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Modulation of cellular redox environment as a novel therapeutic strategy for Parkinson's Disease

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Abbreviation List

6-OHDA	6-hydroxydopamine
AADC	aromatic l-amino acid decarboxylase
AD	Alzheimer's Disease;
ARE	antioxidant responsive element
ATP	adenosine triphosphate
ATP13A2	ATPase 13A2
BSO	buthionine sulfoximine
CHCHD2	coiled-coil-helix-coiled-coil-helix domain containing 2
CNS	central nervous system
EGCG	epigallocatechin-3-gallate
ER	endoplasmic reticulum
ETC	mitochondrial electron transport chain
FBXO7	F-box protein 7
FDA	Food and Drug Administration
G6PD	glucose-6-phosphate dehydrogenase
GIGYF	GRB10 interacting GYF protein 2
GPx	glutathione peroxidase
GR	gluthathione reductase
GS-DAQ	glutathine to form adducts
GSH	glutathione
HCAs	hydroxycinnamic acids
HIF1-α	hypoxia-inducible factor 1-*alpha*
LB's	lewy bodies
L-DOPA	levodopa
LRRK2	leucine-rich repeat kinase 2
MAM	mitochondria-associated membranes
MAO	monoamine oxidase
MPP^+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	mitochondrial deoxyribonucleic acid
mtROS	mitochondrial reactive oxygen species
NADPH	nicotinamide adenine dinucleotide phosphate
NOX	transmembrane NADPH oxidase
Nrf2	nuclear factor erythroid 2–related factor 2
OXPHOS	oxidative phosphorylation
PD	Parkinson's Disease
PGC1-α	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PINK1	PTEN-induced kinase 1
PLA2G6	phospholipase A2 group VI
POLG	DNA polymerase subunit gamma
PPP	pentose phosphate pathway
Prxs	peroxiredoxins
PUFAs	omega-3 polyunsaturated fatty acids
ROS	reactive oxygen species
RNS	reactive nitrogen species
SIRT1	sirtuin 1
SNpc	substantia nigra pars compacta
SOD	superoxide dismutase
sPD	sporadic Parkinson's Disease
TCA	tricarboxylic acid
TFAM	mitochondrial transcription factor A
TFB2m	mitochondrial transcription factor B2
TH	tyrosine hydroxylase
TPP^+	triphenylphosphonium cation
TPx	thioredoxin peroxidase
Trx	thioredoxin
TrxR	thioredoxin reductase
UCH-L1	ubiquitin C-terminal hydrolase L1
UPS	ubiquitin proteasome system
VMAT2	vesicular monoamine transporter 2
VPS35	vacuolar protein sorting-associated protein 35

Abstract

Parkinson's Disease (PD) is an incurable neurodegenerative movement disorder. PD affects 2% of the population above 65 years old; however, with the growing number of senior citizens, PD prevalence is predicted to increase in the following years. Pathologically, PD is characterized by dopaminergic cell neurodegeneration in the *substantia nigra,* resulting in decreased dopamine levels in the nigrostriatal pathway, triggering motor symptoms. Although the pathological mechanisms leading to PD are still unclear, large evidence indicates that oxidative stress plays an important role, not only because it increases with age which is the most significant risk factor for PD development, but also as a result of alterations in several processes, particularly mitochondria dysfunction. The modulation of oxidative stress, especially by using dietary mitochondriotropic antioxidants, represents a promising approach to prevent or treat PD. Although most mitochondria-targeted antioxidants with beneficial effects in PD-associated models have failed to show any therapeutic benefit in clinical trials, several questions remain to be clarified. Hereby, we review the role played by oxidative stress in PD pathogenesis, emphasizing mitochondria as reactive oxygen species (ROS) producers and as targets for oxidative stress-related dysfunctional mechanisms. In addition, we also describe the importance of using dietary-based mitochondria-targeted antioxidants as a valuable strategy to counteract the deleterious effects of ROS in pre-clinical and/or clinical trials of PD, pointing out their significance to slow, and possibly halt, the progression of PD.

Keywords (6-8): Dietary-based antioxidants; metabolism; mitochondria; mitochondria-targeted antioxidants; oxidative stress; Parkinson's Disease.

1. Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's Disease (AD) and the most common disorder affecting motor coordination [1]. PD prevalence ranges from 1 to 2 per 1,000 in unselected populations, and PD affects 2% of the population above 65 years old. Before 50 years of age, PD is rare but reaches a prevalence of 4% in the oldest age groups, pointing out age as the most common risk factor for PD development. The annual prevalence in high-income countries is 14 per 100,000 people in the total population and 160 per 100,000 people over 65 years old [1]. The incidence of PD is 1.5 times higher in males than females [2]. Although the most prevalent PD cases are sporadic forms, which have an unknown cause, around 10-15% of the individuals with PD have a mutation of one or several specific genes, called familial PD form (Table 1) [3]. Currently, at least ten mutated genes have been linked with familial PD, including *α-synuclein* (PARK1), *Parkin* (PARK2), *ubiquitin C-terminal hydrolase L1* (UCH-L1 or PARK5), *PTEN-induced kinase 1* (*PINK1* or PARK6), *DJ-1* (PARK7), *leucine-rich repeat kinase 2* (*LRRK2* or PARK8), *ATPase 13A2* (ATP13A2 or PARK9), *phospholipase A2 group VI* (PLA2G6 or PARK14), *F-Box protein 7* (FBXO7 or PARK15), *GRB10 interacting GYF protein 2* (GIGYF or PARK11) (Table 1) [4,5].

Patients with PD have severe motor symptoms, including resting tremor, muscular rigidity, slowness of movement – *bradykinesia*, postural instability, and difficulty walking and thinking [6]. Patients can also have symptoms not affecting motor coordination, including depression, anxiety, dementia, and sleep abnormalities [6]. Currently, PD is clinically diagnosed based on the motor symptoms of the patients, including the presence of *bradykinesia, combined* with resting tremors or muscular rigidity and the response to specific drugs, including levodopa (L-DOPA), which is the most widely used drug to control PD-associated motor symptoms [7]. However, the diagnosis validation still depends on *postmortem brain* histology [8], which led to the development of several models to study PD pathogenesis and therapeutics over time.

Parkinson's Disease has been known for over 200 years, and despite intense research efforts, effective treatments and timely diagnostic markers are lacking. This review focuses on oxidative stress in PD, particularly on the role of mitochondrial dysfunction in that phenomenon. We also address the use of dietary antioxidant-based therapeutic strategies to counteract the deleterious effects of reactive oxygen species (ROS)

to prevent or treat PD. Although several antioxidants have shown minimal therapeutic benefit, we summarize the molecular and metabolic evidence that novel mitochondria-targeted antioxidants can be multifunctional compounds promoting mitochondrial health. Moreover, we also discuss why antioxidant therapies failed when used in clinical trials, thus showing new insights to achieving novel opportunities for intervention.

2. Oxidative Stress in Parkinson's Disease pathogenesis

Pathologically, PD is characterized by dopaminergic neurodegeneration in *substantia nigra pars compacta (SNpc*), culminating in decreased dopamine levels in the *dorsal striatum* [9]. Although the exact mechanism by which dopaminergic neuronal death occurs is unclear, several contributors to PD pathophysiology have been identified (Figure 1). The widely accepted PD disease mechanisms include accumulation of misfolded protein aggregates, alterations in dopamine metabolism, neuroinflammation, mitochondrial dysfunction, and impaired quality control mechanisms (Figure 1) [10]. The indefinition related with a specific biochemical mechanism for the progressive and complex neurodegenerative cascade makes the oxidative stress an indisputable player in that process [11] (Figure 1). Since oxidative stress is also aging-associated and aging is the most significant risk factor for PD development, it is unclear whether oxidative stress is cause or consequence of detrimental pathways during PD pathogenesis [11]. The oxidative stress theory of aging postulates that reductions in physiologic functions associated with age are caused by a slow and steady accumulation of macromolecules-induced oxidative damage, which increases with age and are associated with organisms' life expectancy [12]. A study based on the aging-time-control coefficient showed that 25 out of 57 molecular processes are controlled by aging [13].

