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Mitochondrial abnormalities in Parkinson's Disease and Alzheimer's Disease; can mitochondria be targeted therapeutically?

Ruby Macdonald[1*], Katy Barnes[1*], Christopher Hastings[1*], Heather Mortiboys[1#]

*joint first author, # corresponding author H.Mortiboys@sheffield.ac.uk

[1] Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, 385a Glossop Road, Sheffield, S10 2HQ

Abstract

Mitochondrial abnormalities have been identified as a central mechanism in multiple neurodegenerative diseases and therefore the mitochondria have been explored as a therapeutic target. This review will focus on the evidence for mitochondrial abnormalities in the two most common neurodegenerative diseases, Parkinson's disease and Alzheimer's disease. In addition, we discuss the main strategies which have been explored in these diseases to target the mitochondria for therapeutic purposes; focusing on mitochondrially targeted anti-oxidants, peptides, modulators of mitochondrial dynamics and phenotypic screening outcomes.

Introduction to mitochondria

The mitochondria are essential organelles to all eukaryotic cells. They are a highly dynamic double membrane-bound structure, containing their own circular, double stranded mitochondrial DNA (mtDNA), distinct from nuclear DNA [1]. Oxidative phosphorylation is the pathway via which mitochondria generate ATP, meeting most of the cells energy requirements. This is carried out by five protein complexes (complexes I-V). During oxidative phosphorylation, an electrochemical gradient is produced between the inner membrane and matrix of the mitochondria, which drives the synthesis of ATP [2,3]. The mitochondria are also essential in other functions such as calcium buffering, steroid hormone synthesis, and apoptosis [1,4]. Mitochondrial functions have been reviewed elsewhere in detail, see Nunnari & Suomalainen (2012) [1]. The mitochondria decrease both in quality and functionality over the course of ageing [5], and mitochondrial dysfunction has been shown in age-related neurodegenerative disorders

such as Parkinson's and Alzheimer's disease. This review will discuss the mitochondrial alterations that have been seen in these diseases and review therapeutics targeting mitochondrial dysfunction.

Mitochondrial alterations in Parkinson's disease

Parkinson's disease (PD) is a progressive, neurodegenerative, motor disorder which affects approximately 1% of the over 60 population [6]. PD is characterised by the degeneration of dopaminergic neurons within the substantia nigra, leading to symptoms of bradykinesia, resting tremor, and muscle rigidity [7]. The disease can also present with non-motor symptoms, such as sleep dysfunction, cognitive impairment, and depression [7]. The nigral neurons, the major cell type affected by PD, are highly susceptible to mitochondrial dysfunction due to high basal rates of oxidative phosphorylation leading to increased oxidative stress [8], and high densities of mitochondria in cultured neuron unmyelinated axons compared to other neuron types [9]. The initial link between mitochondrial dysfunction and PD was founded in the 1980s when recreational drug users were exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which metabolises to MPP+, a complex I inhibitor [10]. This was discovered to produce a Parkinsonian phenotype and nigral neuron loss [10]. Mitochondrial dysfunction has been observed in both sporadic and genetic forms of PD, as well as toxin-induced models of the disease.

Sporadic PD

Research by Schapira et al. (1989) found a decrease in complex I activity in PD substantia nigra tissue [11]. This decrease has been replicated in multiple studies [12–15]. Interestingly, this complex I deficiency in the substantia nigra appears to be specific to PD, as Multiple System Atrophy patients have normal levels of complex I activity [12]. Staining for complex I is variable across the substantia nigra, however PD patients have a higher proportion of neurons showing reduced complex I staining [16]. Complex I is the largest mitochondrial complex containing at least 44 subunits, 7 of which are encoded by mtDNA. The complex transfers electrons from NADH to ubiquinone and translocates protons across the mitochondrial inner membrane [17–19]. Consequences of impaired complex I function include; reduced ATP levels, reactive oxygen species (ROS)

generation, and impaired mitochondrial membrane potential (MMP) leading to calcium-mediated damage [20].

Early studies used samples from patients who had taken levodopa and other PD medications, therefore it is important to show that the complex I deficiency is not a secondary effect of the medication. Platelet samples were collected in a three-phase trial after no medication, after 1-month of carbidopa/levodopa treatment, and after 1-month of carbidopa/levodopa plus selegiline treatment. No changes were observed in complex I, II/III or IV activity after each treatment [21]. This suggests that not only are mitochondrial deficiencies present before drug treatment, but also that the current medications do not improve these mitochondrial abnormalities in peripheral tissue. Therefore, it is plausible to hypothesise that targeting mitochondrial function therapeutically would be beneficial.

Although a reduction in complex I activity has been consistently observed in substantia nigra tissue, mitochondrial complex activity has more differing results in non-CNS tissues such as skeletal muscle, platelets, lymphocytes and fibroblasts [22–27]. Deficiencies shown in other mitochondrial complexes have also been variable. This may be due to disease heterogeneity and the different methodologies used between studies, such as the purification of the mitochondria. The importance of mitochondrial purification has been highlighted by research showing an increasingly significant reduction in complex I activity in PD prefrontal cortex tissue throughout the purification process [28].

Interestingly, although the majority of evidence shows a decrease in complex I activity, blue native gel electrophoresis has shown that protein levels of complex I are unchanged [29]. This suggests that the decrease in activity is not due to reduced levels of complex I, but perhaps due to the modification of its enzymatic properties. It has also been suggested that oxidatively damaged subunits of complex I, lead to misassembly of the complex, and may contribute to its deficiency [30]. However, others have investigated a direct link between complex I deficiency caused by mtDNA changes and parkinsonism; this resulted in no association [31]. This calls into question how complex I (and others) deficiency is caused and if it is a primary or secondary consequence of disease; which is particularly important when targeting it for novel therapeutics.

Oxidative damage may also affect mtDNA, which is particularly susceptible due its proximity to the ROS produced by the mitochondrial complexes [32]. Research has shown that levels of oxidised coenzyme Q-10 and 8-hydroxy-2'-deoxyguanosine are elevated in the cerebral spinal fluid of sporadic PD patients, which may lead to this damage [33]. Several studies have investigated mtDNA with relation to Parkinson's. Many have found somatic mutations accruing over the patient's lifetime to be an important feature of PD, whilst other studies have investigated the role of inherited variation in mtDNA and PD. When considering somatic variation; post-mortem nigral neurons were found to exhibit mtDNA damage in PD patients, as opposed to cortical neurons, which are unaffected [34]. During healthy ageing the mtDNA copy number increases in response to mtDNA deletions increasing, whilst this increase in mtDNA copy number is not seen in those with PD, suggesting impaired mtDNA homeostasis [35]. Several studies have found an increase in mtDNA deletions in substantia nigra from PD patients compared to other brain regions and controls [36–38]. Furthermore, cholinergic neurons from the pedunculopontine nucleus were seen to have significantly increased mtDNA deletions, as well as increased mtDNA copy number in PD patients compared to controls [39]. This recent study calls into question the relative role of mtDNA in different brain regions, an under researched area, where there is still much to be learned from the relative mtDNA copy number and build-up of mtDNA deletions with age in relation to PD.

Further research has shown increases in mtDNA somatic point mutations, particularly in complex IV encoding genes [40]. This was the largest study of acquired mtDNA mutations in post-mortem PD patient tissue and was not limited to one brain region. Interestingly, myocyte enhancer factor 2 D (MEF2D), which binds to mtDNA, might also be affected in PD. MEF2D binds to the section of mtDNA encoding for the complex I subunit NADH dehydrogenase 6 (ND6). If blocked, this leads to reduced complex I activity, increased H_2O_2 and reduced ATP levels [41]. Both reduced MEFD2 and ND6 levels have been observed in post-mortem PD brain tissue [41].

There is some debate within the literature as to the association of inherited variation in mtDNA with PD. Many studies report that haplogroups J and K confer a reduced risk of

PD in various European populations [42–44]. However, in an Australian population, neither haplogroup J nor K was seen to be protective [45]. Furthermore, a two-stage association study showed no association between any of the common European haplogroups and PD, but a meta-analysis did show a reduced risk with haplogroups J, K and T and super-haplogroup JT, as well as an increased risk with super-haplogroup HV [46]. Many of the above studies have undertaken the haplogroup association method which was also utilised to find an association between particular haplogroups (UKJT) and age at onset of PD [47]. Additional controversy is introduced when investigating mtDNA haplogroups and PD associations in different populations, as this can reveal very different results [48,49]. As more sophisticated techniques become available, such as mutational load analysis rather than association studies; these associations between mtDNA haplogroup and PD will become clearer and enable determination of less frequent variants.

As well as functional changes, studies using patient-derived cells from idiopathic PD patients also exhibit alterations in mitochondrial morphology. Post-mortem PD caudate nucleus and skeletal muscle tissue has shown increased variability in mitochondrial morphology compared with healthy controls [50,51]. Additional studies using cellular models of sporadic PD have also shown alterations in mitochondrial morphology. Cytoplasmic hybrid (cybrid) cells are cells in which human neuroblastoma cells (SH-SY5Y) deficient in mtDNA through ethidium bromide exposure are re-populated with mitochondria from sporadic PD patients. These have shown enlarged and swollen mitochondria, with a lower distribution of cristae compared to cybrid cells created using healthy control mitochondria [52]. Cybrid lines containing mitochondria from sporadic PD patients also have reduced MMP and defects in mitochondrial transport, as well as decreased complex I activity and increased ROS [52,53]. However, others using this technique have not found transmission of mitochondrial abnormalities into the resulting cybrids with mtDNA [54]. Furthermore, even in studies identifying transmission of the mitochondrial phenotype, authors did not find any deleterious mtDNA changes to which this phenotype could be attributed [52,53]; indicating further work is required in this area. Peripheral tissues from sporadic PD patients, such as fibroblasts, also show mitochondrial morphology impairments [55]. Neurons derived from sporadic PD patient

fibroblasts can now be generated using induced pluripotent stem cell (iPSC) technologies. Thus far, these have validated the post-mortem and peripheral tissue data showing alterations in mitochondrial function and morphology. For example, Hseih et al. (2016) used iPSC-derived neurons to show a decrease in mitochondrial motility [56].

Familial PD

Although sporadic cases account for the majority of PD, around 10% of cases are familial [57]. Various models have been developed to study familial PD, such as the use of patient fibroblasts, knock-out Drosophila and mice, and iPSC-derived patient neurons. Familial PD models are often less heterogenous than sporadic PD models, due to their specific genetic causes. Therefore, they are highly suited to both studying mitochondrial dysfunction and testing potential therapeutics. These range from specific Leucine-rich repeat kinase 2 (LRRK2) inhibitors, to large scale drug repurposing studies [58,59]. It is beyond the scope of this review to in detail go through the known familial causes of PD; therefore we will concentrate on two genetic types which have clear mitochondrial connections, LRRK2 and parkin/PINK1. Other genetic causes of PD such as alpha-synuclein have also been linked to mitochondrial function, however are beyond the scope of this review and are reviewed elsewhere [60].

LRRK2

LRRK2 mutations are the most common genetic cause of PD, with the most common mutations being a glycine to serine substitution (G2019S) in LRRK2's kinase domain which increases kinase activity [61]. Autosomal dominant mutations can lead to late-onset PD, with polymorphisms in LRRK2 also being a risk factor for sporadic PD [62]. The normal function of LRRK2 seems to be complex, and is cell type specific. Various studies have found a role of LRRK2 in multiple fundamental cellular processes including cytoskeletal maintenance, autophagy and the immune response [61]. For instance, LRRK2 is highly expressed in human immune cells, and LRRK2 levels are increased in both innate and adaptive immune cells in sporadic PD patients [63]. LRRK2 variants in the same alleles as PD influence risk for the inflammatory bowel disease, Crohn's

disease, adding to evidence linking PD, the gut, and inflammation [64]. For example, recent evidence has shown differences in colonic microbiota and microbiota metabolism in sporadic PD, which could potentially be a biomarker for the disease [65]. LRRK2 also has an important role in autophagy and the endolysosomal system. A large scale phosphoproteomics study revealed that LRRK2 regulates a subset of Rab GTPases and identified several endogenous substrates of LRRK2 [66]. In the two years since this discovery many more studies have gone on to investigate the interaction with Rab GTPases and LRRK2, and how this is affected by mutations in LRRK2. Rab GTPases are integral to membrane and vesicular trafficking, for example, Rab8a is important for the fusion and enlargement of lipid droplets [67]. Dysregulation of Rab35 phosphorylation has also been shown to cause neurotoxicity in vivo [68]. Additionally, disruptions in lysosome function and morphology have been shown in primary cortical neurons from G2019S mutant mice [69]. LRRK2 G2019S overexpression produces enlarged lysosomes with reduced degradative capacity [70]. Altered LRRK2 function could negatively influence autophagy and the endolysosomal system, leading to an accumulation of defective mitochondria. However, LRRK2 has also been found at the mitochondrial outer membrane [71], raising the possibility of a more direct role in mitochondrial function.

