is that iPSC-derived neurons are differentiated and tested in two-dimensional monolayer cultures systems, which may not adequately represent the complex signaling and structure of the three-dimensional in vivo environment. Recently, multilayered, midbrain organoid culture systems have been developed from iPSCs that may help circumvent this problem [94–97]. These midbrain organoids contain DA neurons, neuromelanin-producing neurons [94], neural progenitor cells [94–97], astrocytes [96, 97], and oligodendrocytes [97], which when derived from PD patient iPSCs could help us better understand the complex processes underlying DA neuron death.

Finally, there are many aspects of PD pathology that have not been sufficiently modeled by the current iPSC studies. As mentioned previously, no LB formation has been observed in PD iPSC-derived DA neurons. Similarly, neuroinflammation is a clear feature of PD pathology [reviewed in 98]), but the contribution of astrocytes, microglia, and the blood-brain barrier have not been studied in the PD iPSC system. Finally, PD-associated damage is not limited to the nigral DA neurons and the motor system as peripheral non-motor systems also exhibit disease-associated pathology [99–103]. As iPSCs can generate a wide array of cellular subtypes (e.g. astrocytes, microglia, and sensory neurons), future work combining these cell types with DA neurons will hopefully provide a more complete and robust picture of PD pathology, susceptibility patterns, and etiologies.

Conclusion

Human patient-derived iPSCs are another tool in the repertoire to help us better understand PD pathology and the molecular mechanisms underlying neurodegeneration in PD. There are still challenges regarding the use of iPSCs. However, the evolving and improving culturing techniques and differentiation protocols combined with genome editing technologies are opening additional avenues for meticulous investigation of the molecular pathologies underlying PD that could become therapeutic targets. iPSCs offer a unique opportunity to study the development of human diseases, which will add to the wealth of information gained from other available model systems to facilitate the translation of basic science discoveries into clinical treatments.

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Table 1:

Peer-reviewed reports on PD patient-derived iPSCs

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
GBA (Het N370S, Het L444P, and Het RecNcil)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	n.r.	Increased glycosphingolipids; ZFN correction of α -syn protein	[104]
GBA (Het N370S)	DA	Increased extracellular α -syn protein	n.r.	n.r.	Increased BiP/GRP78, calreticulin, PDI, calnexin, and IRE1alpha	No issues	Autophagy/lysosome defects; Abnormal lipid profiles	[56]
GBA (Het N370S)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	No issues	Reduced GBA protein and activity; Increased susceptibility to toxins	[80]
GBA (Het N370S, Het L444P, and Het RecNcil)	DA	n.r.	Abnormal morphology; Decreased respiration; Increased mitoROS; Defective dynamics	n.r.	Increased BiP, XBP1s, phospho-eIF2 α	n.r.	Abnormal NAD metabolism; NAD + precursor rescue	[22]
LRRK2 (G2019S)	DA	Increased α -syn protein	n.r.	Increased NOX1 and MAO-B	n.r.	No issues	Increased susceptibility to toxins; Increased caspase activity; Increased expression of HSPB1	[50]
LRRK2 (G2019S)	DA	n.r.	Impaired respiration; Dysfunctional mobility	n.r.	n.r.	No issues	Increased susceptibility to stress; Pharmacologic rescue	[79]
LRRK2 (G2019S)	NSC	n.r.	n.r.	n.r.	n.r.	Clonal expansion deficiency	Increased susceptibility to proteosomal stress; Nuclear envelope defects	[105]
LRRK2 (G2019S)	DA	Abnormal α -syn protein in cytoplasm	n.r.	n.r.	n.r.	Long-term culture	Impaired autophagy and clearance	[66]
LRRK2 (G2019S)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	No issues	Increased susceptibility to toxins; Increased tau protein; ZFN rescue	[73]
LRRK2 (G2019S)	DA	n.r.	Increased fragmentation; Decreased ATP; Increased mitoROS	n.r.	n.r.	n.r.	Increased lysosome activity; Pharmacologic rescue	[28]