2.1. Disruption of redox circuits in PD pathogenesis

The brain is one of the major organs that generates large amounts of ROS [14]. ROS are generated intracellularly as byproducts of basal metabolism, [15] and can exert beneficial or detrimental effects at the cellular level depending on their concentration and origin, and of antioxidant defense concentrations and activity in different sub-cellular compartments [15]. For instance, electrons derived from different substrates are carried into electron transport chain (ETC) in order to fuel OXPHOS. Electrons leaking from ETC can react

with oxygen, generating superoxide, which is itself a ROS and can generate other ROS [16]. Moreover, NADPH oxidase could product superoxide either as a by-product of ETC or from a dysfunctional variant in the conversion of xanthine dehydrogenase to xanthine oxidase, since in both mechanisms NADPH oxidase transfers electrons from NADPH to molecular oxygen through NOX catalytic subunit [17]. Physiologically, low ROS levels act as a second messenger, modifying oxidation-reduction (redox) states, which culminates in post-translational modifications of kinases and phosphatases and ultimately in the perturbation of processes such as cell survival and proliferation (Figure 2) [15]. ROS-induced modifications can also regulate enzymatic activity, drug detoxification and cellular responses to external perturbations [18], all pivotal in maintaining homeostatic signaling events. In fact, mitochondrial oxidants induce an adaptive response to improve systemic defense, the so-called mitohormesis (Figure 2) [18]. Among the several ROS, hydrogen peroxide (H_2O_2) acts as a second messenger due to its neutral and membrane-permeable capacity allowing its relatively free diffusion from the generation site. Furthermore, H_2O_2 has no unpaired electrons in the last orbital making it comparatively less reactive than other ROS [19].

On the other hand, ROS overproduction resulting from endogenous sources, including transmembrane NADPH oxidases (NOXs) and the mitochondrial ETC, or cumulative environmental exposure, commonly denominated as the exposome, can be detrimental to the cell's well-being. The exposome can involve nutritional cues, and exposure to drugs, toxicants, pollutants, physical (ultraviolet, X-ray, and ionizing radiation) or psychological stressors [18]. The steady-state cellular levels of ROS are a balance between their production and their degradation/removal. Therefore, oxidative stress can result from overproduction of reactive species or by a deficiency of enzymatic and endogenous non-enzymatic antioxidants. The impact of oxidative stress damage on the organism depends on the oxidant type, the place, duration and intensity of its production, the composition and activities of various antioxidants, and repair systems' ability [20]. Classically, the imbalance between ROS and endogenous antioxidants defense mechanisms results in an oxidative stress state, promoting irreversible damage to biomolecules, causing growth arrest and cell death, which are customarily associated with pathological states, including PD, and are currently referred to as oxidative distress [18].

Oxidative distress that occurs in neurons can contribute to PD pathogenesis. Nigral dopaminergic neurons seem to be particularly vulnerable to oxidative stress, possibly due to long axons and millions of synapses, requiring high energy input and hence a high oxygen demand [21]. Moreover, compared with other organs (e.g. liver) the brain has lower antioxidant enzymes activities, namely superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activities. At the same time, the brain contains a high percentage of lipids with unsaturated fatty acids that are prone to lipid peroxidation. In addition, the brain glutathione (GSH) concentration is lower than in other organs, such as the liver, kidney or the small intestine [14]. In fact, cytosolic GSH in neurons is approximately 5 mM compared with 10-11 mM in hepatocytes [22].

Moreover, oxidative distress can result from increased levels of dopamine and its metabolites and mitochondrial dysfunction. On the other hand, antioxidant defense mechanisms in the brain are inadequate, limiting its protective action against ROS [23]. This complex system will be described below in the context of dopaminergic neurons (Figure 3).

