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Mitochondrial homeostasis in cellular models of Parkinson's disease

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Abstract

Mitochondrial function is an important factor in maintaining cellular homeostasis and its dysregulation has become a hallmark for multiple disease conditions. This review aims to synthesise the current knowledge by analysing changes of mitochondrial physiology parameters in Parkinson's disease (PD) and to evaluate the contribution of cellular models of PD in the field. We present a platform for further elucidation of mitochondrial function parameters that may potentiate disease progression.

Keywords - mitochondrial homeostasis, mitochondrial respiration, Parkinson's disease, cellular models of Parkinson's, qualitative analysis

1. Mitochondria and Parkinson's disease

Mitochondria comprise a dynamic organellar network with a central position in maintaining eukaryotic homeostasis. Besides their role in cellular bioenergetics, namely adenosine triphosphate (ATP) synthesis, these organelles support essential metabolic processes, regulation of calcium and reactive oxygen species (ROS) homeostasis as well as a multitude of signalling cascades. The mitochondrial compartments host functional molecular groups which coordinate protein import and sorting, transport of metabolites, mitochondrial DNA (mtDNA) replication and expression, oxidative phosphorylation (OXPHOS) respiratory system, metabolic enzymes protein and organelle quality control mechanisms as well as fusion and fission regulators. Dysfunction in these mitochondrial components leads to impaired homeostasis and has been linked to diseases, of which we shall focus here on Parkinson's disease.

Parkinson's disease (PD) is a progressive neurodegenerative disease characterised primarily by loss of dopaminergic neurons in the nigrostriatal pathway, presenting motor and non-motor clinical phenotypes. It is the most prevalent cause of parkinsonism, a broader clinical syndrome with motor features that include: hypokinesia, bradykinesia, muscle rigidity, joint stiffness, resting tremor, shuffling gait, expressionless face and micrography. Non-motor features comprise constipation, anxiety and depression, REM-sleep behaviour, and olfactory deficits (Poewe et al 2017). The neuropathological hallmark of PD are intracellular protein inclusions called Lewy bodies, consisting predominantly of α-Synuclein. Ageing, environment, and genetic susceptibility are implicated in PD and cellular defects leading to dopaminergic dysfunction are connected with defects in proteostasis, mitochondrial function, vesicle trafficking and lysosomal activity (Figure 1). The distinctiveness of these interactions gives rise to a spectrum of PD neuronal phenotypes that can be unique to individual patients, making development of suitable disease models challenging.

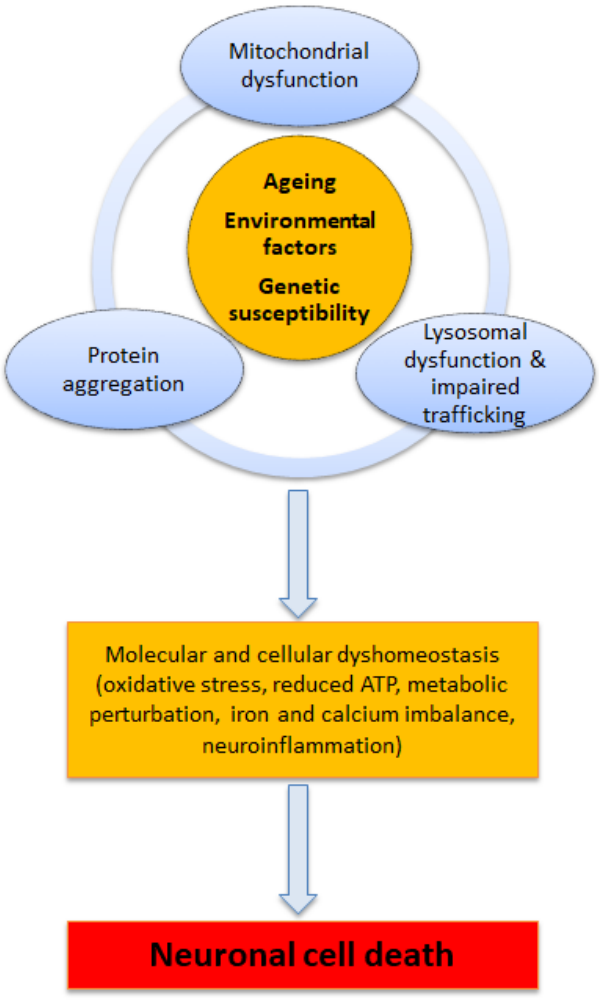

Figure 1. Key cellular defects in PD.

1.1. Respiratory physiology dysfunction as a key feature of PD

The investigation of mitochondria in PD began in early eighties when independent reports revealed that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was the likely cause of permanent parkinsonism in several patients. The parkinsonism phenotype was associated with degeneration of nigrostriatal dopaminergic neurons (Burns et al 1983; Langston et al 1983) and appearance of Lewy bodies in the Substantia Nigra (SN) (Davis et al 1979). It was found that the MPTP metabolite 1-methyl-4-phenylpyridinium (MPP+) can be taken up by dopaminergic transporters (DAT), and inhibit Complex I (CI) of the mitochondrial electron transfer system (ETS) resulting in nigrostriatal degeneration (Ramsay, Singer 1986; Ramsay et al 1986; Salach et al 1984; Vyas et al 1986). Subsequently, it was shown that CI activity was reduced by 20–30% in the SN of patients with sporadic PD (Schapira 2007; Schapira et al 1990), with reports of enzyme complex dysfunction that may affect other tissues as well (Bindoff et al 1989; Mortiboys et al 2008; Müftüoglu et al 2004; Parker et al 1989). In addition, mitochondria from post-mortem tissue, show greater age-dependent accumulation of mtDNA deletions and somatic mosaicism, compared to control subjects (Coxhead et al 2015; Dölle et al 2016; Giannoccaro et al 2017; Grünewald et al 2016).

1.2. Dissecting mitochondrial dysfunction in PD

Parkinsonism is a consistent and predominant feature in 23 monogenic disorders (Klein et al 2018; Marras et al 2016). Many of the identified genes encode proteins that have strong links to mitochondrial function as summarised in Table 1.

Table 1. Key genes linked to mitochondrial dysfunction in PD

Gene	Key biomolecular functions	Key effects of PD mutations on mitochondrial function and cellular homeostasis	References
PINK1 Recessive	**PTEN induced serine/threonine protein kinase 1** - Phosphorylates mitochondrial proteins; - Mediates activation and translocation of Parkin during mitophagy	- Reduced activity of Complexes CI, CII, and CIV - Impaired respiration in the striatum - Decreased respiration and membrane potential - Decreased ATP level and increased ROS - Impaired mitophagy - Impaired synaptic transmission	Amo et al 2011; Clark et al 2006; Gautier et al 2008; Ge et al 2020; Liu et al 2020; Liu et al 2011; Matsumine et al 1997; Morais et al 2009; Park et al 2006; Temelie et al 2018; Valente et al 2001; Yuan et al 2010