With relation to mitochondrial function and morphology; LRRK2 models show some similarities and some differences to sporadic PD. LRRK2 G2019S mutant patient derived fibroblasts show reductions in MMP and cellular ATP levels, however they are distinct from sporadic PD as they show decreased activity in mitochondrial complexes III and IV, as opposed to complex I [72]. LRRK2 G2019S knock-in mice also show differences in mitochondrial complexes, with complex V subunit ATP5A and complex III subunit UQCR2 protein expression increasing in heterozygous mice [73]. LRRK2 does seem to have variable effects on mitochondrial function, with some cell types and studies reporting increased mitochondrial respiration [74], whilst others have reported decreased mitochondrial respiration [72,75]. However, these differences may be due to the media conditions under which the cells were grown, with some studies utilising ‘normal’ culture medium and others utilising media with substrates forcing use of oxidative phosphorylation [76]. Alterations in mitochondrial morphology may be

influenced by an interaction between the mitochondrial fission factor, dynamin-related protein 1 (Drp1), and LRRK2 [77–79]. Evidence shows that in LRRK2 G2019S knock-in mice there are mitochondrial abnormalities which correspond with an arrest in mitochondrial fission [73], as well as similar morphology to that observed in patient LRRK2 G2019S fibroblasts [75].

As discussed above, mitochondria are a major source of ROS in the cell; the detrimental effect of ROS throughout the cell, and specifically in PD, is well documented. However, more recent studies show the need to dissect the ROS pathway in more detail than is currently known. ROS are now known to be important signalling molecules in their own right, rather than simply being destructive to the cell [80]. Their role in PD may be more complex than previously thought, and similar to many other pathways, they may have a protective role which switches at a point in disease progression to being detrimental to the cell. Similar to sporadic PD, familial models also show an increase in ROS and their detrimental effects on proteins and DNA. LRRK2 has been suggested to interact with peroxiredoxin 3 (PRDX3), a mitochondrial antioxidant protein, and mutations in LRRK2 could affect PRDX3's ability to scavenge ROS [81]. Another link between the cells ability to control ROS production and mitochondrial function to maintain cellular energy levels are the uncoupling proteins (UCPs). It is proposed that the upregulation of UCPs, which transport hydrogen ions into the mitochondrial intermembrane space, may be a compensatory mechanism to protect against mitochondrial ROS levels [82]. Interestingly, UCP2 and UCP4 mRNA expression is upregulated in LRRK2 G2019S mutant fibroblasts and SH-SY5Y cells, respectively [74,82]. Current knowledge lacks the full understanding of how mutant LRRK2 causes mitochondrial alterations. Some of the outstanding questions are; is this a cell type specific effect and do all LRRK2 mutations have the same affect? However, that there are mitochondrial abnormalities present in LRRK2 mutant cells and tissue is clear and therefore this represents a viable therapeutic target. An unanswered question remains if the LRRK2 kinase inhibitors which are being developed will also have beneficial effects on mitochondrial function?

Parkin and PINK1

Autosomal recessive mutations in PINK1 and Parkin are causative for early-onset PD. PINK1 is a kinase which is constitutively recycled at the mitochondrial outer membrane; whereas parkin is an E3 ubiquitin ligase which can be recruited to dysfunctional mitochondria. Both parkin and PINK1 have been found to have a crucial role in mitophagy. Mitophagy is the process by which damaged mitochondria are recycled; this is an area of intense study with new mitophagy pathways being elucidated by novel research in various cell types and under different conditions. The most well studied mitophagy pathway is dependent on parkin and PINK1 function; which has been extensively studied in vitro stress-induced situations. These pathways are reviewed elsewhere [83]; here we will focus on the evidence for mitophagy abnormalities in parkin/PINK1 systems which pertain to PD. Much of the PINK1/parkin dependent mitophagy has been delineated in cell lines overexpressing WT parkin with mitochondrial dysfunction induced by global dissipation of the MMP [84–87] and has been reviewed elsewhere [88]. Recent in vivo evidence has suggested that PINK1/parkin mitophagy pathway is not well utilised in many tissue types. McWilliams et al. (2016) developed a mouse model utilising the mito-QC constructs in order to study in vivo mitophagy in a variety of tissues [89]. Further to this work they generated a mito-QC mouse on a PINK1 K/O background, enabling them to study PINK1 dependent mitophagy in vivo in a variety of tissues. This work found that basal mitophagy rates were comparable between WT and PINK1 K/O mice in a variety of tissues including the dopaminergic system. Furthermore, they identified variations in mitophagy rates dependent on the energy status of the tissue selected and studied [90]. The lack of an effect of defects in parkin/PINK1 on basal mitophagy in vivo was provided by the recent studies in Drosophila models [91]. This work raises the issue of the in vivo relevance of PINK1/parkin mitophagy under normal conditions and raises the possibility that this pathway is utilised in a cell type and stress type specific manner. Further work is needed to fully establish the mechanisms of mitophagy which are utilised by the aged dopaminergic system, as well as other tissues affected in PD.

The mitophagy pathway and the role of parkin/PINK1 is extensively studied as discussed above, however, an alternative pathway has also been implicated in parkin/PINK1 PD. This pathway is the degradation of mitochondrial components via

mitochondrial derived vesicles (MDV). Recent work provided a link between the MDV pathway directly to lysosomes and immune/autoimmune responses in PD [92]. This work showed that it is not only in response to stress that the MDV pathway can mediate mitochondrial antigen presentation (MitAP) on the cell surface via major histocompatibility class (MHC) one molecules. Usually, antigens are processed to peptides and presented via MHC by processing of the proteasome system. However, Matheoud et al. (2016) showed this was also possible via MDV's cycling through the lysosome [92]. The team also identified that PINK1/parkin functions as a brake on this pathway. Therefore, without functional PINK1/parkin, MDV's would be available to be processed by the lysosome to peptides and presented as MitAP via MHC on the cell surface, leading to an immune response. The exact role of PINK1/parkin in this pathway is still to be elucidated, however, the MDV pathway to MitAP was shown to be sorting nexin 9 (Snx9) dependent [92].

An area well studied in relation to both PINK1 and parkin mutants is the presence of mitochondrial dysfunction in PINK1 and parkin mutant or knock-down models and patient tissue. Similar to sporadic PD, Parkin mutants show a decrease in mitochondrial complex I activity and a decrease in both MMP and cellular ATP levels. This has been evidenced in both fibroblasts derived from PD patients with parkin mutations [93], parkin knockdown zebrafish embryos [94] and parkin knockout Drosophila models [95]. Furthermore, Pink1 mutant zebrafish show decreases in mitochondrial complex I and III activity [96]. Both parkin and PINK1 mutant patient cells, and cell/animal models have reduced mitochondrial respiration [93,97–99]. Mitochondrial morphological abnormalities have also been reported in both parkin and PINK1 patient cells and models, however both elongation and fragmentation of the mitochondrial network has been observed. Many of these apparently disparate findings occur when comparisons are made between endogenous parkin or PINK1 expression versus overexpression, or the cell culture media conditions vary, enabling the cells to utilise glycolysis or oxidative phosphorylation to predominantly meet the cells energy requirements.

As more work is undertaken in physiologically relevant models; a complex system is elucidated combining roles for PINK1/parkin in mitophagy, MDV's and MitAP,

mitochondrial dysfunction and morphology. Exactly what the major pathway is which triggers cell death in PD remains elusive; with more work needed in both in vivo models and cell models without over expression of PINK1/parkin.

Toxin-induced models

Toxin-induced models have been invaluable in studying mitochondrial alterations in PD. 6-hydroxydopamine (6-OHDA) was the first toxin PD model to be developed. 6-OHDA's structure is similar to dopamine, but with an additional hydroxyl group which leads to oxidative stress in dopaminergic neurons [100]. Mice and rats treated with 6-OHDA show the typical motor defects associated with PD but Lewy bodies are not present [101].

MPTP-induced PD models are also commonly used, and were first developed using non-human primates [102]. These respond to typical PD medication, such as Levodopa, showing the model's clinical utility in developing therapeutics [100]. Since then, MPTP has been utilised in mice [103], C.elegans [104], and zebrafish models of PD [105]. MPTP can cross the blood brain barrier (BBB), where it is metabolised by monoamine oxidase B and forms MPP+, its toxic metabolite. MPP+ enters dopaminergic neurons through dopamine transporters, and inhibits mitochondrial complex I, leading to decreased ATP levels and increased ROS [100]. Interestingly, rats appear to be resistant to MPTP toxicity, due to their high levels of monoamine oxidase at the BBB, which converts MPTP to MPP+. MPP+ is less readily permeable to the brain compared to MPTP, thus conferring this resistance [100]. MPTP-induced models will typically mimic late-stage PD, but not Lewy body pathology. Dopaminergic neuron loss can be altered though different numbers of doses and frequency, though the loss is not progressive [100].

The pesticide rotenone is another complex I inhibitor, which can cross the BBB and cellular membranes to enter the mitochondria [100]. Chronic systemic infusion in rats causes degeneration of dopaminergic neurons, as well as Lewy body-like pathology, which is not seen in other toxin-induced models [106]. However, the reproducibility of rotenone induced dopaminergic loss is low, and there is a high mortality rate [107]. The herbicide paraquat has a very similar structure to MPP+, causes nigral cell loss, and is frequently used in PD models [100]. Paraquat causes redox recycling which yields ROS, principally in the mitochondria [100]. There is epidemiological evidence showing that

exposure to pesticides/herbicides, such as rotenone and paraquat, are a risk factor for PD [108].

The above animal models focus on recapitulating the loss of dopaminergic neurons from the substantia nigra; a recent animal model however, has concentrated on the cholinergic system in PD [109,110]. This model utilises stereotactic injection of lactocystin in the substantia nigra pars compacta, however the authors then concentrate on investigating the effects on the pedunculopontine nucleus, an area which is a promising target for therapy via deep brain stimulation [111].

Overall, mitochondrial dysfunction in PD has been implicated in multiple models, including post-mortem tissue, animal models, and iPSC-derived neurons. These changes, such as reductions in complex I activity and increased ROS, have been evidenced in both familial and sporadic forms of the disease. Targeting these specific changes in mitochondrial function and morphology, such as complex I activity or Drp1-mediated fission may be essential in the development of therapeutics for PD.

Mitochondrial alterations in Alzheimer's disease

Alzheimer's disease (AD) is a progressive, incurable neurodegenerative disease, and the most common cause of dementia worldwide [112]. Common symptoms include a decline in cognitive function, as well as behavioural symptoms such as depression and apathy [113]. Neuropathology of AD is defined by the presence of extracellular plaques composed of amyloid beta (Aβ), and intracellular neurofibrillary tangles containing tau, with profound neuronal loss occurring later in the disease course.

The Amyloid Cascade Hypothesis was first proposed by Hardy and Higgins (1992), and suggests that the accumulation of Aβ is the initial cause of AD pathology, with neurofibrillary tangles, atrophy and cognitive decline occurring as a direct result [114]. However, the extent of Aβ pathology present post-mortem has not been found to correlate well with the clinical progression of the disease [115]. Furthermore, treatments which have targeted the neuropathology have consistently failed in clinical trials [116,117]. This suggests that there are other mechanisms which play a crucial role in the

progression of AD. One such mechanism is mitochondrial dysfunction, which has been indicated in both sporadic (sAD) and familial (fAD) AD, as well as toxin-induced models.

Sporadic AD

Mitochondrial function has been seen to be impaired in sAD; levels of ATP have been seen to be decreased in patient post-mortem tissue [118]. This finding has been replicated in peripheral patient tissue, including fibroblasts [119]. Many studies have found decreased activity of complex IV in sAD patients; in platelets [120–122], fibroblasts, [123] and post mortem tissue [124–126]. Complex IV deficiency has also been seen in patients with mild cognitive impairment (MCI) [127]. It has been proposed that mtDNA deletions, which accumulate with age, may be responsible for complex IV deficiency observed in AD [128]. Changes in mitochondrial genes can be seen early in disease progression in patient blood [129].

Whilst some have suggested that deficiency is specific to complex IV [121,126], others have also seen deficiencies in other complexes. Reduced gene and protein expression of complex I has been seen [130,131], whilst Fisar et al. (2016) observed an increase in complex I activity in sAD platelets [122]. Complex III proteins have also been found to be reduced in AD [132], and recently Armand-Ugon et al. (2017) [133] found expression of subunits from all complexes to be decreased in the entorhinal cortex of AD patients post-mortem.

As well as changes in mitochondrial function, alterations in mitochondrial morphology and distribution have been seen. Perez et al. (2017) found mitochondria in sAD fibroblasts to be reduced in length [119], whilst Wang et al. (2008) [134] saw an increase in the number of fragmented mitochondria. Mitochondria have also been seen to accumulate in the perinuclear region in sAD patient fibroblasts [134], indicating a collapse of the mitochondrial network.