2.1.1. Disruption of redox homeostasis in dopaminergic neurons

2.1.1.1. Pro-oxidant properties of dopamine

SNpc is a vital part of the brain basal ganglia, which predominantly comprises neurons that secrete dopamine, a catecholaminergic neurotransmitter [24]. Taking into account the selective degeneration of dopaminergic neurons of *SNpc*, this suggests dopamine itself may be an oxidative stress source. Dopamine is synthesized from tyrosine by tyrosine hydroxylase (TH) and aromatic l-amino acid decarboxylase (AADC) [25]. After its uptake by vesicular monoamine transporter 2 (VMAT2), dopamine is stored in synaptic vesicles [26]. However, in the presence of an excessive cytosolic amount of dopamine outside of the synaptic vesicle in damaged neurons, that neurotransmitter is metabolized by monoamine oxidase (MAO) generating H_2O_2 or by auto-oxidation to cytotoxic ROS, which in the presence of free Fe^{2+} generates hydroxyl radical ($^{\bullet}OH$), H_2O_2, and dopamine-quinone species (Figure 3). The byproducts of dopamine-quinone species resulting from dopamine oxidation react with GSH to form adducts (GS-DAQ) through the GSH-transferases activity [27]. Subsequently, mitochondrial respiration is inhibited and the permeability transition pore opening occurs in brain mitochondria promoting cell death and neuronal loss [28,29]. Thus, it has been proposed that dopaminergic

cell death-induced by mitochondrial inhibitors depends on dopamine oxidation [27]. In fact, it was demonstrated that dopamine interacts with lipid hydroperoxides produced during peroxidation events leading to the formation of adducts that could account for the selective cytotoxicity of dopaminergic neurons [30]. Moreover, hexanoyl dopamine, an arachidonic acid-derived adduct, causes severe cytotoxicity in human dopaminergic neuroblastoma SH-SY5Y cell line [30]. Since PD also affects non-dopaminergic cells, and not all dopaminergic neurons are equally affected during the disease progression [31], the high toxicity of dopamine can sensitize dopaminergic neurons to oxidative damage.

2.1.1.2. Superoxide dismutases (SODs) activity dysregulation

During cell metabolism, oxygen suffers a series of univalent reduction reactions leading to the production of $^{\bullet}O_2^-$, H_2O_2 and H_2O. Superoxide anion is dismuted by SODs to H_2O_2, which is catalyzed to H_2O by catalase, peroxiredoxins (Prx) or GPx (Figure 3) [32]. SODs are the major antioxidant defense systems, which consist of three isoforms in mammals: the Cu-Zn-dependent SOD (Cu/ZnSOD or SOD1) located in the cytoplasm or the mitochondrial intermembrane space, the manganese-dependent SOD (MnSOD or SOD2) located in the mitochondrial matrix, and the extracellular Cu/ZnSOD (SOD3) [32].

The protective role of SOD1 and SOD2 in both sporadic and genetic PD models has been previously demonstrated [33]. In fact, SOD1 and SOD2 overexpression rescue the toxic effect of products derived from dopamine in human SH-SY5Y cells [34]. Analysis of SODs in PD brains have shown no changes in SOD1 activity, while SOD2 activity was increased [35], suggesting that SOD2 is activated in response to ROS overproduction. However, assays in human SH-SY5Y neuroblastoma cells or drosophila melanogaster-induced PD showed that SOD2 is important to protect against high paraquat concentrations. In contrast, at sub-lethal paraquat concentrations, the over-expression of SOD1 is enough to rescue the paraquat-associated toxicity in drosophila melanogaster [36]. The same occurs when SOD1 is specifically expressed in dopaminergic neurons. These observations indicate that other cytosolic processes inside dopaminergic neurons, namely dopamine oxidation, may amplify ROS toxicity [36]. Defects in SODs activity develop over time in PD, remaining unclear whether this is a later manifestation of deterioration of antioxidant activity or an epigenetic-related phenomenon [37].

2.1.1.3. Thioredoxins and peroxiredoxins system (dys)homeostasis

The thioredoxin system consists of three proteins, namely thioredoxin (Trx), thioredoxin reductase (TrxR), thioredoxin peroxidase (TPx), and nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor [38]. The central core element is Trx, which has an active conserved site Cys-Pro-Gly-Cys, capable of reducing disulfide bonds in proteins [39]. In humans, two different isoforms of Trx are found: a cytosolic isoform (Trx1) and a mitochondrial isoform (Trx2), which can be found in different human body tissues. Trx plays essential roles in regulating redox signals and stress response, modulating several signaling pathways, transcription factors, and immunological response [38].