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
LRRK2 (G2019S)	DA	n.r.	Increased mtDNA damage	n.r.	n.r.	No issues	ZFN rescue	[35]
LRRK2 (G2019S)	DA	n.r.	n.r.	n.r.	n.r.	No issues	DNA methylation changes	[106]
LRRK2 (G2019S)	DA	n.r.	n.r.	n.r.	n.r.	No issues	NGF or LRRK2 kinase inhibitor rescue	[9]
LRRK2 (G2019S)	Sensory	n.r.	n.r.	n.r.	n.r.	No issues	Increased tau levels; Abnormal calcium dynamics; Pharmacologic rescue	[9]
LRRK2 (G2019S)	DA	n.r.	n.r.	n.r.	n.r.	No issues	Increased levels of autophagy genes	[107]
LRRK2 (G2019S)	DA	n.r.	Delayed arrest	n.r.	n.r.	n.r.	Delayed axonal mitophagy; Miro accumulation	[31]
LRRK2 (G2019S)	DA	Increased phospho α -syn protein	n.r.	n.r.	n.r.	Long-term culture	Decreased neuronal firing rate; Increased susceptibility to toxins	[69]
LRRK2 (G2019S)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	No issues	Altered NFKB activation; LRRK2 knock-down decreases α -syn protein but not mRNA	[108]
LRRK2 (G2019S)	DA	n.r.	n.r.	n.r.	Increased nicastrin	n.r.		[58]
LRRK2 (G2019S)	DA	n.r.	Decreased content and distribution; Increased velocity and motility; Decreased respiration	n.r.	n.r.	No issues	Decreased NAD ⁺ levels; Increased sirtuin expression but decreased deacetylase activity; no effect of LRRK2 kinase inhibition	[30]
LRRK2 (G2019S)	Cortical	n.r.	Decreased respiration	n.r.	n.r.	No issues		[30]
LRRK2 (G2019S)	Sensory	n.r.	No issues	n.r.	n.r.	No issues		[30]
LRRK2 (G2019S)	DA	S129 phospho- α -syn correlates with neurite extension	n.r.	n.r.	n.r.	n.r.	Mutation repaired by CRISPR; LRRK2 kinase inhibitor rescued neurite defects	[72]
LRRK2 (I2020T)	DA	n.r.	n.r.	Increased carbonylated proteins	n.r.	No issues	Lowered LRRK2 and phospho-AKT levels; Increased levels of caspase 3; Lowered dopamine release; Autophagy dysfunction; Increased phospho-tau; Increased	[8]

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
							susceptibility to oxidative stress	
OPA1 (p.G488R and p.A495V)	DA	n.r.	Fragmentation; Reduced content; ATP deficits; Respiration impairment	Increased ROS; Decreased BODIPY; Increased carbonylated proteins; Increased SOD1, SOD2, Nrf2, SESTRIN3 and GPX1	n.r.	Reduced	OPA1 expression rescued mitochondrial and oxidative stress phenotypes; Inhibition of necroptosis rescued survival phenotype	[26]
PARKIN (Ex2–4 del & Ex6–7 del)	Mixed Neuron Cultures	Increased α -syn protein	Abnormal morphology; Impaired turnover; Dysregulation of homeostasis	Lowered GSH levels; Increased Nrf2 and NQO1	n.r.	n.r.		[21]
PARKIN (Ex3 del / Ex5 del & Ex3 del / Ex 3 del)	DA	n.r.	No issues	Increased carbonylated proteins; Increased MAO-A and MAO-B	n.r.	No issues	Increased spontaneous dopamine release; Decreased dopamine uptake; Overexpression of parkin rescue	[6]
PARKIN (V324A)	DA	n.r.	n.r.	n.r.	n.r.	Increased apoptosis; Deregulation of AKT signaling pathway	Used progerin to induce phenotypes	[81]
PARKIN (Ex3 del / Ex5 del & Ex3 del / Ex 3 del)	DA	n.r.	n.r.	n.r.	n.r.	n.r.	Reduced microtubule stability; WT parkin rescue	[109]
PARKIN (Ex3, 5, & 6 del)	NPC	n.r.	Increased fragmentation after cytotoxicity	Increased ROS after cytotoxicity	n.r.	No issues	Increased sensitivity to cytotoxicity	[110]
PARKIN (Ex3 del / Ex5 del & Ex3 del / Ex 3 del)	DA	n.r.	n.r.	n.r.	n.r.	No issues	Increase in spontaneous dopamine release; Decrease in dopamine uptake	[10]
PARKIN (del202–203AG and IVS1+1G/A)	DA	n.r.	n.r.	n.r.	n.r.	n.r.	Imbalance in programmed cell death systems	[111]
PARKIN (Ex3del, R42P, Ex3–4del, 1-BP del 255A, R275W, R42P)	DA	Increased α -syn protein and aggregation	Lowered content	n.r.	n.r.	No issues	Impaired DA neuron differentiation; Increased susceptibility to toxicity	[23]
PARKIN (V324A)	DA	Increased α -syn protein and insoluble accumulation	Abnormal morphology; Increased mito stress	n.r.	n.r.	No issues	Upregulation of dopamine; Increased susceptibility to mito stress	[20]