The processes of mitochondrial fusion and fission have also been seen to be impaired in sAD, with changes in the expression of key proteins noted [135–137]. Drp1, involved in mitochondrial fission, was found to be reduced in hippocampal post mortem samples of

sAD [136], a finding replicated in sAD patient fibroblasts [137] and lymphocytes [138]. Drp1 is usually found in the cytoplasm but is recruited to the outer mitochondrial membrane by mitochondrial fission protein (Fis1) and other receptors Mid49, Mff and Mid51, during fission. Localisation of Drp1 to the mitochondria has been found to be reduced [137], suggesting an impairment in the recruitment of Drp1. Drp1 has also been linked to AD pathology; it has been seen to co-localise with Aβ, resulting in abnormal interactions which increase with disease progression [135]. Proteins involved in mitochondrial fusion, such as the mitofusins (Mfn1 and Mfn2) and optic atrophy (OPA1), have also been studied. Decreases in all three main fusion proteins have been seen in post-mortem patient tissue [135,136].

Oxidative stress has also been seen to play an important role in Alzheimer's disease; lipid peroxidation, protein oxidation and DNA oxidation have all been noted in AD as markers of oxidative damage [139]. The mitochondria are a key source of ROS. Damage to the mitochondria, including impairments in the electron transport chain and imbalanced fusion and fission, causes an increased level of ROS, which in turn can contribute to further mitochondrial damage [140]. Increased ROS levels have been noted in sAD fibroblasts [119], which showed an increased accumulation of 8-oxo-guanine, an indicator of oxidative DNA damage [141]. Furthermore, ROS produced by the mitochondria have been seen to trigger the accumulation of Aβ [142].

Familial AD

A small percentage of AD is caused by mutations in the presenilin 1 (PSEN1), presenilin 2 (PSEN2) or amyloid precursor protein (APP) genes. PSEN1 and PSEN2 are localised to the mitochondrial associated membranes (MAMs) [143], whereas PSEN2 in particular modulates Ca^{2+} uptake across the endoplasmic reticulum and the mitochondria [144]. PSEN2 overexpression has been seen to increase the interaction between the two organelles, leading to increased mitochondrial Ca^{2+} uptake [144].

As in sAD, mitochondrial dysfunction has been indicated in genetic forms of AD. Decreased levels of ATP have been seen in various transgenic mouse models

[140,145,146], and in fibroblasts from patients with a PSEN1 mutation [147]. These fibroblasts were also found to show reduced basal and maximal respiration [147]. In PSEN2 knockout mouse embryonic fibroblasts (MEFs), impaired respiratory capacity is seen, with reductions in basal oxygen consumption and spare capacity; a balance towards glycolysis is also noted [148]. Interestingly, respiratory function was restored when human PSEN2 was expressed on the knock-out background, suggesting a key role for PSEN2 in mitochondrial function [148]. As well as decreased ATP levels, various genetic models of fAD have shown reduced MMP, including M17 neuroblastoma cells overexpressing APP [149] and transgenic mouse models [140,150].

Mitochondrial morphology is also affected in fAD. PSEN1 fibroblasts have been seen to have a reduced number of mitochondria [147]. Mitochondria of PSEN2 knockout MEFs have been seen to have less defined cristae [148]; damaged cristae have also been observed in an APP transgenic mouse model [151]. As is also seen in sAD, mitochondria localise around the nucleus in genetic AD models [149].

Mitochondrial quality control mechanisms have also been studied in fAD. For example, mitochondrial transport has been found to be impaired in several fAD mouse models. Anterograde movement is impaired in an APP mouse model [151], whilst both retrograde and anterograde transport have been seen to be impaired in PSEN1 and APP/PSEN1 mouse models [152]. Trushina et al. (2012) also noted that neurons with impaired mitochondrial transport were more susceptible to excitotoxic cell death [152]. Another important process in regulating mitochondrial quality control is mitophagy. An accumulation of damaged mitochondria is often seen in AD, suggesting an impairment in mitophagy. Recently, mitophagy has been studied in PSEN1 patient fibroblasts and iPSC-derived neurons with the same mutation. In both fibroblasts and iPSC-derived neurons, mitochondrial localisation of parkin was seen, suggesting that mitochondria were labelled correctly but unable to be degraded. A reduction in the degradation stage of autophagy was proposed, to account for the accumulation of damaged mitochondria [153].

Similar to sAD, increased oxidative stress has been indicated in fAD. Increased ROS levels have been observed in several fAD transgenic mouse models [140,146,150].

Increased oxidative DNA damage has also been seen in mice expressing mutated APP from 6 months of age, becoming more pronounced at 24 months [150]. Interestingly, fAD lymphocytes have been seen to respond differently to oxidative stress than sAD lymphocytes. When treated with 2-deoxy-D-ribose (2dRib), which induces oxidative stress, PSEN1 cells proved to be more resistant, with a lower rate of apoptosis and lower mitochondrial membrane depolarisation compared to sAD cells [154].

Toxin induced models of AD

As well as sAD and fAD, mitochondrial dysfunction is also seen in toxin-induced models of AD. Administration of scopolamine has been seen to induce several key features of AD, including cognitive impairments and the accumulation of Aβ [155]. This model also exhibits increased oxidative stress [156,157], and mitochondria with a higher vulnerability to swelling and membrane potential dissipation [158].

Another toxin induced model of AD is the administration of streptozotocin, which has been seen to induce cognitive impairments [159], as well as the accumulation of both Aβ and hyper-phosphorylated tau [160]. This model also demonstrates decreased activity of complex I [161] and complex IV [160], an increase in Drp1 protein expression [161], and decreased MMP [160].

Treatment with Aβ also induces AD-like phenotypes. Cells treated with Aβ show mitochondrial defects, including a decrease in MMP, fragmentation of the mitochondria, and generation of ROS [162].

There is a substantial amount of evidence showing mitochondrial dysfunction plays a key role in Alzheimer's disease, in both sAD and fAD, as well as in toxin induced models of AD. Alterations in mitochondrial function, as well as morphology and mechanisms of quality control, have been demonstrated in various models of AD. With many treatments focussed on the neuropathology of AD being unsuccessful, mitochondrial dysfunction provides a new target for the treatment of AD.

Mitochondrial targeted Antioxidants as a therapeutic strategy for neurodegeneration

As outlined above, mitochondrial abnormalities are well characterised in both PD and AD. As a result, several approaches have been utilised to address the mitochondria as a therapeutic target. One of the major pathways harnessed by potential therapeutics is the antioxidant pathway. Antioxidants are compounds that inhibit the oxidation of other molecules. Exogenous antioxidants, such as vitamins, carotenoids and flavonoids, obtained from the diet or synthetically have long been used to promote good health or as treatments. These antioxidants are often distributed ubiquitously throughout the body and typically localised predominantly in the cytosol [163]. However, antioxidant therapeutic strategies for PD and AD have focused on developing mitochondrial targeted antioxidants.

One of the most studied mitochondrially targeted antioxidant compounds is mitoquinone (MitoQ), consisting of a modified ubiquinone conjugated to a triphenylphosphonium (TPP). TPP conjugation is a comprehensively established approach to develop mitochondrial targeted species [164]. Physiochemical factors allow for TPP conjugated compounds to directly penetrate lipid bilayers and accumulate at the negatively charged mitochondrial membrane [164]. MitoQ exerts direct antioxidant action by scavenging superoxide, peroxyl, and peroxynitrite ROS [164]. Furthermore, once oxidised, MitoQ is continually recycled to its antioxidant ubiquinol form [164]. MitoQ has also been found to be protective in both MPP^+ and 6-OHDA toxin induced PD in in vitro experiments. MitoQ reduces mitochondrial fragmentation and translocation of Bax when used to pre-treat SH-SY5Y neuroblastoma cells exposed to 50 µM of 6-OHDA [165]. Furthermore, MitoQ treatment of MPTP treated N27 cells reduces toxicity, improves MMP and reduces apoptotic markers. Treatment of MPTP exposed mice with MitoQ reversed the loss of tyrsosine hydroxylase and MMP and reduced the activation of caspase 3. Additionally, this treatment regime translated into improved motor function [166]. The numerous studies of MitoQ both in vitro and in vivo models of PD led to MitoQ being tested in a clinical trial for PD. Unfortunately, MitoQ failed to show any therapeutic effect in a 128 patient double blind 12 month human trial at either 40 mg and 80 mg per day dose [167].

However, MitoQ was shown to be effective in a clinical trial preventing liver damage in hepatitis C patients [168].

MitoQ has also been tested in both in vitro and in vivo models of AD. N2a cells pre-treated with MitoQ showed reduced hydrogen peroxide levels after Aβ treatment, under these conditions ATP levels and MMP were also shown to be improved [169]. In a transgenic mouse model expressing three human mutant genes of APP, PSEN1, and tau, the treatment with MitoQ showed an improved behavioural phenotype[170]. Additionally, isolated MitoQ treated transgenic mice brains showed reduced lipid peroxidation (an indicator of ROS exposure), reduced Aβ burden and reduced caspase activation.

Skulachev (SkQ1) antioxidants are similar to MitoQ, however they involve the use of conjugated mitochondrial targeted motifs, like rhodamine and TPP, to plastiquinone [171]. Much like ubiquinone, plastiquinone acts as an antioxidant by quenching superoxide. Using a rat model with an inherited over production of free radicals that present AD like pathology (OXYS model), SkQ1 supplemented via the diet was found to accumulate in neuronal mitochondria [172]. Furthermore, SkQ1 supplementation reduces Aβ levels and tau hyperphosporylation in addition to improving memory and learning behaviours [172,173].

MitoApo, similarly to MitoQ, is a TPP conjugated form of the organic compound apocynin. Apocynin is an inhibitor NADPH oxidase and thereby acts as an antioxidant by preventing NADPH oxidase from converting O_2 into superoxide. MitoApo has been found to protect primary cortical neurons against peroxide shock, in addition to protection from 6-OHDA treatment in Lund Human Mesencephalic (LUHMES) cells [174]. In a preclinical animal model of PD, MitoApo exhibited strong neuroprotective effects against MPP^+, attenuating glial cell activation and improving motor function [175].

Melatonin is a direct scavenger of many ROS species; hydroxyls, peroxyls, free radicals, peroxylnitrites and other nitrous oxides under physiological conditions [176]. This direct ROS scavenging action, coupled with evidence that melatonin is mitochondrially localised [177], makes melatonin an attractive mitochondrial therapy for neurodegenerative diseases. Melatonin is also produced endogenously, therefore the

direct antioxidant effect of melatonin is enhanced by its ability to induce antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione (GSH), and inhibit the action of many pro-oxidant pathways [178]. In a 6-OHDA lesion rodent model, treatment with melatonin for 7 days via osmotic pump ameliorated the reduced respiratory chain enzymes activity in nigral tissue, in addition to improving motor behaviour. Co-administration of melatonin with MPTP in a mouse model abolished any dopaminergic cell loss [179].

Melatonin has been shown to inhibit Aβ induced ROS production in vivo. Aβ-induced phospholipid damage was shown to be mitigated by melatonin treatment [180]. In mitochondria isolated from APP/PSEN1 transgenic mice, treatment with melatonin reduced Aβ levels, and improved MMP and ATP production [181].

Peptide strategies

Outside of the TPP conjugation strategy for creating mitochondrial targeting compounds, Sezto-Schiller (SS) tetrapeptides have been used to create mitochondrial targeted antioxidants. SS tetrapeptides contain an aromatic cationic sequence which produces preferential localisation to the inner mitochondrial membrane. However, this localisation method does not seem to be wholly based on the MMP. These SS tetrapeptides have been studied in an MPTP treated mouse PD model [182] and were found to have neuro-protective properties. Mice pre-treated with SS-31 and SS20 half an hour before a series of MPTP intraperitoneal injections showed reduced dopamine depletion and greater survival of dopaminergic neurons in the substantia nigra pars compacta. Isolated mitochondria from the MPTP treated mice have reduced oxygen consumption and reduced ATP production; treatment with SS-31 and SS-20 attenuate these reductions [182]. Regarding AD models, SS-31 reduces the toxicity of Aβ. Aβ toxicity in N2a cells causes reduced ATP and MMP, and increased ROS production; pre-treatment with SS-31 improves all of these parameters [169]. In addition, in N2a cells overexpressing APP, SS-31 improved neurite outgrowth [169].

Strategies to manipulate mitochondrial quality control and dynamics

As outlined above, alterations in mitochondrial morphology and dynamics are features of several PD and AD models and patient tissue. As a result, attempts have been made to manipulate the mitochondrial quality control processes as therapeutic targets for AD and PD.

Some familial forms of PD are caused by mutations in PINK1 which results in reduced kinase activity [183], . Kinetin, an adenosine N^6-furfuryladenine moiety, has been found to mirror PINK1 action, increasing Parkin recruitment to damaged mitochondria; which leads to reduced apoptosis in human derived dopaminergic neurons [184]. More recently, however, an in vivo study in an alpha synuclein rodent model found no positive effect of kinetin treatment [185].