The enzyme responsible for catalyzing the reduction of the disulfide at the Trx active site is TrxR. In humans, there are three different TrxR: TrxR1 locates in the cytosol, TrxR2 locates in mitochondria and thioredoxin-glutaredoxin reductase (TrxR3) [40]. While TrxR1 and TrxR2 are expressed in all mammalian cells and tissues, including the brain, TrxR3 is expressed in testis [38]. Trx acts as the reducing agent for peroxiredoxins and others proteins, namely p53, nuclear factor erythroid 2–related factor 2 (Nrf2), hypoxia-inducible factor 1-alpha (HIF1-α), and others [41].

The system dependence of Trx is also linked with TPx, which cooperatively can reduce lipid peroxides and H_2O_2 (Figure 3). TPx is part of a family of proteins called Prx [42]. The Prx family are thiol-dependent peroxidases promoting the reduction of H_2O_2, $ONOO^-$ and alkyl hydroperoxides. Prx has a conserved active site cysteine, called the peroxidatic cysteine, which reacts with peroxide forming a cysteine sulfenic acid (R-SOH) and releases the corresponding alcohol or water [43]. However, some of the Prx contain also a second cysteine, called the resolving cysteine, which attacks the R-SOH to form a water molecule and a disulfide bond in the protein. In humans, there are six isoforms of Prx (Prx1-6) and their expression in central nervous system (CNS) is cell type-specific and localize in different cellular compartments [21]

Trx and Prx induction contributes to brain tolerance to toxic insults. The thioredoxin/ thioredoxin reductase (Trx/TrxR) system protects dopaminergic cells against toxicity induced by MPP^+, 6-OHDA and paraquat [27]. Work performed in patients with sporadic PD (sPD) showed that oxidative forms of DJ-1 protein protect both culture cells and *substantia nigra* of mice from oxidative stress by inducing Trx1 gene expression

via the transcription factor Nrf2 [44]. Incubation of immortalized rat dopaminergic cells with auranofin, an inhibitor of TrxR, in the presence of sub-toxic concentrations of PD-associated toxins, including 6-OHDA and paraquat resulted in H_2O_2 increased levels, mitochondrial dysfunction, and oxidative stress-induced cell death [45,46].

Overexpression of peroxiredoxin 1, 2 and 4 (Prx1/2/4) protects against dopaminergic cell death induced by 6-OHDA, while the silencing of the mitochondrial isoforms, Prx3 and Prx5 increased the sensitivity to MPP^+. Dopaminergic neuronal cells treated with 6-OHDA lead to ROS generation, which causes oxidative modification of Prx1, while Prdx1 overexpression protects neuronal cells against 6-OHDA-induced cell death by means of ROS scavenging [47]. Moreover, brain tissues from PD patients show increased levels of Prdx2 [48] and Prdx3 [49]. Prdx2 overexpression attenuated 6-OHDA neurotoxicity in *in vitro* and *in vivo* models of PD through antiapoptotic effects, namely by inhibiting caspase-dependent apoptosis and suppressing of apoptosis signal-regulating kinase signaling cascade [50].

[51]. Glutathione metabolism is regulated by the glutathione cycle, in which glutamate, the most abundant excitatory transmitter in the CNS, is added and released during this cycle [52]. GSH can react with $^{\bullet}O_2^-$, NO, $ONOO^-$ and hydroxyl radical, this latter is only scavenged by GSH since there is no other known antioxidant system against hydroxyl radical (Figure 3). Moreover, GSH works as an electron donor to reduce H_2O_2 or other peroxides by GPx [53]. The brain has a relatively high level of GPx when compared with catalase. However, the *substantia nigra* contains lower GSH levels than other brain parts, including *cortex*, *cerebellum*, *hippocampus,* and *striatum* [54]. H_2O_2 is reduced to H_2O by GPx using GSH, which is oxidized to GSH disulfide (GSSG) (Figure 3). The latter is reduced back by GSH reductase with NADPH to GSH and is reused as a GPx substrate [53].