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
PARKIN (c. 255delA)	DA	Increased phospho- α -syn	n.r.	n.r.	n.r.	No issues	Increased susceptibility to neurotoxins	[69]
PARKIN (V324A)	DA	n.r.	n.r.	Increased mitoROS	n.r.	No issues	Used telomerase inhibition to induce phenotypes; Increased DNA damage	[82]
PARKIN (Ex2-4 del & Ex6-7 del)	DA	n.r.	Impaired mitophagy; Disrupted quality control	n.r.	n.r.	No issues		[32]
PARKIN (Ex2-4 del & Ex6-7 del)	DA	n.r.	Impaired mitophagy	Increased ROS production	n.r.	No issues	Increased apoptosis	[33]
PARKIN (c. 1072delT, p.A324fsX110)	DA	n.r.	Reduced complex I activity; Increased branching and fragmentation	n.r.	n.r.	No issues		[29]
PARKIN (Ex3 del / Ex5 del, Ex3 del / Ex 3 del, Ex3 del / R42P)	DA	n.r.	n.r.	n.r.	n.r.	No issues	Activation of D1 receptors causes oscillatory activities; WT parkin rescue	[112]
PARKIN (Ex2-4 del & Ex6-7 del)	DA	n.r.	Increased fragmentation	n.r.	n.r.	n.r.		[25]
PARKIN (Ex2-4 del & Ex6-7 del)	DA	n.r.	n.r.	n.r.	n.r.	No issues	Increased soluble epoxide hydrolase (sEH); Increased basal apoptosis; sEH inhibitor protected against apoptosis	[113]
PINK1 (Q456X)	DA	n.r.	Reduced mtDNA	n.r.	n.r.	No issues	Increase in PGC1 α expression	[114]
PINK1 (Q456X)	DA	n.r.	Impaired respiration	Increased mitoROS after cytotoxicity; Reduced GSH after cytotoxicity	n.r.	No issues	Increased susceptibility to cellular stress	[79]
PINK1 (Q456X)	DA	n.r.	n.r.	n.r.	n.r.	Increased apoptosis; Dereglulation of AKT signaling pathway	Used progerin to induce phenotypes	[81]
PINK1 (-Ex7/del & -Ex7)	DA	n.r.	Increased fragmentation	n.r.	n.r.	No issues	Increased LRRK2 expression; Reversed with WT PINK1	[24]
PINK1 (Q456X)	DA	Increased α -syn protein and insoluble accumulation	Abnormal morphology; Increased mito stress	n.r.	n.r.	No issues	Upregulation of dopamine; Increased	[20]