PRKN (Parkin) Recessive	**E3 ubiquitin protein ligase Parkin** - Ubiquitinates multiple substrates for proteasome-dependent protein degradation; - Regulates mitophagy by ubiquitination of mitochondrial proteins	- Reduced activity of Complexes CI and CII - Impaired mitophagy	Damiano et al 2014; Lehmann et al 2016
DJ-1 Recessive	**Parkinson's disease protein 7** Multifunctional protein protecting against oxidative stress and regulating mitochondrial homeostasis - Protein and nucleotide glycase; - Cysteine protease -Redox regulated chaperone - Parkin S-nitrosylation	- Reduced ATP synthesis and respiration - Increased ROS, reduced membrane potential, higher transition-pore opening (cell death) - Respiratory Complex stability, mitochondrial quality control, maintenance of cell redox balance; - Accelerates accumulation and aggregation of α-Synuclein in mice	Bonifati et al 2003; Giaime et al 2012; Klein, Lohmann-Hedrich 2007; Ozawa et al 2020; Xu et al 2017
LRRK2 Dominant	**Leucine rich repeat serine/threonine protein kinase 2** - Serine threonine protein kinase which phosphorylates a broad range of proteins. - GTPase activity - Scaffolding protein	- Decreased mitochondrial membrane potential and cellular ATP level - Modulates mitochondrial dynamics - Modulates cellular trafficking	Abeliovich, Gitler 2016; Mortiboys et al 2010; Xinglong Wang et al 2012
SNCA (α-Synuclein) Dominant	**Alpha-Synuclein** - Cellular trafficking - Roles in synaptic vesicle formation, docking and neurotransmitter release	- Impairment of CI-dependent respiration - Inhibition of mitochondrial protein import, - Mitochondrial membrane depolarisation - Mitochondrial fragmentation	Ludtmann et al 2018; Shahmoradian et al 2019; Wang et al 2019; Zambon et al 2019

VPS35 Dominant	**Vacuolar protein sorting-associated protein 35** - Component of the retromer cargo selective complex involved in endosomal trafficking and lysosomal degradation pathway	- Impaired mitochondrial dynamics and function - Mitochondrial fragmentation - Enhanced turnover of the mitochondrial DRP1 complexes - Defects in the CI and supercomplex assembly - Bioenergetic deficits - Impaired autophagy	Wang et al 2016; Zhou et al 2017
ATP13A2 Recessive	**Polyamine-transporting ATPase 13A2** - Lysosomal ATPase (P-type) - Regulates cellular homeostasis of cations	- Increased cellular sensitivity to extracellular zinc - Decreased ATP synthesis rates, - Higher frequency of mitochondrial DNA lesions - Increased respiration - Increased fragmentation of the mitochondrial network	Grünewald et al 2012
FBXO7 Recessive	**F-box only protein 7** - E3 ubiquitin protein ligase - Parkin recruitment to mitochondria	- Impaired mitochondrial clearance, - Proteasomal malfunctioning - Increased ROS and PARP overactivation (cell death)	Burchell et al 2013; Delgado-Camprubi et al 2017; Ilyin et al 2000; Shojaee et al 2008
CHCHD2 Dominant	**Coiled-coil-helix-coiled-coil-helix (CHCH) domain – containing protein 2** - Transcription factor - Proposed role in OXPHOS regulation, - Modulates cell death signalling	- Modifies mitochondrial reticular morphology Reduced oxidative phosphorylation - Apoptosis induction via cytochrome *c* destabilization - Impaired respiration - Increased mitochondrial ROS production	Cornelissen et al 2020; Lee et al 2018; Meng et al 2017

To illustrate how the proteins encoded by these genes interact with each other and the respiratory Complexes we have created a visual network for protein-protein interactions (Figure 2).

The network was built using input protein products for the genes in Table 1 PINK1, Parkin (PARK2), DJ-1 (PARK7), LRRK2, SNCA, VPS35, ATP13A2, FBXO7 and *CHCHD2*. The protein products of the genes in Table 1 were uploaded to InnateDB (Breuer et al 2013) (http://www.innatedb.com) pathway analysis tool and mapped to Pathway ID-2912,

source database: KEGG, $p < 1.0E-5$. The other genes (139) that were part of the Pathway ID-2912, were then used as an input for STRINGdb with the initial 9 genes, organism *Homo sapiens*, to generate the protein-protein interaction network. This protein network illustrated the complexity of interaction between the proteins linked to mitochondrial homeostasis found to be mutated in PD and the mitochondrial respiratory Complexes. Thus, the relevant PD mutations are likely to impact on many aspects of mitochondrial physiology. The PD models that we are discussing further will be instrumental in detailing these interactions and their effects on mitochondrial dysfunction in PD.

Genome Wide Association Studies (GWAS) have uncovered increasing numbers of PD risk alleles which remain to be studied in detail (Blauwendraat et al 2020; Chang et al 2017; Nalls et al 2014). As we go forward, idiopathic PD samples including primary fibroblasts, iPSc and iPSc derived neurons are more readily available to elucidate etiopathological roles of the mitochondria in PD providing a test-bed for potential therapeutic strategies.

To provide a platform for ongoing and future work we have reviewed and compared studies of mitochondrial physiology in cellular PD models and compared these with animal models of PD to identify common features that may be investigated as PD risk factors.

2. Mitochondrial homeostasis parameters

Given the importance of consolidating and disseminating protocols for mitochondrial homeostasis dysfunction and neurodegeneration (Burbulla, Krüger 2012; Fang et al 2017; Joshi, Bakowska 2011; Lampl et al 2015), here we have considered parameters that are typically used to assess PD phenotypes and we have summarised common assays employed for these analyses.

2.1. Mitochondrial respiration

Oxygen consumption by the mitochondria is one of the physiological parameters used to characterise the health status of mitochondrial preparations from cell models or tissue samples from animal models. Mitochondrial respiratory physiology has mostly been interrogated with two platforms, the Oroboros O2k (Doerrier et al 2018) and Seahorse XF (Gu et al 2021) whose advantages and experimental capabilities have been previously compared (Horan et al 2012; Zdrazilova et al 2021; Gnaiger 2021). By permeabilization of cells, purification of mitochondria and specific combinations of respiration substrates and inhibitors these allow detailed dissection of mitochondrial physiology states (Gnaiger et al 2020) as well as differentiating between oxidative phosphorylation and glycolysis. Typically, mitochondrial function is assessed through basal respiration, response to endogenous substrates (ROUTINE) in saturating substrate and ADP conditions (OXPHOS), respiration uncoupled from ATP synthesis (LEAK) and residual oxygen capacity (*Rox*) (Gnaiger 2020).