Kinetin was found to be neuro-protective in an AD model induced by aluminium chloride and D-galactose treatment [186]. Kinetin was co-administered with aluminium chloride and D-galactose at three doses. Wei et al. (2017) observed dose dependant activity with kinetin co-administration improving performance in Morris water maze [186]. This dose dependant effect also translated into increased activity of key antioxidant enzymes GSH, SOD and catalase (CAT). Furthermore, kinetin was shown to significantly reduce Aβ deposition induced by the aluminium chloride and D-galactose treatment [186]. Whilst the specific mechanism of kinetin has yet to be elucidated, this research raises key insights to the neuroprotective effects of increased mitophagy.

Drp1 inhibitors have been explored as a therapeutic avenue in both PD and AD. Mdivi-1, mitochondrial division inhibitor 1, is a quinazolinone that allosterically binds to Drp1 and prevents the self-assembly of ring structures by inhibiting GTPase activity; therefore reducing the fission activity of Drp1 [187]. Mdivi-1 improves dopamine release and neuronal survival in an in vivo MPTP mouse model [188]. In an A53T-alpha- synuclein rat model of PD, mdivi-1 treatment prevented motor defects and loss of neurons [189]. Furthermore, mdivi-1 reduced mitochondrial fragmentation and lipid peroxidation, in addition to significantly improving the mitochondrial spare respiratory capacity in isolated A53T synaptosomes [189]. Recently, the ability of mdivi-1 to effect mitochondrial morphology has been called into question with alternative mechanisms being identified therefore the mdivi-1 literature must be interpreted with caution[190]. Curiously, Bordt et

al. (2017) failed to observe any mitochondrial morphology effects in primary neurons and COS-7 cells but verified that mdivi-1 was an inhibitor, although weakly (K_i>1.2mM), of the GTPase activity of Drp1 [190]. Furthermore, in this study Bordt et al. (2017) report that mdivi-1 acts as a reversible complex I inhibitor at concentrations greater than 25 μM in primary neurons by a yet to be elucidated mechanism. Whilst Bordt et al. (2017) raise a valid caution of the use of mdivi-1, most of published data using mdivi-1 reports it as an inhibitor of Drp1 as determined by analysis of mitochondrial morphology, with mdivi-1 being protective in PD and AD models [187–189]. Other compounds which inhibit Drp1 function have also been found to have protective effects in in vitro models [191]. Rationally designed peptides which inhibit 40% and 50% of the GTPase activity of Drp1 have been used in an MPP^+ in vitro model of PD [192]. The peptide P110 inhibited Drp1 mitochondrial translocation in SH-SY5Y cells treated with MPP^+ and CCCP [192]. Furthermore, P110 prevented an increase in the production of mitochondrial superoxide species and prevented a drop in the MMP upon exposure to MPP^+ [192].

Mdivi-1 has been studied in an Alzheimer's cybrid cell model in which SH-SY5Y cells are depleted of endogenous mtDNA and replaced with mitochondria from sporadic Alzheimer's patients [193]. The SH-SY5Y cybrids have reduced ATP output and a highly fragmented mitochondrial network. Mdivi-1 treatment blocked mitochondrial fragmentation, improved ATP production, MMP, complex IV activity, and supressed ROS production [193]. Confirming a morphology effect of mdivi-1, the cybrids treated with mdivi-1 differed morphologically from the untreated cybrids in having longer and denser mitochondria. In N2a neuronal cultures exposed to Aβ42 peptide there is increased production of hydrogen peroxide, whilst the mdivi-1 pre-treated and post-treated cells reduced hydrogen peroxide production to control levels [194]. The mdivi-1 treated cells also showed improved ATP production and cell viability. The effects of mdivi-1 have also been explored in vivo in CRND8 mice, an amyloid precursor line [195]. Primary neuronal cultures from CRND8 mice treated with mdivi-1 showed significantly reduced amount of fractured mitochondria and increased MMP and ATP output [195]. The mice rapidly acquire amyloid pathology impairments in their behaviour; dosing with mdivi-1 improved behaviour in the spontaneous alteration task in a Y-maze apparatus [195].

On review, it seems that Drp1 inhibition-based therapies may seem promising but there are many caveats to be taken with such an approach. There is little understanding of the effects of chronic exposure of Drp1 modulating species or effects in off-target tissues.

Deep brain stimulation strategies on mitochondrial disorders

Deep brain stimulation (DBS) has emerged as a strategic surgical treatment for patients with PD and other movement disorders. Lately its application has been extended to a wider range of neuropsychiatric disorders. In 2016, Kim et al. observed that DBS of the nucleus accumbens in adrenocorticotropic hormone treated rats resulted in greater mitochondrial function compared to the untreated control [196]. This finding suggests that there is scope to use DBS directly to modulate mitochondrial function, however it exists as a monolith and an open exciting avenue of mitochondrial research. Clinically, DBS has been used with positive results on at least four patients with mitochondrial specific disorders. DBS was found to be beneficial in a 41 year old male with multiple mtDNA deletions leading to striatal necrosis [197]. The treatment was found to have persisting effect after two years [197]. In 2012, a 49 year old male with a rapidly progressive Parkinson-dystonia syndrome with multiple mtDNA deletions also responded well to DBS [198]. An immediate therapeutic effect was found with DBS treatment of a patient with mitochondrial encephalopathy which remained stable for three years [199]. Martinez-Ramirez et al. (2016) reported a case of DBS treatment on a patient with a biopsy proven complex I deficiency suffering from myoclonus and dystonia [200]. The effect of the DBS treatment was immediate, with symptoms being improved six months after DBS, however, a regression was observed 12 months post-DBS. Whilst these four case studies show promise in the treatment of mitochondrial disorders, it is unclear if the DBS was acting directly on the mitochondria of the patients.

Phenotypic drug screens for compounds which improve mitochondria function

Finally, we and others have carried out compound screens to identify compounds which improve mitochondrial function in PD and AD. We carried out the first compound screen in patient derived fibroblasts of PD patients (with parkin mutations) using MMP as the primary read out [59]. In a screening cascade which included secondary assays

investigating cellular ATP levels, toxicity screening, and expanded concentration response curves; we identified a group of compounds which improved mitochondrial function in parkin mutant patient fibroblasts [59]. A high proportion of these compounds had a common structural feature of a steroid backbone. Furthermore, we investigated the effect of two compounds on the individual activity of the respiratory chain enzymes, and found a large increase in the activity of all complexes; not just complex I, which is reduced in parkin mutant patient fibroblasts [59]. One of the compounds is already in clinical use for primary biliary cirrhosis, ursodeoxycholic acid (UDCA). Next, we investigated the effects of UDCA in other forms of PD; we found an increase in cellular ATP levels in fibroblasts from patients with G2019S LRRK2 mutations, as well as people who have the G2019S mutation and who do not yet have PD symptoms [72]. In this study, we also found a protective effect of UDCA in an in vivo Drosophila model of G2019S LRRK2 [72]. Others have also tested UDCA and the related compound TUDCA in PD models. TUDCA is protective in C. elegans models of PD [201]. UDCA treatment in a rat rotenone PD model was shown to normalise ATP content, increase striatal dopamine content, reduce expression of apoptotic markers and alter mitochondrial morphology by electron microscopy [202].

Both UDCA and TUDCA have also been tested in AD models [203,204]. TUDCA treatment reduces apoptosis in AD mutant neuroblastoma cells via a p53 mechanism [203]. In two different AD mouse models, TUDCA treatment reduces Aβ pathology and prevents cognitive impairment [204].

Phenotypic screens differ greatly from the classical compound screens undertaken by the pharmaceutical industry. Phenotypic screening has some advantages in that this can be performed in disease relevant models, such as patient derived cells, and may lead to the identification of many compounds with the ability to modulate a particular pathway; for example, those associated with the mitochondria. The difficulty is then being able to identify the target by which the compound is positively modulating the pathway. If successful, however, this can lead to the identification of novel therapeutic targets which can then be screened in a more classical way. Figure 1 outlines an example pathway of how a phenotypic screen could be undertaken and how the

successful drug candidates could be taken forward through the drug discovery pipeline. Phenotypic screening is being utilised more often by academic groups with expertise in the complex biology and models which are required to make phenotypic screening as beneficial as possible. In light of the literature surrounding the role of autophagy and mitophagy in PD; several recently published screens have investigated modulators of these processes [205,206]. One study even identifying the mechanism of action of an autophagy modulator being complex I inhibition. This once again highlights the overlap between mitochondrial function and the autophagy/lysosomal pathways.

In conclusion, mitochondrial abnormalities are a feature of both sporadic and familial forms of PD and AD. Several approaches have been taken to target these mitochondrial abnormalities therapeutically, some directly targeting mitochondria and some via an indirect mechanism. Several of these approaches are promising avenues to explore further in addition to novel compound screening approaches targeting mitochondrial abnormalities.

[1] Nunnari J, Suomalainen A. (2012) Mitochondria: in sickness and in health. Cell. **148**, 1145–59.

[2] Berg JM, Tymoczko JL, Stryer L. (2012) Biochemistry. 7th ed. Basingstoke: W.H. Freeman; 2012.

[3] Hames B, Hooper N. (1997) Instant notes in biochemistry [Internet]. 4th ed. Garland Science, Taylor & Francis Group; 1997. Available from: http://onlinelibrary.wiley.com/doi/10.1016/0307-4412(97)90108-4/abstract

[4] Miller WL. (2013) Steroid hormone synthesis in mitochondria. Mol. Cell. Endocrinol. [Internet]. **379**, 62–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23628605

[5] Payne BAI, Chinnery PF. (2015) Mitochondrial dysfunction in aging: Much progress but many unresolved questions. Biochim. Biophys. Acta [Internet]. **1847**, 1347–1353. Available from:

711 https://www.sciencedirect.com/science/article/pii/S0005272815001097

712 [6] de Lau LM, Breteler MM. (2006) Epidemiology of Parkinson's disease. Lancet
713 Neurol. **5**, 525–535.

714 [7] Kalia L V, Lang AE. (2015) Parkinson's disease. Lancet [Internet]. **386**, 896–912.
715 Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673614613933

716 [8] Haddad D, Nakamura K. (2015) Understanding the susceptibility of dopamine
717 neurons to mitochondrial stressors in Parkinson's disease. FEBS Lett. [Internet].
718 **589**, 3702–3713. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26526613

719 [9] Pacelli C, Giguère N, Bourque MJ, Lévesque M, Slack RS, Trudeau LÉ. (2015)
720 Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key
721 Contributors to the Vulnerability of Dopamine Neurons. Curr. Biol. [Internet]. **25**,
722 2349–2360. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26320949

723 [10] Ballard PA, Tetrud JW, Langston JW. (1985) Permanent human parkinsonism
724 due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): seven cases.
725 Neurology [Internet]. **35**, 949–56. Available from:
726 http://www.ncbi.nlm.nih.gov/pubmed/3874373

727 [11] Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. (1989)
728 Mitochondrial complex I deficiency in Parkinson's disease. Lancet [Internet]. **1**,
729 1269. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2566813

730 [12] Schapira AH, Mann VM, Cooper JM, Dexter D, Daniel SE, Jenner P, et al. (1990)
731 Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency
732 in Parkinson's disease. J. Neurochem. [Internet]. **55**, 2142–5. Available from:
733 http://www.ncbi.nlm.nih.gov/pubmed/2121905

734 [13] Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. (1990)
735 Mitochondrial complex I deficiency in Parkinson's disease. J. Neurochem.
736 [Internet]. **54**, 823–7. Available from:
737 http://www.ncbi.nlm.nih.gov/pubmed/2154550

738 [14] Mann VM, Cooper JM, Krige D, Daniel SE, Schapira AH, Marsden CD. (1992)

739 Brain, skeletal muscle and platelet homogenate mitochondrial function in
740 Parkinson's disease. Brain [Internet]. **115**, 333–42. Available from:
741 http://www.ncbi.nlm.nih.gov/pubmed/1606472

742 [15] Janetzky B, Hauck S, Youdim MB, Riederer P, Jellinger K, Pantucek F, et al.
743 (1994) Unaltered aconitase activity, but decreased complex I activity in substantia
744 nigra pars compacta of patients with Parkinson's disease. Neurosci. Lett.
745 [Internet]. **169**, 126–8. Available from:
746 http://www.ncbi.nlm.nih.gov/pubmed/8047266

747 [16] Hattori N, Ikebe S, Tanaka M, Ozawa T, Mizuno Y. (1993) Immunohistochemical
748 studies on complexes I, II, III, and IV of mitochondria in Parkinson's disease. Adv.
749 Neurol. [Internet]. **60**, 292–6. Available from:
750 http://www.ncbi.nlm.nih.gov/pubmed/8380521

751 [17] Murray J, Zhang B, Taylor SW, Oglesbee D, Fahy E, Marusich MF, et al. (2003)
752 The Subunit Composition of the Human NADH Dehydrogenase Obtained by
753 Rapid One-step Immunopurification. J. Biol. Chem. [Internet]. **278**, 13619–13622.
754 Available from: http://www.ncbi.nlm.nih.gov/pubmed/12611891

755 [18] Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, Walker JE. (2006) Bovine
756 Complex I Is a Complex of 45 Different Subunits. J. Biol. Chem. [Internet]. **281**,
757 32724–32727. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16950771