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
							susceptibility to mito stress	
PINK1 (Q456X)	DA	n.r.	n.r.	Increased mitoROS	n.r.	No issues	Used telomerase inhibitor to induce phenotype; increased DNA damage	[82]
PINK1 (p.C388R/p.C388R)	DA	n.r.	Disrupted mitochondrial quality control	n.r.	n.r.	No issues		[32]
SNCA (A53T)	Cortical	n.r.	n.r.	n.r.	Increased Nicastrin, BiP, and PDI	n.r.	Increased nitrosative stress	[55]
SNCA (A53T)	Cortical	Increased α -syn protein	n.r.	n.r.	n.r.	No issues	ZFN correction	[71]
SNCA (A53T)	Cortical	n.r.	n.r.	n.r.	n.r.	n.r.	Perturbed protein translation	[58]
SNCA (G209A)	DA	Increased phospho- α -syn; α -syn aggregation	n.r.	n.r.	n.r.	No issues	Synaptic defects	[7]
SNCA (A53T)	DA	No issues	Decreased membrane potential; Fragmentation; Decreased volume	n.r.	n.r.	No issues		[27]
SNCA (triplication)	DA	Increased α -syn protein	n.r.	Increased MAO-A and HMOX2	n.r.	No issues	Increase in protein-aggregation genes; Increased susceptibility to oxidative stress	[49]
SNCA (triplication)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	No issues		[115]
SNCA (triplication)	NPC	Increased α -syn protein	Decreased function; Altered energy balance and lowered ATP	Increased basal ROS; Increased ROS production	n.r.	Reduced proliferative capacity	Increased susceptibility to stress; Partial rescue with shRNA	[116]
SNCA (triplication)	DA	Increased α -syn protein	n.r.	n.r.	n.r.	n.r.	Impaired differentiation; Delayed maturation; Lowered neuronal activity; Increased autophagic flux	[117]
SNCA (triplication)	Sensory	Increased α -syn protein	n.r.	n.r.	n.r.	No issues		[9]
SNCA (triplication)	DA	Increased phospho- α -syn	n.r.	n.r.	n.r.	Long-term culture	Increased susceptibility to neurotoxins	[69]
SNCA (triplication)	Cortical	Increased α -syn protein	n.r.	n.r.	Increase in ER stress & UPR genes	No issues	CRISPR rescue	[57]
SNCA (triplication)	NPC	Increased α -syn protein	n.r.	n.r.	n.r.	n.r.	DNA damage	[118]

Gene	Cell Type	α -syn	Mitochondria	Oxidative Stress	ER & UPR	Viability	Other	Ref
SNCA (triplication)	DA	n.r.	n.r.	n.r.	n.r.	n.r.	Many differentially expressed genes including SNCA, PARK2, PINK1	[119]
SNCA (triplication)	DA	Increased α -syn protein	Decreased membrane potential; Fragmentation; Decreased volume	n.r.	n.r.	No issues		[27]
SNCA (triplication)	Cortical	Increased α -syn aggregation	Decreased membrane potential	Increased redox index	n.r.	Increased cell death at basal	Abnormal NADH levels; CRISPR correction	[67]
Sporadic (no known mutation)	DA	No issues	n.r.	n.r.	n.r.	Long-term culture	Impaired autophagy; Defective autophagosome clearance	[66]
Sporadic (no known mutation)	DA	n.r.	n.r.	n.r.	n.r.	No issues	DNA methylation changes	[106]
Sporadic (no known mutation)	DA	Increased phospho- α -syn	n.r.	n.r.	n.r.	Long-term culture		[69]
Sporadic (LRRK2 RS1491923)	DA	No issues	No issues	n.r.	n.r.	No issues	No effect of LRRK2 kinase inhibition	[120]

Abbreviations: α -syn, alpha-synuclein; CRISPR, clustered regularly interspaced short palindromic repeats; DA, dopaminergic; del, deletion; ER, endoplasmic reticulum; Ex, exon; GBA, glucocerebrosidase; HET, heterozygous; LRRK2, leucine-rich repeat kinase 2; MAO-A/B, monoamine oxidase A/B; mitoROS, mitochondrial reactive oxygen species; NAD⁺, nicotinamide adenine dinucleotide; NGF, nerve growth factor; NPC, neural progenitor cells; n.r., not reported; NSC, neural stem cells; Ref, reference; UPR, unfolded protein response; WT, wildtype; ZFN, zinc finger nuclease