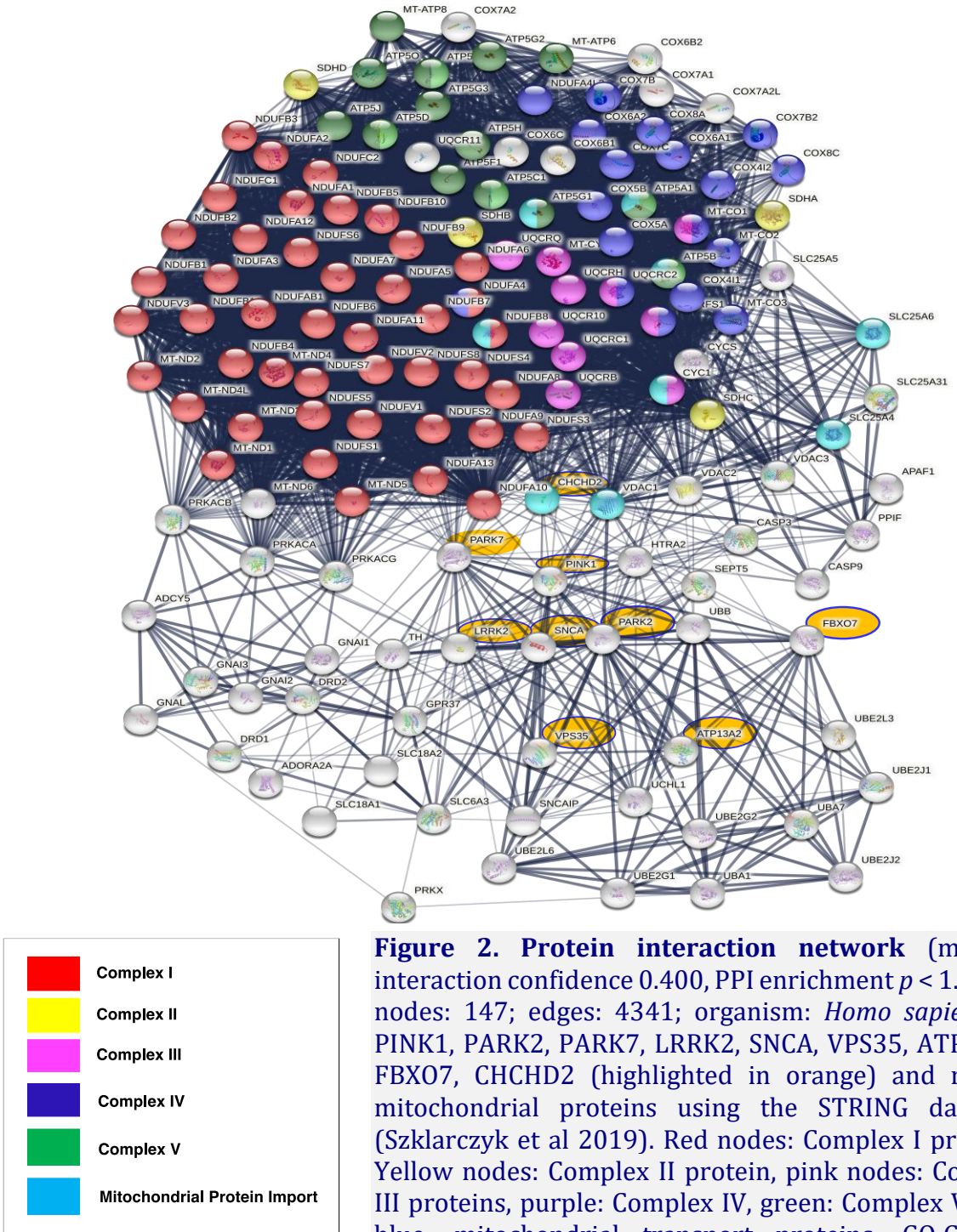

Figure 2. Protein interaction network (medium interaction confidence 0.400, PPI enrichment $p < 1.0e-16$; nodes: 147; edges: 4341; organism: *Homo sapiens*) of PINK1, PARK2, PARK7, LRRK2, SNCA, VPS35, ATP13A2, FBXO7, CHCHD2 (highlighted in orange) and related mitochondrial proteins using the STRING database (Szklarczyk et al 2019). Red nodes: Complex I proteins, Yellow nodes: Complex II protein, pink nodes: Complex III proteins, purple: Complex IV, green: Complex V, light blue: mitochondrial transport proteins. GO:Cellular components was used for functional enrichment of the proteins. GO:0005747- mitochondrial respiratory Complex I; GO:0045277- Complex IV; GO:0045261-proton-transporting ATP synthase complex; GO:0005749- Complex II; GO:0005750- Complex III; GO:0005753- ATP synthase and Reactome pathways, HSA-1268020- mitochondrial protein import.

In PD models a decrease in oxygen consumption is typically connected with the disease. However, sometimes the disease condition is linked to enhanced respiration and oxygen consumption (see analysis below and attached tables). Biochemical assays can be used to measure individual mitochondrial complexes' activities (Barrientos et al 2009) however, respirometry is now the gold standard to evaluate mitochondrial function.

2.2. Adenosine triphosphate

Adenosine triphosphate (ATP) measurement is important to assess mitochondrial function for *in vivo* and *in vitro* models. A variety of colorimetric, fluorometric and luminescence assays are used for these measurements (Fujikawa, Yoshida 2010). These provide information about the total ATP in the sample and can't distinguish the ATP produced from glycolysis versus oxidative phosphorylation. More recently developed genetically-encoded fluorescent reporters are able to detect changes in ATP at the mitochondrial level (Imamura et al 2009).

2.3. Mitochondrial membrane potential

Mitochondrial membrane potential describes the difference in charge across the mitochondrial inner membrane, regulating ion transport and protein import. Together with the pH gradient across the inner membrane, it is the driving force of ATP synthesis during the oxidative phosphorylation process. Fluctuations in mitochondrial potential need to be interpreted in conjunction with other mitochondrial physiology parameters, e.g. respiration, in order to address mitochondrial health (Nicholls, Budd 2000). However significant membrane depolarisation is typically correlated with cellular death (Bock, Tait 2020). The mitochondrial potential is determined by using membrane permeable fluorescent probes which accumulate in the mitochondrial matrix proportionally to the mitochondrial potential Fluorophores employed for this purpose include TMRM, JC-1, TMRE, Rhodamine 123, which can be evaluated using flow cytometry, fluorometry and fluorescence microscopy for detailed subcellular and temporal changes in mitochondrial potential

2.4. Mitochondrial dynamics

Mitochondria are dynamic organelles that regulate their structure-function through fission, fusion, exchange of components, transport, biogenesis and degradation. The bioenergetic and metabolic status of the mitochondria are intertwined with these processes (Picard et al 2013; Van Laar, Berman 2013) and have been associated with disease in both cellular and animal models of PD.

Mitochondrial morphology is typically assessed alongside mitochondrial number and size and more recently branching and elongation states are evaluated through form factor and aspect ratio measurements (Antony et al 2020; Burbulla, Krüger 2012).

2.5. Reactive oxygen species

Reactive oxygen species (ROS) produced in oxidative metabolism have a two-sided contribution to mitochondria and cellular homeostasis. They can act as physiological signalling molecules and at the same time their excess presents a significant challenge to

which the mitochondria can respond with a range antioxidant defences (Andreyev et al 2015; Andreyev et al 2005). Mitochondrial stress including oxidative damage itself can cause an imbalance between ROS production and removal. This can give rise to net ROS production with consequent increase in lipid, protein and DNA oxidation products and connects with neurodegenerative disease etiopathology (Lin, Beal 2006). Measurements of mitochondrial reactive oxygen species are typically undertaken with superoxide redox-sensitive probes (mito-HEt, MitoSOX, Dihydrorhodamine 123) or redox sensitive fluorescent proteins targeted to mitochondria (e.g. reduction-oxidation-sensitive GFP probes). Membrane permeable fluorescent probes present difficulties in specific measurement of mitochondrially derived ROS rather than general cellular ROS, whereas the main challenges of GFP probes are linked to their redox and pH related sensitivity.

3. Mitochondrial homeostasis in animal models of PD

3.1. Toxicological models

6-hydroxydopamine (6-OHDA), or oxidopamine, is a product of the dopamine metabolism which can be taken up by dopaminergic neurons via the DAT receptors and has also been found at elevated levels in the urine of PD patients (Andrew et al 1993). As a synthetic neurotoxin it is widely used to generate rodent models of Parkinson's disease (PD). Unilateral injection of 6-OHDA in SN and medial forebrain bundle (MFB) in rat (Costall et al 1976; Simmonds, Uretsky 1970; Tieu 2011; Ungerstedt 1968) and mouse (Thiele et al 2012) causes dopaminergic neuronal death and motor behaviour defects including ipsilateral circling behaviour. Mechanistically, 6-OHDA is understood to cause the death of dopaminergic neurons in rats through inhibition of the mitochondrial ETS Complexes CI and CIV and through the production of free radicals (Ferger et al 2001; Glinka, Youdim 1995). High-resolution respirometry data from mitochondria in the SN of male Sprague-Dawley rats which had been injected with 6-OHDA in the MFB found that CI activity was decreased along with the respiratory control ratio (RCR) in a time-dependent manner (Kupsch et al 2014). These observations tally with the finding that 6-OHDA caused oxidative stress in the striatum (Smith, Cass 2007) in agreement with evidence for oxidative stress as a common feature in PD patients.