758 [19] Fernández-Vizarra E, Tiranti V, Zeviani M. (2009) Assembly of the oxidative
759 phosphorylation system in humans: what we have learned by studying its defects.
760 Biochim. Biophys. Acta [Internet]. **1793**, 200–11. Available from:
761 http://www.ncbi.nlm.nih.gov/pubmed/18620006

762 [20] Greenamyre JT, Sherer TB, Betarbet R, Panov A V. (2001) Complex I and
763 Parkinson's Disease. IUBMB Life [Internet]. **52**, 135–141. Available from:
764 http://www.ncbi.nlm.nih.gov/pubmed/11798025

765 [21] Shults CW, Nasirian F, Ward DM, Nakano K, Pay M, Hill LR, et al. (1995)
766 Carbidopa/levodopa and selegiline do not affect platelet mitochondrial function in

767 early parkinsonism. Neurology [Internet]. **45**, 344–8. Available from:
768 http://www.ncbi.nlm.nih.gov/pubmed/7854537

769 [22] Yoshino H, Nakagawa-Hattori Y, Kondo T, Mizuno Y. (1992) Mitochondrial
770 complex I and II activities of lymphocytes and platelets in Parkinson's disease. J.
771 Neural Transm. Park. Dis. Dement. Sect. [Internet]. **4**, 27–34. Available from:
772 http://www.ncbi.nlm.nih.gov/pubmed/1347219

773 [23] Winkler-Stuck K, Wiedemann FR, Wallesch CW, Kunz WS. (2004) Effect of
774 coenzyme Q10 on the mitochondrial function of skin fibroblasts from Parkinson
775 patients. J. Neurol. Sci. [Internet]. **220**, 41–8. Available from:
776 http://linkinghub.elsevier.com/retrieve/pii/S0022510X04000322

777 [24] Martín MA, Molina JA, Jiménez-Jiménez FJ, Benito-León J, Ortí-Pareja M,
778 Campos Y, et al. (1996) Respiratory-chain enzyme activities in isolated
779 mitochondria of lymphocytes from untreated Parkinson's disease patients. Grupo-
780 Centro de Trastornos del Movimiento. Neurology [Internet]. **46**, 1343–6. Available
781 from: http://www.ncbi.nlm.nih.gov/pubmed/8628479

782 [25] Blake CI, Spitz E, Leehey M, Hoffer BJ, Boyson SJ. (1997) Platelet mitochondrial
783 respiratory chain function in Parkinson's disease. Mov. Disord. [Internet]. **12**, 3–8.
784 Available from: http://www.ncbi.nlm.nih.gov/pubmed/8990047

785 [26] Anderson JJ, Bravi D, Ferrari R, Davis TL, Baronti F, Chase TN, et al. (1993) No
786 evidence for altered muscle mitochondrial function in Parkinson's disease. J.
787 Neurol. Neurosurg. Psychiatry [Internet]. **56**, 477–80. Available from:
788 http://www.ncbi.nlm.nih.gov/pubmed/8505638

789 [27] Shoffner JM, Watts RL, Juncos JL, Torroni A, Wallace DC. (1991) Mitochondrial
790 oxidative phosphorylation defects in parkinson's disease. Ann. Neurol. [Internet].
791 **30**, 332–339. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1952821

792 [28] Parker WD, Parks JK, Swerdlow RH. (2008) Complex I deficiency in Parkinson's
793 disease frontal cortex. Brain Res. [Internet]. **1189**, 215–218. Available from:
794 http://www.ncbi.nlm.nih.gov/pubmed/18061150

[29] Schägger H. (1995) Quantification of oxidative phosphorylation enzymes after blue native electrophoresis and two-dimensional resolution: normal complex I protein amounts in Parkinson's disease conflict with reduced catalytic activities. Electrophoresis [Internet]. **16**, 763–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7588559

[30] Keeney PM, Xie J, Capaldi RA, Bennett JP. (2006) Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J. Neurosci. [Internet]. **26**, 5256–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16687518

[31] Palin EJH, Paetau A, Suomalainen A. (2013) Mesencephalic complex I deficiency does not correlate with parkinsonism in mitochondrial DNA maintenance disorders. Brain. **136**, 2379–2392.

[32] Yakes FM, Van Houten B. (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc. Natl. Acad. Sci. U. S. A. [Internet]. **94**, 514–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9012815

[33] Isobe C, Abe T, Terayama Y. (2010) Levels of reduced and oxidized coenzymeQ-10 and 8-hydroxy-2′-deoxyguanosine in the cerebrospinal fluid of patients with living Parkinson's disease demonstrate that mitochondrial oxidative damage and/or oxidative DNA damage contributes to the neurodegenera. Neurosci. Lett. [Internet]. **469**, 159–163. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19944739

[34] Sanders LH, McCoy J, Hu X, Mastroberardino PG, Dickinson BC, Chang CJ, et al. (2014) Mitochondrial DNA damage: Molecular marker of vulnerable nigral neurons in Parkinson's disease. Neurobiol. Dis. [Internet]. **70**, 214–223. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24981012

[35] Dölle C, Flønes I, Nido GS, Miletic H, Osuagwu N, Kristoffersen S, et al. (2016) Defective mitochondrial DNA homeostasis in the substantia nigra in Parkinson disease. Nat. Commun. [Internet]. **7**, 13548. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/27874000

[36] Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat. Genet. **38**, 515–517.

[37] Nido GS, Dölle C, Flønes I, Tuppen HA, Alves G, Tysnes OB, et al. (2018) Ultradeep mapping of neuronal mitochondrial deletions in Parkinson's disease. Neurobiol. Aging [Internet]. **63**, 120–127. Available from: https://doi.org/10.1016/j.neurobiolaging.2017.10.024

[38] Reeve AK, Krishnan KJ, Elson JL, Morris CM, Bender A, Lightowlers RN, et al. (2008) Nature of Mitochondrial DNA Deletions in Substantia Nigra Neurons. Am J Hum Genet. **82**, 228–235.

[39] Bury AG, Pyle A, Elson JL, Greaves L, Morris CM, Hudson G, et al. (2017) Mitochondrial DNA changes in pedunculopontine cholinergic neurons in Parkinson disease. Ann. Neurol. **82**, 1016–1021.

[40] Coxhead J, Kurzawa-Akanbi M, Hussain R, Pyle A, Chinnery P, Hudson G. (2016) Somatic mtDNA variation is an important component of Parkinson's disease. Neurobiol. Aging [Internet]. **38**, 217.e1-217.e6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26639157

[41] She H, Yang Q, Shepherd K, Smith Y, Miller G, Testa C, et al. (2011) Direct regulation of complex I by mitochondrial MEF2D is disrupted in a mouse model of Parkinson disease and in human patients. J. Clin. Invest. [Internet]. **121**, 930–940. Available from: http://www.jci.org/articles/view/43871

[42] van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, et al. (2003) Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am. J. Hum. Genet. [Internet]. **72**, 804–11. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1180345&tool=pmcentrez&rendertype=abstract

[43] Ghezzi D, Marelli C, Achilli A, Goldwurm S, Pezzoli G, Barone P, et al. (2005)

Mitochondrial DNA haplogroup K is associated with a lower risk of parkinson's disease in Italians. Eur. J. Hum. Genet. **13**, 748–752.

[44] Gaweda-Walerych K, Maruszak A, Safranow K, Bialecka M, Klodowska-Duda G, Czyzewski K, et al. (2008) Mitochondrial DNA haplogroups and subhaplogroups are associated with Parkinson's disease risk in a Polish PD cohort. J. Neural Transm. **115**, 1521–1526.

[45] Mehta P, Mellick GD, Rowe DB, Halliday GM, Jones MM, Manwaring N, et al. (2009) Mitochondrial DNA haplogroups J and K are not protective for Parkinson's disease in the Australian community. Mov Disord. **24**, 290–292.

[46] Hudson G, Nalls M, Evans JR, Breen DP, Winder-Rhodes S, Morrison KE, et al. (2013) Two-stage association study and meta-analysis of mitochondrial DNA variants in Parkinson disease. Neurology [Internet]. **80**, 2042–2048. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23645593

[47] Georgiou A, Demetriou CA, Heraclides A, Christou YP, Leonidou E, Loukaides P, et al. (2017) Mitochondrial superclusters influence age of onset of Parkinson's disease in a gender specific manner in the Cypriot population: A case-control study. PLoS One. **12**, 1–11.

[48] Liou CW, Chuang JH, Chen JB, Tiao MM, Wang PW, Huang ST, et al. (2016) Mitochondrial DNA variants as genetic risk factors for Parkinson disease. Eur. J. Neurol. **23**, 1289–1300.

[49] Chen YF, Chen WJ, Lin XZ, Zhang QJ, Cai JP, Liou CW, et al. (2015) Mitochondrial DNA haplogroups and the risk of sporadic Parkinson's disease in Han Chinese. Chin. Med. J. (Engl). **128**, 1748–1754.

[50] Lach B, Grimes D, Benoit B, Minkiewicz-Janda A. (1992) Caudate nucleus pathology in Parkinson's disease: ultrastructural and biochemical findings in biopsy material. Acta Neuropathol. [Internet]. **83**, 352–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1374203

[51] Ahlqvist G, Landin S, Wroblewski R. (1975) Ultrastructure of skeletal muscle in

patients with Parkinson's disease and upper motor lesions. Lab. Invest. [Internet]. **32**, 673–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1127883

[52] Trimmer PA, Swerdlow RH, Parks JK, Keeney P, Bennett JP, Miller SW, et al. (2000) Abnormal Mitochondrial Morphology in Sporadic Parkinson's and Alzheimer's Disease Cybrid Cell Lines. Exp. Neurol. [Internet]. **162**, 37–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10716887

[53] Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, et al. (1996) Origin and functional consequences of the complex I defect in Parkinson's disease. Ann. Neurol. [Internet]. **40**, 663–71. Available from: http://doi.wiley.com/10.1002/ana.410400417

[54] Aomi Y, Chen CS, Nakada K, Ito S, Isobe K, Murakami H, et al. (2001) Cytoplasmic transfer of platelet mtDNA from elderly patients with Parkinson's disease to mtDNA-less HeLa cells restores complete mitochondrial respiratory function. Biochem. Biophys. Res. Commun. **280**, 265–273.

[55] Smith GA, Jansson J, Rocha EM, Osborn T, Hallett PJ, Isacson O. (2016) Fibroblast Biomarkers of Sporadic Parkinson's Disease and LRRK2 Kinase Inhibition. Mol. Neurobiol. [Internet]. **53**, 5161–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26399642

[56] Hsieh CH, Shaltouki A, Gonzalez AE, Bettencourt da Cruz A, Burbulla LF, St. Lawrence E, et al. (2016) Functional Impairment in Miro Degradation and Mitophagy Is a Shared Feature in Familial and Sporadic Parkinson's Disease. Cell Stem Cell. **19**, 709–724.

[57] Thomas B, Beal MF. (2007) Parkinson's disease. Hum. Mol. Genet. [Internet]. **16**, R183–R194. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17911161

[58] Taymans J-M, Greggio E. (2016) LRRK2 Kinase Inhibition as a Therapeutic Strategy for Parkinson's Disease, Where Do We Stand? Curr. Neuropharmacol. [Internet]. **14**, 214–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26517051

[59] Mortiboys H, Aasly J, Bandmann O. (2013) Ursocholanic acid rescues mitochondrial function in common forms of familial Parkinson's disease. Brain [Internet]. **136**, 3038–3050. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24000005

[60] Faustini G, Bono F, Valerio A, Pizzi M, Spano P, Bellucci A. (2017) Mitochondria and α-synuclein: Friends or foes in the pathogenesis of Parkinson's disease? Genes (Basel). **8**, 377.