Since its discovery as a PD inducing toxin, **MPTP** has been used to develop animal models of disease. While rats have been largely resistant to MPTP toxicity, MPTP animal models that have been successfully used in PD research include mice and non-human primates such as squirrel monkeys and macaques (Riachi et al 1990). In mouse models, the inhibition of CI in brain mitochondria resulted in reduced ATP levels, an in increase in oxidative stress and the loss of DA neurons in the SN (Nicklas et al 1985; Petroske et al 2001).

Rotenone is a widely used pesticide in aquatic environments. Like MPTP it is highly lipophilic, enabling its transport across the blood brain barrier (BBB). Like MPTP it also inhibits the catalytic activity of the mitochondrial CI enzyme, a characteristic pathology in PD patients (Betarbet et al 2000; Parker et al 1989; Schapira et al 1990), although there is now evidence for a CI independent activity of rotenone (Choi et al 2008). Alongside CI inhibition, rotenone administration to rats also resulted in decreased levels of DA and its

metabolites in the striata (Heikkila et al 1985). However, damage to brain regions not including the SN had also been recorded, suggesting that rotenone may be unsuitable for producing animal models of PD (Ferrante et al 1997; Höglinger et al 2003). Another significant issue with rotenone rat models was the inconsistent response to the toxin, which often resulted in high rates of mortality (Antkiewicz-Michaluk et al 2003; Fleming et al 2004). However as a systemic inhibitor of mitochondrial function it is capable of inducing PD-like pathology across several animal models (Cicchetti et al 2010; Greenamyre et al 2010).

Paraquat is a herbicide that was first suggested as a toxin that could induce PD due to the structural similarities that it shares with MPP+. Investigations into paraquat as an environmental risk factor for PD have largely used mice as the animal model. It was shown in mice that paraquat treatment can cause a dose- and age-dependent decrease of DA neuron numbers in the SN, and a decline in the density of striatal DA nerve terminals (Brooks et al 1999; McCormack et al 2002). Paraquat mice models have replicated increased presence of α-Synuclein fibrils, the up-regulation of α-Synuclein protein levels and the formation of aggregates that contain α-Synuclein (Manning-Bog et al 2002). In striatal mitochondria isolated from paraquat-treated Sprague-Dawley rats LEAK (state 4) respiration was significantly increased while the respiratory control ratio was significantly decreased in comparison with control rats (Czerniczyniec et al 2015).

The most significant drawback of toxicologic models of PD are that they are acute models of disease contrary to the typical evolution of PD which takes place over many years, the high variability of the results between different murine strains that are administered the toxins (Fornai et al 2005) and the variability in reproducing PD features including loss of DA neurons (Miller 2007), appearance of Lewy bodies and motor and non-motor behavioural changes (Meredith, Rademacher 2011; Vingill et al 2018). Although animal toxicological models are still in use for studying various aspects of PD etiopathology, they focus less on mitochondrial homeostasis.

3.2. Transgenic models

The discoveries in the genetics of PD have led to development of genetic murine models harbouring genetic modifications related to PD. However, these models do not fully recapitulate the PD characteristics and present rather mild phenotypes (Airavaara et al 2020; Terzioglu, Galter 2008). To complement these, other animal models of PD, particularly using *D. melanogaster*, have been successfully employed to address mitochondrial homeostasis alongside behavioural and other mechanistic PD characteristics (Hewitt, Whitworth 2017).

Much of the work done to elucidate the mitophagy pathway, within which **PINK1** is a crucial enzyme, was completed using *D. melanogaster* (Clark et al 2006; Park et al 2006; Yang et al 2006). These first studies reported that in *D. melanogaster Pink1* loss of function mutants present the PD phenotypes of dysfunctional mitochondria and locomotive defects associated with DA neurons degeneration. Further studies have reported impaired synaptic transmission, defects in mitochondrial fission and decreased ATP levels arising from reduced Complex I and Complex IV(CIV) activity (Liu et al 2011; Morais et al 2009). When *Pink1* models of PD in mice have been investigated, the fidelity

of the phenotype to PD in humans has had mixed results. RNAi silencing of the *Pink1* gene in mice aged 6 months failed to cause a significant decrease in the number of TH-positive neurons in the SN (Zhou et al 2007). Building upon this, it has been reported that the *Pink1* null mice had no changes to the number of DA neurons or striatal DA content, but there was a significant decrease in the evoked release of DA (Kitada et al 2009). Impaired mitochondrial respiration was observed in the striatum of *Pink1* null mice (Gautier et al 2008) and similarly to *D. melanogaster Pink1* mutants which present reduced respiratory activity linked to Complex I and Complex II (Liu et al 2020). Interestingly the *Pink1* loss of function accelerates in vivo neurodegenerative phenotypes induced by mitochondrial stress triggered by the expression of an unfolded protein in the mitochondrial matrix, namely a truncated form of ornithine transcarbamylase (deltaOTC) (Moisoi et al 2014).

Investigations into the role of the ***Parkin*** gene, in PD were also first conducted in *D. melanogaster*, concurrently with the *Pink1* studies (Clark et al 2006; Greene et al 2003; Park et al 2006; Yang et al 2006). Many of the studies that have investigated *Parkin* loss of function mutants as a model of PD in *D. melanogaster* have observed mitochondrial dysfunction. *Parkin* loss-of-function *D. melanogaster* had significantly decreased CI and CII activity when measured as a function of oxygen flux by high resolution respirometry (Lehmann et al 2016). In mitochondria isolated from the striata of 9 months old *Parkin* null mice OXPHOS (state 3) respiration was significantly decreased while in 24 months old mice respiratory reserve was instead significantly decreased on Complex I substrates, both as detected by high resolution respirometry (Damiano et al 2014).

Multiple studies have reported motor defects in **DJ-1** loss of function murine models of PD as well as altered DA metabolism, however a loss of DA neurons and formation of Lewy bodies has been more difficult to replicate (Chen et al 2005; Goldberg et al 2005; Kim et al 2005; Rousseaux et al 2012). A recent study reported that DJ-1 deficiency in mice accelerated the accumulation and aggregation of the key Lewy body component, α-Synuclein (Xu et al 2017). In mitochondria isolated from the cortex of DJ-1 null mice aged either 3 months or 24 – 26 months, there were no significant differences in OXPHOS or LEAK respiration for Complex I, Complex II or Complex III/IV (Giaime et al 2012).

Murine models addressing **α-Synuclein** pathology range from transgenic mice overexpressing human α-Synuclein with PD relevant mutations, overexpression of human α-Synuclein mutants, through viral delivery of transgenes directly to the brain and more recently, delivery to the brain of exogenous human α-Synuclein in oligomeric or preformed fibrils (PFF) forms (Airavaara et al 2020; Carta et al 2020; Terzioglu, Galter 2008). In transgenic mice, A53T α-Synuclein mutant induces defects in the autophagic clearance of mitochondria (Chen et al 2015). Other mitochondrial roles for α-Synuclein in PD have been derived mainly from cellular models, as detailed below, and are consistent with the finding that Lewy bodies from patients with PD contained fragmented mitochondria crowded with lipids and lysosomes as well as α-Synuclein (Shahmoradian et al 2019).

Additional PD murine models have mutations affecting mitochondrial function and molecular quality control. Loss of function of the mitochondrial protease HtrA2, situated in the intermembrane space, induces a reduction in mitochondrial respiration,

accumulation of oxidative stress markers and accumulation of unfolded proteins in the mitochondria. These correlate with sustained upregulation of the integrated stress signalling specifically in the brain, which contributes to neurodegeneration (Moisoi et al 2009). Mitochondrial protein quality control is an important emerging area of interest, addressed throughout the toxicological and genetic models with an aim to identify how quality control mechanisms contribute to PD etiopathogenesis and may be targeted to ameliorate disease phenotypes (Hu et al 2019; Lautenschläger et al 2020).

An interesting transgenic model to address mitochondrial homeostasis in PD is represented by the 'MitoPark' mouse model with deletion of the mitochondrial transcription factor A (TFAM) in dopaminergic neurons. Although the model does not present a mutation of a PD gene, it is inducing mitochondrial dysfunction and PD phenotypes rendering the model as a tool to further etiopathology mechanistic studies in the field (Beckstead, Howell 2021; Ekstrand et al 2007; Ekstrand, Galter 2009; Galter et al 2010).

Despite the differences in how the murine models are being developed which will influence the interpretation of the results, these are still being employed to investigate 'whole body' aspects of PD etiopathology and responses to treatments.

4. Mitochondrial homeostasis in cellular models of PD

Given the experimental challenges and the extensive time required for use of animal models to study mechanistic details of mitochondrial dysfunction in PD, an extensive range of cellular PD models has been developed. These comprise cell lines as well as primary neuronal and iPSc neuronal models undergoing combinations of PD related toxin treatments as well as relevant genetic manipulations.

PD treatments (Enogieru et al 2019; Fonseca-Fonseca et al 2019; Iglesias-González et al 2012; Imamura et al 2006; Ma et al 2019) While in animal models MPTP itself is used as an inducer of PD etiopathology, in cellular models of dopaminergic neurons presenting the DAT transporter, like SH-SY5Y, its metabolite MPP+ is employed. In addition, inhibitors of mitochondrial function particularly those demonstrated to induce PD phenotypes in animal models, e.g. rotenone, are also employed as PD relevant toxins in cellular models. MPP+ and rotenone are primarily linked to inducing a dysfunction in the CI linked respiration. However, some studies have shown that these drugs are toxic in the absence of CI functionality (Choi et al 2008). Whether CI inhibition leads to compensatory mechanisms that are influenced by MPP+ and Rotenone is not fully addressed.

6-OHDA is successfully employed as a PD-relevant toxin in cellular models, both for its dopaminergic link as well as its oxidative stress-inducing properties. Generic oxidative stressors like H_2O_2 have also been used in cellular PD models.

More recently models of α-Synuclein seeding/exposure to oligomers or preformed fibrils (PFFs) have been developed to address the mitochondrial toxicity of the different types of protein structures (Mahul-Mellier et al 2020; Tapias et al 2017; Zambon et al 2019).

PD phenotypes are evaluated by a wide range of assays determining cell viability (e.g. MTS MTT, Alamar Blue), accumulation of ROS and redox profile (ROS-DCF-DA,

Amplex red, reduced glutathione content, malondialdehyde, carbonylated proteins, catalase and superoxide dismutase activities), autophagy/mitophagy markers, formation of protein aggregates, electrophysiological properties of neuronal cells, Ca^{2+} /Mg^{2+} imaging, alongside the mitochondrial homeostasis parameters specified above.

4.1. Murine primary neurons

Primary neuronal cultures from mice and rats offer unique possibilities to study molecular mechanisms of neurodegeneration given their neurochemical properties correlated to the specific cell type. Primary neuronal cultures can be derived from selected brain regions leading to development of specific cellular models, e.g. dopaminergic neurons, cortical neurons, striatal neurons, hippocampus neurons. A strong advantage of these cultures is that they can be derived from transgenic animals providing homogenous genetic cellular models. However, primary neuronal cultures have disadvantages including the limited number of cells that can be obtained in one preparation, while the preparation and maintenance of the cultures are not trivial Moreover, numerous potential variations in preparation may affect the neuronal physiology including the mitochondrial homeostasis leading to heterogeneity in the experimental results. In addition, the primary neuronal cultures are typically derived from embryonic stage or newly born animals questioning their appropriateness for age related neurodegenerative diseases.

Despite these drawbacks, primary neuronal cultures are a common and very useful tool for PD studies including for analysis of mitochondrial function parameters and provide valuable information on mitochondrial physiology.

Thus, treatment with rotenone of dopaminergic neurons induces decreased respiration, enhanced ROS and decreased mitochondrial potential (Radad et al 2006, 2015; Zhu et al 2019). The decrease in mitochondrial respiration following rotenone treatment is also reported in cortical cultures. Interestingly, loss of Parkin induces a decrease in mitochondrial respiration in striatal neurons but not cortical neurons (Damiano et al 2014).

More recently primary neurons have been used in the context of α-Synuclein pathology analysis using exogenous addition of α-Synuclein. Contrary to monomeric α-Synuclein, pathological α-Synuclein forms (oligomeric, dopamine-modified and phosphorylated), bind with high affinity to mitochondria, resulting in mitochondrial membrane depolarisation and impaired cellular respiration (Di Maio et al 2016; Wang et al 2019). Models of α-Synuclein seeding/exposure to preformed fibrils (PFFs) demonstrated that mitochondrial dysfunction develops as a consequence of the interaction between the newly formed α-Synuclein aggregates and mitochondria, resulting in reduced basal and maximal respiration, coupled with diminished spare respiratory capacity (Mahul-Mellier et al 2020; Tapias et al 2017; Zambon et al 2019). However, it appears that the effects of α-Synuclein pathology on mitochondrial respiration occur primarily at later stages of LB formation and maturation (Burtscher et al 2020; Mahul-Mellier et al 2020).

4.2. SH-SY5Y

SH-SY5Y have gained ground as a popular cell model for PD (Xicoy et al 2017). Developed from a metastatic neuroblastoma the cell line (Biedler, Schachner 1978) has been shown to present tyrosine hydroxylase activity (Ross, Biedler 1985) and consequent dopaminergic phenotypes. The cell line is broadly used as a PD model in differentiated or nondifferentiated conditions. A variety of protocols have been reported for culturing of the cells as well as for differentiation (Xicoy et al 2017), which makes it difficult to cross-compare data between studies.

MPP+ treatment in differentiated SH-SY5Y (Risiglione et al 2020) has demonstrated a profound effect on decreasing coupled respiration and increasing the LEAK respiration indicating significant damage at the level of the inner membrane. However, the study did not account for loss of mitochondrial mass. Treatment of SH-SY5Y with 6-OHDA is shown to reduce NADH-linked mitochondrial respiration but detailed characterisation of mitochondrial physiological changes under 6-OHDA has not been performed (Iglesias-González et al 2012). A protective effect for antioxidant enzymes was correlated with increasing ROS, a result of 6-OHDA treatment, thereby uncoupling respiration and phosphorylation.