[61] Wallings R, Manzoni C, Bandopadhyay R. (2015) Cellular processes associated with LRRK2 function and dysfunction. FEBS J. [Internet]. **282**, 2806–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25899482

[62] Lee J-W, Cannon JR. (2015) LRRK2 mutations and neurotoxicant susceptibility. Exp. Biol. Med. [Internet]. **240**, 752–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25888648

[63] Cook DA, Kannarkat GT, Cintron AF, Butkovich LM, Fraser KB, Chang J, et al. (2017) LRRK2 levels in immune cells are increased in Parkinson's disease. NPJ Park. Dis. [Internet]. **3**, 11. Available from: http://www.nature.com/articles/s41531-017-0010-8

[64] Hui KY, Fernandez-Hernandez H, Hu J, Schaffner A, Pankratz N, Hsu N-Y, et al. (2018) Functional variants in the LRRK2 gene confer shared effects on risk for Crohn's disease and Parkinson's disease. Sci. Transl. Med. [Internet]. **10**, eaai7795. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29321258

[65] Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, Goeser F, et al. (2017) Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. Genome Med. [Internet]. **9**, 39. Available from: http://genomemedicine.biomedcentral.com/articles/10.1186/s13073-017-0428-y

[66] Steger M, Tonelli F, Ito G, Davies P, Trost M, Vetter M, et al. (2016) Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a

936 subset of Rab GTPases. Elife [Internet]. **5**, e12813. Available from:
937 http://www.ncbi.nlm.nih.gov/pubmed/26824392

938 [67] Yu M, Arshad M, Wang W, Zhao D, Xu L, Zhou L. (2018) LRRK2 mediated Rab8a
939 phosphorylation promotes lipid storage. Lipids Health Dis. [Internet]. **17**, 34.
940 Available from: http://www.ncbi.nlm.nih.gov/pubmed/29482628

941 [68] Jeong GR, Jang E-H, Bae JR, Jun S, Kang HC, Park C-H, et al. (2018)
942 Dysregulated phosphorylation of Rab GTPases by LRRK2 induces
943 neurodegeneration. Mol. Neurodegener. [Internet]. **13**, 8. Available from:
944 http://www.ncbi.nlm.nih.gov/pubmed/29439717

945 [69] Schapansky J, Khasnavis S, DeAndrade MP, Nardozzi JD, Falkson SR, Boyd JD,
946 et al. (2018) Familial knockin mutation of LRRK2 causes lysosomal dysfunction
947 and accumulation of endogenous insoluble α-synuclein in neurons. Neurobiol.
948 Dis. [Internet]. **111**, 26–35. Available from:
949 https://www.sciencedirect.com/science/article/pii/S0969996117302838?via%3Dih
950 ub

951 [70] Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, et
952 al. (2015) Pathogenic LRRK2 mutations, through increased kinase activity,
953 produce enlarged lysosomes with reduced degradative capacity and increase
954 ATP13A2 expression. Hum. Mol. Genet. [Internet]. **24**, 6013–6028. Available
955 from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddv314

956 [71] Biskup S, Moore DJ, Celsi F, Higashi S, West AB, Andrabi SA, et al. (2006)
957 Localization of LRRK2 to membranous and vesicular structures in mammalian
958 brain. Ann. Neurol. [Internet]. **60**, 557–569. Available from:
959 http://www.ncbi.nlm.nih.gov/pubmed/17120249

960 [72] Mortiboys H, Furmston R, Bronstad G, Aasly J, Elliott C, Bandmann O. (2015)
961 UDCA exerts beneficial effect on mitochondrial dysfunction in LRRK2(G2019S)
962 carriers and in vivo. Neurology [Internet]. **85**, 846–52. Available from:
963 http://www.ncbi.nlm.nih.gov/pubmed/26253449

[73] Yue M, Hinkle KM, Davies P, Trushina E, Fiesel FC, Christenson TA, et al. (2015) Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice. Neurobiol. Dis. [Internet]. **78**, 172–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25836420

[74] Papkovskaia TD, Chau K-Y, Inesta-Vaquera F, Papkovsky DB, Healy DG, Nishio K, et al. (2012) G2019S leucine-rich repeat kinase 2 causes uncoupling protein-mediated mitochondrial depolarization. Hum. Mol. Genet. [Internet]. **21**, 4201–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22736029

[75] Mortiboys H, Johansen KK, Aasly JO, Bandmann O. (2010) Mitochondrial impairment in patients with Parkinson disease with the G2019S mutation in LRRK2. Neurology [Internet]. **75**, 2017–2020. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21115957

[76] Johns DR, Hurko O, Attardi G, Chomyn A. (1996) Respiration and Growth Defects in Transmitochondrial Cell Lines Carrying the 11778 Mutation Associated with Leber ' s Hereditary Optic Neuropathy *. **271**, 13155–13161.

[77] Wang X, Yan MH, Fujioka H, Liu J, Wilson-Delfosse A, Chen SG, et al. (2012) LRRK2 regulates mitochondrial dynamics and function through direct interaction with DLP1. Hum. Mol. Genet. [Internet]. **21**, 1931–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22228096

[78] Uo T, Dworzak J, Kinoshita C, Inman DM, Kinoshita Y, Horner PJ, et al. (2009) Drp1 levels constitutively regulate mitochondrial dynamics and cell survival in cortical neurons. Exp. Neurol. **218**, 274–285.

[79] Su YC, Qi X. (2013) Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by LRRK2 G2019S mutation. Hum. Mol. Genet. [Internet]. **22**, 4545–4561. Available from: https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/hmg/22/22/10.1093_hmg_ddt301/1/ddt301.pdf?Expires=1501149701&Signature=GTX~AN-RSruFqDCcTelG~9IgjpthkkWU1dXp4008wXR6y6IQw954uQuqx0XhNFJWK6SIbQEnJMwz-CvxLG2pT2ZHIkZIysjMzRM9ow9tVbvTljPXTsvC

[80] D'Autréaux B, Toledano MB. (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell Biol. [Internet]. **8**, 813–824. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17848967

[81] Angeles DC, Gan B-H, Onstead L, Zhao Y, Lim K-L, Dachsel J, et al. (2011) Mutations in LRRK2 increase phosphorylation of peroxiredoxin 3 exacerbating oxidative stress-induced neuronal death. Hum. Mutat. [Internet]. **32**, 1390–7. Available from: http://doi.wiley.com/10.1002/humu.21582

[82] Grünewald A, Arns B, Meier B, Brockmann K, Tadic V, Klein C. (2014) Does uncoupling protein 2 expression qualify as marker of disease status in LRRK2-associated Parkinson's disease? Antioxid. Redox Signal. [Internet]. **20**, 1955–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24251413

[83] Chu CT. (2018) Multiple pathways for mitophagy: A neurodegenerative conundrum for Parkinson's disease. Neurosci. Lett.

[84] McLelland G-L, Goiran T, Yi W, Dorval G, Chen CX, Lauinger ND, et al. (2018) Mfn2 ubiquitination by PINK1/parkin gates the p97-dependent release of ER from mitochondria to drive mitophagy. Elife. **7**, e32866.

[85] Hasson SA, Kane LA, Yamano K, Huang C, Danielle A, Buehler E, et al. (2013) High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. Nature. **504**, 291–295.

[86] Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. (2010) Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J. Cell Biol. **191**, 933–942.

[87] Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. (2010) PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. **8**.

[88] Pickrell AM, Youle RJ. (2015) The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron [Internet]. **85**, 257–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25611507

[89] McWilliams TG, Prescott AR, Allen GFG, Tamjar J, Munson MJ, Thomson C, et al. (2016) Mito-QC illuminates mitophagy and mitochondrial architecture in vivo. J. Cell Biol. **214**, 333–345.

[90] McWilliams TG, Prescott AR, Montava-Garriga L, Ball G, Singh F, Barini E, et al. (2018) Basal Mitophagy Occurs Independently of PINK1 in Mouse Tissues of High Metabolic Demand. Cell Metab. , 439–449.

[91] Lee JJ, Sanchez-Martinez A, Zarate AM, Benincá C, Mayor U, Clague MJ, et al. (2018) Basal mitophagy is widespread in Drosophila but minimally affected by loss of Pink1 or parkin. J. Cell Biol. **217**, 1613–1622.

[92] Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, et al. (2016) Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation. Cell. **166**, 314–327.

[93] Mortiboys H, Thomas KJ, Koopman WJH, Klaffke S, Abou-Sleiman P, Olpin S, et al. (2008) Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. Ann. Neurol. [Internet]. **64**, 555–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19067348

[94] Flinn L, Mortiboys H, Volkmann K, Koster RW, Ingham PW, Bandmann O. (2009) Complex I deficiency and dopaminergic neuronal cell loss in parkin-deficient zebrafish (Danio rerio). Brain [Internet]. **132**, 1613–1623. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19439422

[95] Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. (2003) Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. Proc. Natl. Acad. Sci. U. S. A. [Internet]. **100**, 4078–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12642658

[96] Flinn LJ, Keatinge M, Bretaud S, Mortiboys H, Matsui H, De Felice E, et al. (2013) TigarB causes mitochondrial dysfunction and neuronal loss in PINK1 deficiency. Ann. Neurol. [Internet]. **74**, 837–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24027110

[97] Haylett W, Swart C, Van Der Westhuizen F, Van Dyk H, Van Der Merwe L, Van Der Merwe C, et al. (2016) Altered mitochondrial respiration and other features of mitochondrial function in parkin-mutant fibroblasts from Parkinson's disease patients. Parkinsons. Dis. **2016**.

[98] Stevens DA, Lee Y, Kang HC, Lee BD, Lee Y, Bower A, et al. (2015) Parkin loss leads to PARIS-dependent declines in mitochondrial mass and respiration. Proc. Natl. Acad. Sci. **112**, 11696–11701.

[99] Stauch KL, Villeneuve LM, Purnell PR, Ottemann BM, Fox HS. (2018) Loss of Pink1 modulates synaptic mitochondrial bioenergetics in the rat striatum prior to motor symptoms: concomitant complex I respiratory defects and increased complex II-mediated respiration. Proteomics Clin Appl. **10**, 1205–1217.

[100] Martinez TN, Greenamyre JT. (2012) Toxin models of mitochondrial dysfunction in Parkinson's disease. Antioxid. Redox Signal. [Internet]. **16**, 920–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21554057

[101] Tieu K. (2011) A guide to neurotoxic animal models of Parkinson's disease. Cold Spring Harb. Perspect. Med. [Internet]. **1**, a009316. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22229125

[102] Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ. (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc. Natl. Acad. Sci. U. S. A. [Internet]. **80**, 4546–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6192438

[103] Meredith GE, Rademacher DJ. (2011) MPTP mouse models of Parkinson's disease: an update. J. Parkinsons. Dis. [Internet]. **1**, 19–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23275799

[104] Braungart E, Gerlach M, Riederer P, Baumeister R, Hoener MC. (2004) Caenorhabditis elegans MPP+ Model of Parkinson's Disease for High-Throughput Drug Screenings. Neurodegener. Dis. [Internet]. **1**, 175–183. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/16908987

[105] Sarath Babu N, Murthy CLN, Kakara S, Sharma R, Brahmendra Swamy C V., Idris MM. (2016) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced Parkinson's disease in zebrafish. Proteomics [Internet]. **16**, 1407–1420. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26959078

[106] Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov A V., Greenamyre JT. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. [Internet]. **3**, 1301–1306. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11100151

[107] Bové J, Prou D, Perier C, Przedborski S. (2005) Toxin-induced models of Parkinson's disease. NeuroRx [Internet]. **2**, 484–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16389312

[108] Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. (2011) Rotenone, paraquat, and Parkinson's disease. Environ. Health Perspect. [Internet]. **119**, 866–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21269927

[109] Pienaar IS, Gartside SE, Sharma P, Paola V De, Gretenkord S, Withers D, et al. (2015) Pharmacogenetic stimulation of cholinergic pedunculopontine neurons reverses motor deficits in a rat model of Parkinson ' s disease. Mol. Neurodegener. [Internet]. , 1–22. Available from: http://dx.doi.org/10.1186/s13024-015-0044-5

[110] Pienaar IS, Harrison IF, Elson JL, Bury A, Woll P, Simon AK, et al. (2015) An animal model mimicking pedunculopontine nucleus cholinergic degeneration in Parkinson's disease. Brain Struct. Funct. [Internet]. **220**, 479–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24292256

[111] Elson JL, Kochaj R, Reynolds R, Pienaar IS. (2017) Temporal-Spatial Profiling of Pedunculopontine Galanin-Cholinergic Neurons in the Lactacystin Rat Model of Parkinson's Disease. Neurotox. Res. **34**, 16–31.

[112] Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. (2007) How common are the “common” neurologic disorders? Neurology. **68**, 326–337.

[113] Herrup K. (2015) The case for rejecting the amyloid cascade hypothesis. Nat. Neurosci. **18**, 794–799.

[114] Hardy J, Higgins G. (1992) Alzheimer’s disease: the amyloid cascade hypothesis. Science (80-.). [Internet]. **256**, 184–185. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1566067

[115] Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. (2009) Age, Neuropathology, and Dementia. N. Engl. J. Med. [Internet]. **360**, 2302–2309. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0806142

[116] Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. (2014) Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer’s Disease. N. Engl. J. Med. [Internet]. **370**, 311–321. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1312889

[117] Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. (2013) A Phase 3 Trial of Semagacestat for Treatment of Alzheimer’s Disease. N. Engl. J. Med. [Internet]. **369**, 341–350. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1210951

[118] Hoyer S. (1992) Oxidative energy metabolism in Alzheimer brain - Studies in early-onset and late-onset cases. Mol. Chem. Neuropathol. **16**, 207–224.

[119] Pérez MJ, Ponce DP, Osorio-Fuentealba C, Behrens MI, Quintanilla RA. (2017) Mitochondrial bioenergetics is altered in fibroblasts from patients with sporadic Alzheimer’s disease. Front. Neurosci. **11**.