In SH-SY5Y PINK1/Parkin downregulation and overexpression of loss of function disease mutations are the most relevant genetic transformations employed for this model (Supplementary File 1). Also in SH-SY5Y, the R492X mutation overexpression appears to have a dominant effect in inducing mitochondrial dysfunction and oxidative stress, particularly in the presence of MPP+ (Yuan et al 2010). Although DJ-1 has multiple roles in maintaining cellular function there is now evidence for a role in S-nitrosylation of Parkin. Thus, denitrosylation of Parkin due to DJ-1 loss of function has negative consequences on mitochondrial function reducing ATP synthesis and respiration (Ozawa et al 2020). PD-associated mutations in F-box only protein (FBXO7) have been linked to disruption in mitochondrial homeostasis. SH-SY5Y genetically modified to achieve FBXO7 loss of function have been used to demonstrate that FBXO7 deficiency is linked to mitochondrial dysfunction increased ROS and consequent PARP overactivation which contributes to cell death (Delgado-Camprubi et al 2017).

SH-SY5Ys are also used to produce cybrids by fusing dopaminergic cells depleted of mtDNA with human platelets from PD patients. The cybrids recapitulate mitochondrial dysfunction observed in PD human samples providing an additional model to study PD (Cronin-Furman et al 2019; Keeney et al 2006).

4.3. Mouse embryonic fibroblasts

Immortalised mouse embryonic fibroblasts (MEF) offer the opportunity of high numbers of cells to employ in parallel for different experimental approaches.

Pink1 KO MEF have been used to demonstrate that mitochondrial dysfunction in PD is not due to proton leak but defects of the electron transfer system with consequences on decreased mitochondrial potential, ATP levels and increased ROS production. Mitochondrial impairments are more pronounced when the cells were grown in galactose rather than glucose medium (Amo et al 2011). We have also shown independently in MEF

that *Pink1* KO results in impaired respiration, reduced ATP levels, increased ROS and decreased mitochondrial potential (Temelie et al 2018). However, the individual activity of the respiratory Complexes did not appear to be affected by *Pink1* loss of function (Heeman et al 2011). The data in MEF are consistent with results on mitochondrial impairment in the brains of *Pink1* KO mice (Gispert et al 2009).

DJ-1 loss of function in KO primary MEF, does not appear to affect mitochondrial respiration but increases ROS production, contributes to reduced mitochondrial potential and leads to higher mitochondrial transition-pore opening, rendering the cells more susceptible to death following oxidative stress (Giaime et al 2012). The DJ-1 effect on mitochondrial physiology has shown some differences in immortalised MEF, with reduced respiration in the DJ1-KO while the other mitochondrial features were consistent with data in the primary cells (Krebiehl et al 2010).

Lrrk2 mutant MEF have been obtained through genetic manipulation *in vitro* rather than from transgenic mice and shows the Lrrk2 kinase activity sustains mitochondrial function via tethering of mitochondria to the ER (Toyofuku et al 2020).

4.4. Human fibroblasts

A number of studies have reported decreased respiratory activity in fibroblasts from PD patients with *Parkin* mutations (Mortiboys et al 2008; Pacelli et al 2011). In contrast, some studies reported higher mitochondrial respiratory rates in *Parkin*-mutant fibroblasts, while exhibiting more fragmented mitochondrial networks and ultrastructural abnormalities (Haylett et al 2016). Zanelatti et al reported higher respiratory activity, reduced ATP levels and mitochondrial potential. While the mitochondrial size did not appear affected, a peculiar mitochondrial network with "chain-like" structures was observed in mutant fibroblasts (Zanellati et al 2015). In human fibroblasts (HF) with *Parkin* mutations, Grunewald et al observed an overall decrease in the ATP level, increased oxidative stress associated with enhanced mitochondrial mass and higher sensitivity to oxidative stress (Grünewald et al 2010). The high variability between the different fibroblast lines made it difficult to find significant differences between controls and PD (González-Casacuberta et al 2019). Respiratory system dysfunction is also identified in fibroblasts from patients with *PINK1* mutations (Hoepken et al 2007).

The impaired ETS Complex assembly in genetic PD together with reduced mitochondrial potential has been reported in samples with *DJ-1* mutation (Di Nottia et al 2017; Krebiehl et al 2010). *VPS35* mutations result in defective mitochondrial function (Wang et al 2016; Zhou et al 2017), whereas inefficient response to mitochondrial challenges was seen in fibroblasts with *LRRK2* mutation G2019S, suggesting compromised bioenergetic function (Juárez-Flores et al 2018). Fibroblasts from non-manifesting carriers of *LRRK2* mutation showed an increase in mitochondrial network in standard growing conditions (glucose) and an improvement of mitochondrial dynamics under mitochondrial challenging conditions (galactose), while in PD patients carrying the same mutation, mitochondrial dynamic pattern is similar to controls (glucose condition)

and there were less branched networks and shorter mitochondria with galactose (Juárez-Flores et al 2018).

CHCHD2 encodes a protein that modulates mitochondrial function in conjunction with the ALS/FTD-associated gene CHCHD10. CHCHD2 accumulates in damaged mitochondria and regulates CHCHD10 oligomerisation and has been linked to PD (Huang et al 2018; Liu et al 2020). The *CHCHD2* disease-causing mutations in PD patient fibroblasts induce fragmentation of the mitochondrial reticular morphology and results in reduced activity of Complexes CI and CIV (Lee et al 2018), as well as accumulation in an insoluble form in the intermembrane space and apoptosis induction via cytochrome *c* destabilization, impaired respiration and increased mitochondrial ROS production (Cornelissen et al 2020; Meng et al 2017).

Studies have focussed on idiopathic PD (IPD) patient stratification based on identification of pathological mechanisms linked to mitochondrial homeostasis in peripheral tissues using dermal fibroblasts. These demonstrate high variability in mitochondrial parameters between patients. Thus only a small number of IPD samples present significant mitochondrial dysfunction in skin fibroblasts (Carling et al 2020) as reflected in ATP production, IPD mitochondria present morphometric changes leading to reduced depolarisation by FCCP (Antony et al 2020) and mitochondrial bioenergetics are changed more significantly by metabolic stress in IPD cases versus control (Milanese et al 2019). Deus et al have shown that idiopathic PD fibroblasts present hyperpolarised mitochondria associated with reduced ATP and enhanced ROS (Deus et al 2020), while Ambrosi et al demonstrated proteolytic and bioenergetic deficits in IPD fibroblasts (Ambrosi et al 2014).

Human fibroblasts show a circadian mitochondrial and glycolytic activity (Pacelli et al 2019). This has impacted on how mitochondrial function appears in PD versus control samples. Thus, human fibroblasts with mutated *Parkin* present mitochondrial dysfunction and reduced respiration that is evident when the cell culture is synchronised, while these differences are not evident under basal asynchronous conditions. This may explain some of the difficulties in observing significant differences in patient primary fibroblasts.

Baltimore Longitudinal Study of Aging (BLSA) clearly indicated that, if health status and biopsy conditions are controlled, the replicative lifespan of fibroblasts in culture does not correlate with donor age (Cristofalo et al 1998). Despite the fact that mitochondrial dysfunction has been listed as one of nine hallmarks of ageing (López-Otín et al 2013), to our knowledge there is no study dedicated to investigate the effect of age of donor on mitochondrial dysfunction in HF PD. Antony et al recently included age and gender of PD patients as variables in their morphometric analysis, showing no significant covariates affecting TMRM fluorescence, and no significant difference in mitochondrial branching between male and female subjects (Antony et al 2020). A recent study of healthy subjects provided evidence for a functional specialization of human dermal fibroblasts and identifies the partial loss of cellular identity as an important age-related change in the human dermis (Solé-Boldo et al 2020). More studies to come may focus on potential correlation of mitochondrial dysfunction in HF with age of PD patients as well as with severity of the disease.

4.5. iPSc derived neurons

In recent years iPSc technologies have allowed the development of human neuronal models to avoid the impact of the genetic differences between murine animal models and human neurons. These have been developed either from fibroblasts of patients with idiopathic PD or with characterised mutations through genetic modifications approaches including CRISP-R-Cas9 editing to produce KO lines.

iPSc derived dopaminergic neurons with *α-Synuclein A53T* mutation as well as *α-Synuclein* triplication cause impairment in several mitochondrial function parameters, including respiration (basal, maximal, spare capacity), reduction in mitochondrial potential and change in mitochondrial morphology associated with decreased DRP1 phosphorylation (Zambon et al 2019). Interestingly non-differentiated iPSc did not present the mitochondrial respiration dysfunction. Additional mechanistic findings from this study were perturbation of lipid biology, enhanced ER stress and autophagic dysfunction in the PD models. iPSc derived neuroepithelial cells genetically engineered to harbour *α-Synuclein A53T* and *α-Synuclein A30P* mutations also present reduced energy performance, reflected in lower basal respiration and ATP level (Arias-Fuenzalida et al 2017).

iPSc derived dopaminergic neurons with Parkin loss of function show no change in respiration with glucose as a substrate, but reduced respiration with lactate (Bogetofte et al 2019). Interestingly iPSc derived neurons show strong mitochondrial dysfunction phenotypes at the end stage of differentiation, when the metabolic shift from glycolysis to oxidative phosphorylation has completed, consisting of decreased ATP levels, decreased mitochondrial potential, increased mitochondrial fragmentation, and increased mitochondrial ROS production (Schwartzentruber et al 2020).

LRRK2 G2019S iPSc-derived neurons analysis has demonstrated that dopaminergic neurons present an enhanced number of mitochondrial abnormalities when compared with glutamatergic and sensory neurons including decreased respiration, and trafficking abnormalities (Schwab et al 2017). Decreased respiration in LRRK2 iPSc neurons has been observed in another independent study which reported an increase in respiratory activity in iPSc derived neurons harbouring *PINK1 Q456X* mutation (Cooper et al 2012). Mutation of another component of the vesicle trafficking machinery VPS35 has been found to lead to decreased respiration and mitochondrial potential, increased ROS and defective trafficking and consequent impaired mitophagy (Hanss et al 2020).

5. Qualitative analysis of mitochondrial homeostasis parameters

Here we evaluated mitochondrial parameters in cellular PD models and compared them with those obtained in animal PD models to address to which extent the data converge. Mitochondrial homeostasis is assessed and reported differently by different research groups. Therefore, we have employed a scoring system to compare and classify data from experimental studies relative to controls in each study. For mitochondrial respiration parameters, ATP, ROS and mitochondrial potential we have scored ‘1’ for an increase in the disease model versus control, ‘-1’ for a decrease in the disease model

versus control and '0' for no change. For mitochondrial fragmentation we have scored '-1' for more fragmented or damaged mitochondrial network '1' for less fragmented and higher mitochondrial network. The controls have a score of '0'.

We have focused on studies that present mitochondrial respiration data and we have considered that mitochondrial respiration was changed (reduced or enhanced) if one mitochondrial parameter out of the several studied in the original publication presented a significant change. The papers considered for the analysis were searched in PUBMED using combinations of the keywords 'mouse embryonic fibroblasts', 'SH-SY5Y', 'human fibroblasts', 'iPSc neurons', 'primary neurons' 'Parkinson's disease' 'Parkinson's models', 'mitochondria', 'mitochondrial respiration'. The main inclusion criterion was that the study had analysed mitochondrial respiration. Studies bringing additional data on the key selected models have been added to strengthen the characterisation of these models. The scores are recorded in the Supplementary File 1. The data were processed with GraphPad Prism.

Our qualitative summary of mitochondrial homeostasis parameters shows that the parameters analysed most often in PD murine brains are mitochondrial respiratory activity, ATP level and accumulation of oxidative species. These show a consistent decrease of respiratory activity, reduced ATP and increased oxidative species (Figure 3A). For primary neuronal cultures studies analysing mitochondrial respiration, have also addressed oxidative stress markers and mitochondrial potential (Figure 3B). SH-SY5Y models of PD show consistent decrease of respiratory activity, reduced ATP and increased oxidative species. These are accompanied by mitochondrial potential reduction and mitochondrial fragmentation (Figure 3C).

Mitochondrial homeostasis parameters show that in MEFs PD models there is consistent decrease of respiratory activity, reduced ATP and increased oxidative species. These are accompanied by mitochondrial potential reduction and mitochondrial fragmentation (Figure 3D)

The qualitative analysis of mitochondrial parameters in HF follows the same pattern as in other cellular models of PD (Figure 3E and F). However, there is much higher variability in the data and increasingly the mitochondrial properties of these samples are observed in parallel in glucose versus galactose conditions to highlight the impact of the disease mutation or treatment in predominantly glycolytic versus oxidative phosphorylation metabolic conditions. When galactose is used instead of glucose, to create mitochondria-challenging conditions, HF showed stronger decrease in the mitochondrial parameters respiration (González-Casacuberta et al 2019; Milanese et al 2019), and mitochondrial membrane potential (Lee et al 2018), as well as stronger increase in fragmentation (Juárez-Flores et al 2018; Lee et al 2018) and ROS production (Juárez-Flores et al 2018). This data might suggest that PD derived HF have lower oxidative capacity to cope with an extra metabolic requirement such as the galactose condition as compared to HF from healthy individuals. In addition, the direction of the change in mitochondrial parameters depends also of the protein mutated, thus for example the loss-of function mutations in Parkin, impact not only the process of mitophagy but can also affect the physiological regulation of mitochondrial dynamics,

which can influence mitochondrial activity, e.g. augmented fusion of mitochondrial network resulting in increased respiratory activity (Zanellati et al 2015).

iPSc derived PD models appear to have generally higher sensitivity to cellular stressors affecting mitochondrial activity or to PD toxins (Cooper et al 2012) while the details of mitochondrial function present much higher variability and appear to be strongly dependent on the PD mutation as well as the neuronal type the iPSc have been processed into (Figure 3G). The increase in respiration in one study was explained as compensation for decreased mitochondrial inner membrane integrity, evidenced by increased proton leakage, in attempt to compensate for less efficient ATP production (Harjuhaahto et al 2020).