[120] Parker WD, Filley CM, Parks JK. (1990) Cytochrome oxidase deficiency in Alzheimer’s disease. Neurology [Internet]. **40**, 1302–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2166249

[121] Parker WD, Mahr NJ, Filley CM, Parks JK, Hughes D, Young DA, et al. (1994)

1133 Reduced platelet cytochrome c oxidase activity in Alzheimer's disease.
1134 Neurology. **44**, 1086–90.

1135 [122] Fisar Z, Hroudova J, Hansikova H, Spacilova J, Lelkova P, Wenchich L, et al.
1136 (2016) Mitochondrial Respiration in the Platelets of Patients with Alzheimer's
1137 Disease. Curr Alzheimer Res [Internet]. **13**, 930–941. Available from:
1138 http://www.ncbi.nlm.nih.gov/pubmed/26971932

1139 [123] Curti D, Rognoni F, Gasparini L, Cattaneo A, Paolillo M, Racchi M, et al. (1997)
1140 Oxidative metabolism in cultured fibroblasts derived from sporadic Alzheimer's
1141 disease (AD) patients. Neurosci. Lett. **236**, 13–16.

1142 [124] Kish SJ, Bergeron C, Rajput A, Dozic S, Mastrogiacomo F, Chang LJ, et al.
1143 (1992) Brain Cytochrome Oxidase in Alzheimer's Disease. J. Neurochem. **59**,
1144 776–779.

1145 [125] Mutisya EM, Bowling AC, Beal MF. (1994) Cortical cytochrome oxidase activity is
1146 reduced in Alzheimer's disease. J. Neurochem. [Internet]. **63**, 2179–84. Available
1147 from: http://www.ncbi.nlm.nih.gov/pubmed/7964738

1148 [126] Maurer I, Zierz S, Möller HJ. (2000) A selective defect of cytochrome c oxidase is
1149 present in brain of Alzheimer disease patients. Neurobiol. Aging. **21**, 455–462.

1150 [127] Burbaeva GSH, Boksha IS, Savushkina OK, Turishcheva MS, Tereshkina EB,
1151 Starodubtseva LI, et al. (2012) Platelet cytochrome c-oxidase and glutamine
1152 synthetase-like protein in patients with mild cognitive impairment. Zhurnal Nevrol.
1153 i Psihiatr. Im. S.S. Korsakova. **112**, 55–58.

1154 [128] Krishnan KJ, Ratnaike TE, De Gruyter HLM, Jaros E, Turnbull DM. (2012)
1155 Mitochondrial DNA deletions cause the biochemical defect observed in
1156 Alzheimer's disease. Neurobiol. Aging. **33**, 2210–2214.

1157 [129] Lunnon K, Keohane A, Pidsley R, Newhouse S, Riddoch-Contreras J, Thubron
1158 EB, et al. (2017) Mitochondrial genes are altered in blood early in Alzheimer's
1159 disease. Neurobiol. Aging. **53**, 36–47.

1160 [130] Fukuyama R, Hatanpää K, Rapoport SI, Chandrasekaran K. (1996) Gene

expression of ND4, a subunit of complex I of oxidative phosphorylation in mitochondria, is decreased in temporal cortex of brains of Alzheimer's disease patients. Brain Res. **713**, 290–293.

[131] Kim SH, Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. (2001) The reduction of NADH - Ubiquinone oxidoreductase 24- and 75-kDa subunits in brains of patients with Down syndrome and Alzheimer's disease. Life Sci. **68**, 2741–2750.

[132] Kim SH, Vlkolinsky R, Cairns N, Lubec G. (2000) Decreased levels of complex III core protein 1 and complex V beta chain in brains from patients with Alzheimer's disease and Down syndrome. Cell. Mol. Life Sci. **57**, 1810–1816.

[133] Armand-Ugon M, Ansoleaga B, Berjaoui S FI. (2017) Reduced Mitochondrial Activity is Early and Steady in the Entorhinal Cortex but it is Mainly Unmodified in the Frontal Cortex in Alzheimer's Disease. Curr Alzheimer Res. **14**, 1327–1334.

[134] Wang X, Su B, Fujioka H, Zhu X. (2008) Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. Am. J. Pathol. **173**, 470–482.

[135] Manczak M, Calkins MJ, Reddy PH. (2011) Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: Implications for neuronal damage. Hum. Mol. Genet. **20**, 2495–2509.

[136] Wang X, Su B, Lee H -g., Li X, Perry G, Smith MA, et al. (2009) Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Disease. J. Neurosci. [Internet]. **29**, 9090–9103. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1357-09.2009

[137] Martín-Maestro P, Gargini R, García E, Perry G, Avila J, García-Escudero V. (2017) Slower Dynamics and Aged Mitochondria in Sporadic Alzheimer's Disease. Oxid. Med. Cell. Longev. [Internet]. **2017**, 1–14. Available from: https://www.hindawi.com/journals/omcl/2017/9302761/

[138] Wang S, Song J, Tan M, Albers KM, Jia J. (2012) Mitochondrial fission proteins in

peripheral blood lymphocytes are potential biomarkers for Alzheimer's disease. Eur. J. Neurol. [Internet]. **19**, 1015–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22340708

[139] Wang X, Wang W, Li L, Perry G, Lee H-G, Zhu X. (2014) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim. Biophys. Acta [Internet]. **1842**, 1240–1247. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24189435

[140] Hauptmann S, Scherping I, Dröse S, Brandt U, Schulz KL, Jendrach M, et al. (2009) Mitochondrial dysfunction: An early event in Alzheimer pathology accumulates with age in AD transgenic mice. Neurobiol. Aging. **30**, 1574–1586.

[141] Ramamoorthy M, Sykora P, Scheibye-Knudsen M, Dunn C, Kasmer C, Zhang Y, et al. (2012) Sporadic Alzheimer disease fibroblasts display an oxidative stress phenotype. Free Radic. Biol. Med. **53**, 1371–1380.

[142] Leuner K, Schütt T, Kurz C, Eckert SH, Schiller C, Occhipinti A, et al. (2012) Mitochondrion-Derived Reactive Oxygen Species Lead to Enhanced Amyloid Beta Formation. Antioxid. Redox Signal. [Internet]. **16**, 1421–1433. Available from: http://online.liebertpub.com/doi/abs/10.1089/ars.2011.4173

[143] Area-Gomez E, De Groof AJC, Boldogh I, Bird TD, Gibson GE, Koehler CM, et al. (2009) Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria. Am. J. Pathol. **175**, 1810–1816.

[144] Zampese E, Fasolato C, Kipanyula MJ, Bortolozzi M, Pozzan T, Pizzo P. (2011) Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca2+ cross-talk. Proc. Natl. Acad. Sci. [Internet]. **108**, 2777–2782. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1100735108

[145] Zhang C, Rissman RA, Feng J. (2015) Characterization of ATP alternations in an Alzheimer's disease transgenic mouse model. J. Alzheimer's Dis. **44**, 375–378.

[146] Dixit S, Fessel JP, Harrison FE. (2017) Mitochondrial dysfunction in the APP/PSEN1 mouse model of Alzheimer's disease and a novel protective role for

ascorbate. Free Radic. Biol. Med. **112**, 515–523.

[147] Gray NE, Quinn JF. (2015) Alterations in mitochondrial number and function in Alzheimer's disease fibroblasts. Metab. Brain Dis. **30**, 1275–1278.

[148] Contino S, Porporato PE, Bird M, Marinangeli C, Opsomer R, Sonveaux P, et al. (2017) Presenilin 2-dependent maintenance of mitochondrial oxidative capacity and morphology. Front. Physiol. **8**.

[149] Wang X, Su B, Siedlak SL, Moreira PI, Fujioka H, Wang Y, et al. (2008) Amyloid- overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. Proc. Natl. Acad. Sci. [Internet]. **105**, 19318–19323. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0804871105

[150] Rönnbäck A, Pavlov PF, Mansory M, Gonze P, Marlière N, Winblad B, et al. (2016) Mitochondrial dysfunction in a transgenic mouse model expressing human amyloid precursor protein (APP) with the Arctic mutation. J. Neurochem. **136**, 497–502.

[151] Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. (2011) Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. Hum. Mol. Genet. **20**, 4515–4529.

[152] Trushina E, Nemutlu E, Zhang S, Christensen T, Camp J, Mesa J, et al. (2012) Defects in mitochondrial dynamics and metabolomic signatures of evolving energetic stress in mouse models of familial alzheimer's disease. PLoS One. **7**.

[153] Martín-Maestro P, Gargini R, A. Sproul A, García E, Antón LC, Noggle S, et al. (2017) Mitophagy Failure in Fibroblasts and iPSC-Derived Neurons of Alzheimer's Disease-Associated Presenilin 1 Mutation. Front. Mol. Neurosci. [Internet]. **10**. Available from: http://journal.frontiersin.org/article/10.3389/fnmol.2017.00291/full

[154] Wojsiat J, Laskowska-Kaszub K, Alquézar C, Białopiotrowicz E, Esteras N, Zdioruk M, et al. (2017) Familial Alzheimer's Disease Lymphocytes Respond

Differently Than Sporadic Cells to Oxidative Stress: Upregulated p53-p21 Signaling Linked with Presenilin 1 Mutants. Mol. Neurobiol. **54**, 5683–5698.

[155] Choi DY, Lee YJ, Lee SY, Lee YM, Lee HH, Choi IS, et al. (2012) Attenuation of scopolamine-induced cognitive dysfunction by obovatol. Arch. Pharm. Res. **35**, 1279–1286.

[156] El-Khadragy M, Al-Olayan E, Moneim A. (2014) Neuroprotective Effects of Citrus reticulata in Scopolamine-Induced Dementia Oxidative Stress in Rats. CNS Neurol. Disord. - Drug Targets [Internet]. **13**, 684–690. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1871-5273&volume=13&issue=4&spage=684

[157] Pachauri SD, Tota S, Khandelwal K, Verma PRP, Nath C, Hanif K, et al. (2012) Protective effect of fruits of Morinda citrifolia L. on scopolamine induced memory impairment in mice: A behavioral, biochemical and cerebral blood flow study. J. Ethnopharmacol. **139**, 34–41.

[158] Wong-Guerra M, Jiménez-Martin J, Pardo-Andreu GL, Fonseca-Fonseca LA, Souza DO, de Assis AM, et al. (2017) Mitochondrial involvement in memory impairment induced by scopolamine in rats. Neurol. Res. **39**, 649–659.

[159] Mehla J, Pahuja M, Gupta YK. (2013) Streptozotocin-induced sporadic Alzheimer's Disease: Selection of appropriate dose. J. Alzheimer's Dis. **33**, 17–21.

[160] Correia SC, Santos RX, Santos MS, Casadesus G, Lamanna JC, Perry G, et al. (2013) Mitochondrial abnormalities in a streptozotocin-induced rat model of sporadic Alzheimer's disease. Curr. Alzheimer Res. [Internet]. **10**, 406–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23061885

[161] Paidi RK, Nthenge-Ngumbau DN, Singh R, Kankanala T, Mehta H, Mohanakumar KP. (2015) Mitochondrial Deficits Accompany Cognitive Decline Following Single Bilateral Intracerebroventricular Streptozotocin. Curr. Alzheimer Res. [Internet]. **12**, 785–795. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26159195

[162] Sarkar P, Zaja I, Bienengraeber M, Rarick KR, Terashvili M, Canfield S, et al. (2014) Epoxyeicosatrienoic acids pretreatment improves amyloid -induced mitochondrial dysfunction in cultured rat hippocampal astrocytes. AJP Hear. Circ. Physiol. [Internet]. **306**, H475–H484. Available from: http://ajpheart.physiology.org/cgi/doi/10.1152/ajpheart.00001.2013

[163] Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. (2014) Mitochondria-targeted antioxidants for treatment of Parkinson's disease: preclinical and clinical outcomes. Biochim. Biophys. Acta [Internet]. **1842**, 1282–94. Available from: http://linkinghub.elsevier.com/retrieve/pii/S092544391300286X

[164] Murphy MP. (2008) Targeting lipophilic cations to mitochondria [Internet]. Biochim. Biophys. Acta - Bioenerg. **1777**, 1028–1031. Available from: https://ac.els-cdn.com/S0005272808000790/1-s2.0-S0005272808000790-main.pdf?_tid=7fa42b30-fc4a-11e7-a93f-00000aacb360&acdnat=1516278436_445803690df8f11b2f3258d9f07dd131

[165] Solesio ME, Prime TA, Logan A, Murphy MP, del Mar Arroyo-Jimenez M, Jordán J, et al. (2013) The mitochondria-targeted anti-oxidant MitoQ reduces aspects of mitochondrial fission in the 6-OHDA cell model of Parkinson's disease. Biochim. Biophys. Acta - Mol. Basis Dis. [Internet]. **1832**, 174–182. Available from: https://ac.els-cdn.com/S0925443912001706/1-s2.0-S0925443912001706-main.pdf?_tid=9c4a9c1e-fc4b-11e7-a670-00000aab0f26&acdnat=1516278921_60edd6e79b5428f1d14ded8dd9d1af0b

[166] Ghosh A, Chandran K, Kalivendi S V, Joseph J, Antholine WE, Hillard CJ, et al. (2010) Neuroprotection by a mitochondria-targeted drug in a Parkinson's disease model. Free Radic. Biol. Med. [Internet]. **49**, 1674–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20828611

[167] Snow BJ, Rolfe FL, Lockhart MM, Frampton CM, O'Sullivan JD, Fung V, et al. (2010) A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's

disease. Mov. Disord. [Internet]. **25**, 1670–1674. Available from: http://doi.wiley.com/10.1002/mds.23148

[168] Gane EJ, Weilert F, Orr DW, Keogh GF, Gibson M, Lockhart MM, et al. (2010) The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. Liver Int. [Internet]. **30**, 1019–1026. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20492507

[169] Manczak M, Mao P, Calkins MJ, Cornea A, Reddy AP, Murphy MP, et al. (2010) Mitochondria-targeted antioxidants protect against amyloid-beta toxicity in Alzheimer's disease neurons. J. Alzheimers. Dis. [Internet]. **20 Suppl 2**, S609-31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20463406

[170] McManus MJ, Murphy MP, Franklin JL. (2011) The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease. J. Neurosci. [Internet]. **31**, 15703–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22049413

[171] Korshunova GA, Shishkina A V., Skulachev M V. (2017) Design, synthesis, and some aspects of the biological activity of mitochondria-targeted antioxidants. Biochem. **82**, 760–777.