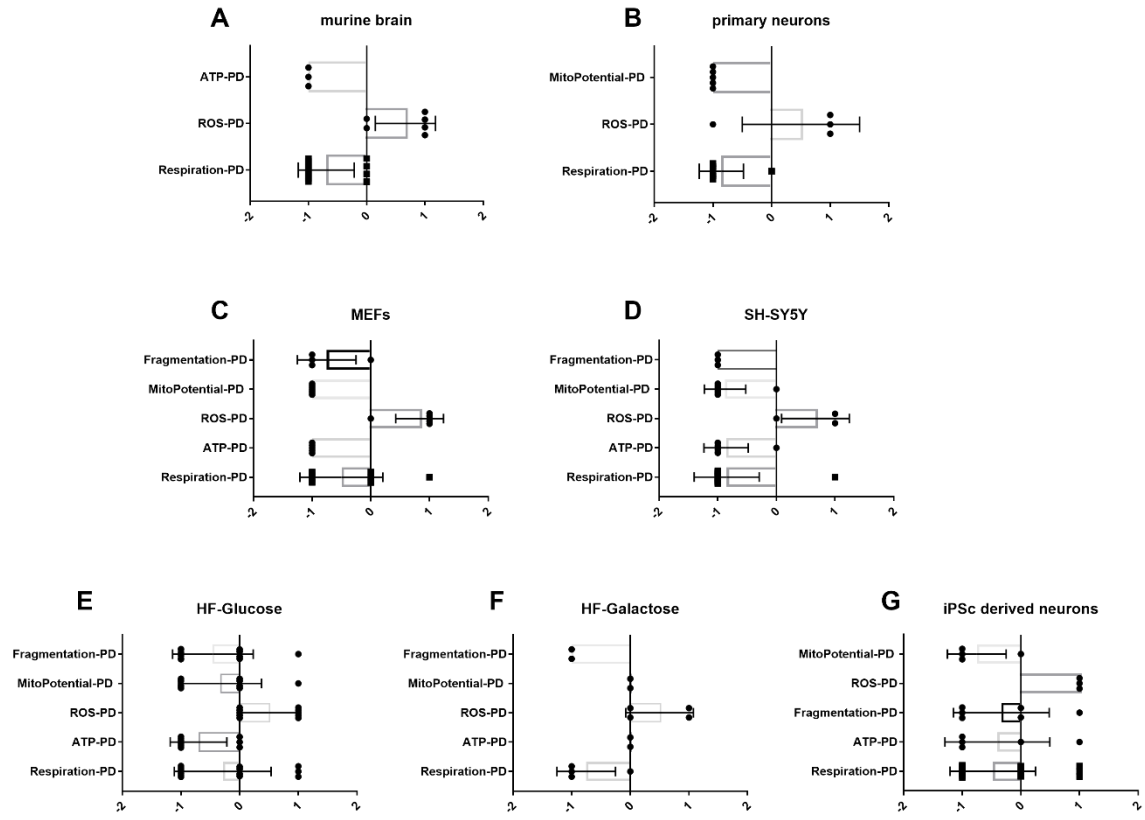

Figure 3. Variation of mitochondrial homeostasis parameters in models of PD represented as mean+/-SD versus the 'zero' line as control. The analysis includes data from (A) murine brain (15 studies), (B) primary neurons (9 studies) (C) SH-SY5Y (13 studies), (D) mouse embryonic fibroblast (MEF) (7 studies) (E) human fibroblasts in glucose media (17 studies) (F) human fibroblast in galactose media (4 studies) and (G) iPSc derived neurons (9 studies).

Information provided by different disease models has largely been complementary and has offered a panoramic prospective on the mitochondrial contribution to mechanisms of dysfunction in PD. The direction of change for these parameters in a PD context is fairly consistent between the different models. However, the results present variability given that most models have their own experimental characteristics which include cell type, passage (even subcloning) status, culture media, treatment variations, diverse methods of genetic manipulations even for the same gene etc. The variability in mitochondrial parameters has also been addressed in the literature, with different explanations or comments. One of plausible explanations is that, in most cell types, mitochondria perform at the basal respiratory levels, and that subtle changes can be observed only when they need to use their "reserve respiratory capacity" to increase substrate oxidation and/or ATP synthesis (e.g. mitochondria challenging conditions) (Rossignol et al 2003). It is also possible that the presence of variability in our analysis could be a result of the activation of different compensatory mechanisms that may depend on the model or mutation type (e.g. increased respiration to compensate for decreased coupling and inefficient ATP production, increase in mitophagy/ autophagy). Consequently, employing more than one model for the study of a particular condition may give complementary answers to the specific research question.

In modelling PD, immortalised cell lines offer the advantage of being able to use large amounts of cells that can be manipulated genetically and pharmacologically to address mechanistic details of disease and to investigate novel pharmacological approaches to tackle cellular dysfunction. Despite differences between individual studies, the data indicate that PD models present reduced mitochondrial respiration activity, reduced ATP levels, reduced mitochondrial potential and enhanced ROS typically together with mitochondria fragmentation. The qualitative analysis presented here indicates that SH-SY5Ys and MEF provide relatively consistent results which correlate well with the data obtained in the brain of murine PD models. Human fibroblasts from PD patients whether idiopathic or harbouring genetic mutation reflect best the high individual variability of mitochondrial function parameters. Similarly, the results of iPSc derived neurons reflect the variability of the models and present additional experimental challenges to maintain, rendering the experiments expensive.

6. Perspectives

Mitochondrial dysfunction plays a central, multifaceted role in PD pathogenesis, and the number of models, described herein, provided valuable information on the role of mitochondrial dysfunction in PD. However, to date, the findings obtained in a variety of PD models failed to result in the development of successful treatment strategies targeting mitochondrial pathways, and clinical trials targeting mitochondrial dysfunction and oxidative stress have not yet demonstrated significant beneficial effects. One of the reasons may be that it seems unlikely that any model can fully recapitulate the complexity of the human disease. For instance, cultured cells, as reduced systems, can be used to provide an answer to specific questions, clarify signalling pathways and resolve mechanistic details. Cellular PD models have significant disadvantages in studying mitochondrial function, due to the high rate of glycolytic metabolism (cell lines) or mixed

glycolytic and oxidative metabolism (primary fibroblasts), which is different from neurons. Furthermore, more complex models (iPSCs-derived neurons), even though they recapitulate PD pathogenicity at an individual basis, including genetic and epigenetic factors, such as aging and environmental insults, fail in the assessment of the contribution of non-neuronal cell populations to the pathogenic process. The use of animal models (toxic or genetic) has strengths in reproducing the complex interplay between different neural and non-neural cells in disease pathogenesis. Unfortunately, animal models also have limitations. Thus, toxin-induced models fail to replicate the chronic course of the disease in the aged animals, whereas genetic models fail to reproduce some of the important hallmarks of PD including the influence of aging, epigenetic, and disease-modifying factors characteristic of PD etiopathology. Choosing a 'best model' to recapitulate disease particularly for a disease with multifactorial etiopathology like PD is still challenging as each of them offers advantages and disadvantages. Nevertheless, each model can contribute to deciphering the mechanisms that play a role of mitochondrial dysfunction in PD pathogenesis and help identify the relevant pieces of the puzzle of mitochondria-triggered neuronal demise. It is worth mentioning that, in recent years, several targets for therapeutic interventions have been recognized — specific organelles (e.g. mitochondria or lysosomes), or disease — associated proteins (e.g. β-glucocerebrosidase or LRRK2). This concept, may, in the future, result in the approach where specific subgroups of patients with PD could be identified, enabling a more personalized therapeutic approach.

Thus, establishing mechanistic details in cell lines and validating such data in patient HF or iPSc derived neurons can perhaps give an integrated view on the disease aspect that is investigated. Addressing the disease condition and pharmacological approaches at whole body level may still require the use of animal models. However, we have seen now significant development of human cellular models, including patient derived, to be able to include more of these in PD studies. Despite the fact that none of the models can fully reproduce the complex pattern of *in vivo* human PD, it can be still stated that they can provide better understanding of molecular mechanisms and risk factors responsible for neuronal demise in PD and help identify reliable markers of the disease process. Therefore, for the time being, studies in animal models still have a valuable role in PD research, although the knowledge gained with cellular models, especially those derived from iPSCs that would allow a more personalized approach, may help to develop better targeted disease-modifying therapeutic strategies.

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