[172] Stefanova NA, Muraleva NA, Maksimova KY, Rudnitskaya EA, Kiseleva E, Telegina D V, et al. (2016) An antioxidant specifically targeting mitochondria delays progression of Alzheimer's disease-like pathology. Aging (Albany. NY). **8**, 2713–2733.

[173] Silachev D, Plotnikov E, Zorova L, Pevzner I, Sumbatyan N, Korshunova G, et al. (2015) Neuroprotective Effects of Mitochondria-Targeted Plastoquinone and Thymoquinone in a Rat Model of Brain Ischemia/Reperfusion Injury. Molecules [Internet]. **20**, 14487–14503. Available from: http://www.mdpi.com/1420-3049/20/8/14487/

[174] Brenza TM, Ghaisas S, Ramirez JEV, Harischandra D, Anantharam V,

Kalyanaraman B, et al. (2017) Neuronal protection against oxidative insult by polyanhydride nanoparticle-based mitochondria-targeted antioxidant therapy. Nanomedicine Nanotechnology, Biol. Med. [Internet]. **13**, 809–820. Available from: https://ac.els-cdn.com/S1549963416301745/1-s2.0-S1549963416301745-main.pdf?_tid=862438a0-fc05-11e7-898a-00000aacb35e&acdnat=1516248812_c4308954ac057a072c78617b404492a3

[175] Ghosh A, Langley MR, Harischandra DS, Neal ML, Jin H, Anantharam V, et al. (2016) Mitoapocynin Treatment Protects Against Neuroinflammation and Dopaminergic Neurodegeneration in a Preclinical Animal Model of Parkinson's Disease. J. Neuroimmune Pharmacol. [Internet]. **11**, 259–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26838361

[176] Reiter RJ, Mayo JC, Tan D-X, Sainz RM, Alatorre-Jimenez M, Qin L. (2016) Melatonin as an antioxidant: under promises but over delivers. J. Pineal Res. [Internet]. **61**, 253–278. Available from: http://doi.wiley.com/10.1111/jpi.12360

[177] Tan D-X, Manchester LC, Liu X, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ. (2013) Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. J. Pineal Res. [Internet]. **54**, 127–138. Available from: http://doi.wiley.com/10.1111/jpi.12026

[178] Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, et al. (2004) Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal Res. [Internet]. **36**, 1–9. Available from: http://doi.wiley.com/10.1046/j.1600-079X.2003.00092.x

[179] Antolin I, Mayo JC, Sainz RM, del Brio M de los A, Herrera F, Martin V, et al. (2002) Protective effect of melatonin in a chronic experimental model of Parkinson’s disease. Brain Res. [Internet]. **943**, 163–173. Available from: www.elsevier.com

[180] Rosales-Corral SA, Lopez-Armas G, Cruz-Ramos J, Melnikov VG, Tan D-X, Manchester LC, et al. (2012) Alterations in Lipid Levels of Mitochondrial

Membranes Induced by Amyloid-β: A Protective Role of Melatonin. Int. J. Alzheimers. Dis. [Internet]. **2012**, 459806. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22666620

[181] Dong W, Huang F, Fan W, Cheng S, Chen Y, Zhang W, et al. (2010) Differential effects of melatonin on amyloid-β peptide 25-35-induced mitochondrial dysfunction in hippocampal neurons at different stages of culture. J. Pineal Res. [Internet]. **48**, 117–125. Available from: http://doi.wiley.com/10.1111/j.1600-079X.2009.00734.x

[182] Yang L, Zhao K, Calingasan NY, Luo G, Szeto HH, Beal MF. (2009) Mitochondria targeted peptides protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. Antioxid. Redox Signal. [Internet]. **11**, 2095–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19203217

[183] Aerts L, Craessaerts K, De Strooper B, Morais VA. (2015) PINK1 kinase catalytic activity is regulated by phosphorylation on serines 228 and 402. J. Biol. Chem. [Internet]. **290**, 2798–811. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25527497

[184] Hertz NT, Berthet A, Sos ML, Thorn KS, Burlingame AL, Nakamura K, et al. (2013) A neo-substrate that amplifies catalytic activity of parkinson's-disease-related kinase PINK1. Cell [Internet]. **154**, 737–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23953109

[185] Orr AL, Rutaganira FU, de Roulet D, Huang EJ, Hertz NT, Shokat KM, et al. (2017) Long-term oral kinetin does not protect against α-synuclein-induced neurodegeneration in rodent models of Parkinson's disease. Neurochem. Int. [Internet]. **109**, 106–116. Available from: https://ac.els-cdn.com/S0197018617300372/1-s2.0-S0197018617300372-main.pdf?_tid=1c246e0e-0009-11e8-92b0-00000aacb35f&acdnat=1516690165_377363b34eafca887fbb40d53b2d2220

[186] Wei Y, Liu D, Zheng Y, Li H, Hao C, Ouyang W. (2017) Protective effects of kinetin against aluminum chloride and D-galactose induced cognitive impairment

and oxidative damage in mouse. Brain Res. Bull. [Internet]. **134**, 262–272. Available from: www.elsevier.com/locate/brainresbull

[187] Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, et al. (2008) Chemical Inhibition of the Mitochondrial Division Dynamin Reveals Its Role in Bax/Bak-Dependent Mitochondrial Outer Membrane Permeabilization. Dev. Cell [Internet]. **14**, 193–204. Available from: http://www.cell.com/developmental-cell/pdf/S1534-5807(07)00475-3.pdf

[188] Rappold PM, Cui M, Grima JC, Fan RZ, de Mesy-Bentley KL, Chen L, et al. (2014) Drp1 inhibition attenuates neurotoxicity and dopamine release deficits in vivo. Nat. Commun. [Internet]. **5**, 5244. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25370169

[189] Bido S, Soria FN, Fan RZ, Bezard E, Tieu K. (2017) Mitochondrial division inhibitor-1 is neuroprotective in the A53T-α-synuclein rat model of Parkinson's disease. Sci. Rep. [Internet]. **7**, 7495. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28790323

[190] Bordt EA, Clerc P, Roelofs BA, Saladino AJ, Tretter L, Adam-Vizi V, et al. (2017) The Putative Drp1 Inhibitor mdivi-1 Is a Reversible Mitochondrial Complex I Inhibitor that Modulates Reactive Oxygen Species. Dev. Cell [Internet]. **40**, 583–594.e6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1534580717301168

[191] Mallat A, Uchiyama LF, Lewis SC, Fredenburg RA, Terada Y, Ji N, et al. (2018) Discovery and characterization of selective small molecule inhibitors of the mammalian mitochondrial division dynamin, DRP1. Biochem. Biophys. Res. Commun. **499**, 556–562.

[192] Qi X, Qvit N, Su Y-C, Mochly-Rosen D. (2013) A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. J. Cell Sci. [Internet]. **126**, 789–802. Available from: http://jcs.biologists.org/content/joces/126/3/789.full.pdf

[193] Gan X, Huang S, Wu L, Wang Y, Hu G, Li G, et al. (2014) Inhibition of ERK-DLP1

signaling and mitochondrial division alleviates mitochondrial dysfunction in Alzheimer's disease cybrid cell. Biochim. Biophys. Acta [Internet]. **1842**, 220–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24252614

[194] Reddy PH, Manczak M, Yin X. (2017) Mitochondria-Division Inhibitor 1 Protects Against Amyloid-β induced Mitochondrial Fragmentation and Synaptic Damage in Alzheimer's Disease. J. Alzheimers. Dis. [Internet]. **58**, 147–162. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28409745

[195] Wang W, Yin J, Ma X, Zhao F, Siedlak SL, Wang Z, et al. (2017) Inhibition of mitochondrial fragmentation protects against Alzheimer's disease in rodent model. Hum. Mol. Genet. **26**, 4118–4131.

[196] Kim Y, McGee S, Czeczor JK, Walker AJ, Kale RP, Kouzani AZ, et al. (2016) Nucleus accumbens deep-brain stimulation efficacy in ACTH-pretreated rats: alterations in mitochondrial function relate to antidepressant-like effects. Transl. Psychiatry [Internet]. **6**, e842. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27327257

[197] Aniello MS, Martino D, Petruzzella V, Eleopra R, Mancuso M, Dell'Aglio R, et al. (2008) Bilateral striatal necrosis, dystonia and multiple mitochondrial DNA deletions: Case study and effect of deep brain stimulation. Mov. Disord. [Internet]. **23**, 114–118. Available from: https://doi.org/10.1002/mds.21760

[198] Pelzer E, Pauls AKM, Binder E, Brunn A, Fink GR, Timmermann L. (2012) Deep brain stimulation in rapidly progressive parkinson-dystonia syndrome due to mitochondrial disorder. Parkinsonism Relat. Disord. [Internet]. **18**, 672–674. Available from: http://dx.doi.org/10.1016/j.parkreldis.2011.10.012

[199] Norbert K, Endre P, Istvan B, Jozsef J, Ferenc N, Hajnalka M. (2006) Neurosurgical treatment of tremor in mitochondrial encephalopathy. Mov. Disord. **21**, 2227–2230.

[200] Martinez-Ramirez D, Hack N, Vasquez ML, Morita H, Giugni JC, Wolf JM, et al. (2016) Deep Brain Stimulation in a Case of Mitochondrial Disease. Mov. Disord.

Clin. Pract. [Internet]. **3**, 139–145. Available from: http://doi.wiley.com/10.1002/mdc3.12241

[201] Ved R, Saha S, Westlund B, Perier C, Burnam L, Sluder A, et al. (2005) Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of α-synuclein, parkin, and DJ-1 in Caenorhabditis elegans. J. Biol. Chem. **280**, 42655–42668.

[202] Abdelkader NF, Safar MM, Salem HA. (2016) Ursodeoxycholic Acid Ameliorates Apoptotic Cascade in the Rotenone Model of Parkinson's Disease: Modulation of Mitochondrial Perturbations. Mol. Neurobiol. [Internet]. **53**, 810–7. Available from: http://link.springer.com/10.1007/s12035-014-9043-8

[203] Ramalho RM, Borralho PM, Castro RE, Sola S, Steer CJ, Rodrigues MP. (2006) Tauroursodeoxycholic acid modulates p53-mediated apoptosis in Alzheimer ' s disease mutant neuroblastoma cells. J Neurochem. **98**, 1610–1618.

[204] Lo AC, Callaerts-Vegh Z, Nunes AF, Rodrigues CMP, D'Hooge R. (2013) Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice. Neurobiol. Dis. **50**, 21–29.

[205] Zhang Y, Nguyen DT, Olzomer EM, Poon GP, Cole NJ, Puvanendran A, et al. (2017) Rescue of Pink1 Deficiency by Stress-Dependent Activation of Autophagy. Cell Chem. Biol. **24**, 471–480.e4.

[206] Robke L, Futamura Y, Konstantinidis G, Wilke J, Aono H, Mahmoud Z, et al. (2018) Discovery of the novel autophagy inhibitor aumitin that targets mitochondrial complex I. Chem. Sci. [Internet]. **9**, 3014–3022. Available from: http://dx.doi.org/10.1039/C7SC05040B

Figure 1. Example phenotypic screening pipeline. This figure outlines the various stages which a phenotypic screen could progress by. Stage 1 is the identification, testing of robustness and assessing suitability of the screening assay. This stage can

often be the most time consuming stage to set up. Stage 2 is the primary screen, considerations at this stage include through put, size of library and number of times the screen will be run. Stage 3 is the secondary screening phase which includes excluding false positives, toxicity testing and validating a positive effect on the pathway via an alternative methodology. Stage 4 is the tertiary screening, in depth characterisation of the pathway in other model systems which is coupled with the often extensive and time consuming target identification step. Step 5 then is the hit to lead optimisation and structure activity relationship with medicinal chemistry input. This step could involve designing a complete new screen dependent on the target identified in step 4. This pathway is simply a representation of the steps which could be undertaken in phenotypic screening and does not depict the only pathway to follow.