Decreased GSH levels have been considered one of the earliest biochemical alterations associated with

PD, since the loss of GSH occurs in incidental Lewy Bodies (LBs) disease, which is considered a pre-occurring PD phase in which non motor symptoms are present. A decrease in GSH levels by 40-50% has been reported in autopsied brain PD patients [55]. Accordingly, depletion of GSH promotes nigrostriatal degeneration in PD mice model [27]. Furthermore, dopaminergic neurons treated with buthionine sulfoximine (BSO), an inhibitor of GSSG reductase, showed decreased GSH levels and increased neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP^+) and 6-OHDA [56,57]. In addition, decreased catalase and GSH levels were demonstrated in blood and *postmortem* brain PD patients [58]. A clinical trials phase II using GSH to slow down the progression of PD symptoms was already completed with no results released so far (NCT02424708).

2.2. Mitochondria as a ROS producer and its involvement in Parkinson's Disease pathogenesis

Mitochondria are cellular organelles that regulate important anabolic and catabolic pathways [59]. Although one of the main functions of mitochondria is adenosine triphosphate (ATP) production through oxidative phosphorylation (OXPHOS), that organelle also plays essential roles in calcium homeostasis, biogenesis of iron-sulfur clusters, heme and steroid synthesis, fatty acids catabolism, redox regulation of cellular signaling, and regulation of programmed cell death [60]. Moreover, mitochondria have essential roles in other cellular processes, including autophagy, and cellular proliferation and differentiation [61]. According to specific internal and external cues, including oxygen availability or oxidative stress, and because of their nature, dynamic mitochondrial distribution, structure, and function can be modified [62], impacting cell physiology. Consequently, alterations in cell's redox circuits impact mitochondrial morphology and function at different levels (Figure 4), which may have pathophysiological consequences [63].

Approximately 2-4% of the oxygen consumed by mitochondria is redirected to form ${}^{\bullet}O_2^-$ [14]. Mitochondrial complexes I and III are primary sources of mitochondrial ${}^{\bullet}O_2^-$ [64] and the main target of ROS-induced oxidative stress, leading to ATP production inhibition and uncontrolled ROS generation. Subsequently, the vicious cycle between the defects in the ETC and ROS production drives the uncontrolled oxidative stress that may underlie PD pathogenesis, especially in the progressive dopaminergic neuronal

degeneration [65]. In this sense, ROS appears as the trigger point and the one significant consequence of mitochondrial dysfunction that occurs in PD (Figure 4).

Mitochondrial dysfunction can also result from environmental toxins exposure, implicated in sPD epidemiology. This increased awareness of environmental exposure associated with the risk of developing PD later in life through mitochondrial damage and augmented oxidative stress (Figure 4) [66].

MPTP-induced parkinsonism in intravenous drug users [67,68] was the first report showing that mitochondrial dysfunction is related to PD symptoms [69]. MPTP penetrates the blood brain barrier (BBB), being converted into its toxic form MPP^+ by glial MAO [70]. MPP^+ interferes with mitochondrial complex I of respiratory chain activity in dopaminergic neurons, causing selective neurodegeneration in human and mouse *SNpc* [67,71]. Rotenone is a well-studied insecticide presenting neuronal toxicity. Rotenone is highly lipophilic and can easily cross biological membranes, including BBB [72], leading to dopaminergic neurodegeneration. Rotenone, a mitochondrial complex I inhibitor, results in decreased ATP levels, leading to electron leakage that can form $^{\bullet}O_2^-$ [72]. Currently, rotenone treatment is used as a PD model due to its ability to reproduce parkinsonism-like features [73], including the accumulation and aggregation of specific hallmarks, such as α-synuclein, α-synuclein- and polyubiquitin-positive LBs and Lewy neurites, combined with apomorphine-responsive behavior deficits, early and sustained activation of microglia, oxidative modification and translocation of DJ1 into mitochondria *in vivo*, impairment of ubiquitin-proteasome system (UPS), iron accumulation in the *substantia nigra* through a mechanism involving transferrin and transferrin receptor 2, α-synuclein pathology in enteric neurons and functional deficits in gastrointestinal function, including gastroparesis [74]. The decreased activity of mitochondrial complex I in neurons from idiopathic PD patients were associated with mitochondrial DNA (mtDNA) depletion and decreased in mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFB2m) protein contents [75,76]. Furthermore, decreased complex I expression has also been shown in several brain regions of PD [77-79]. Complex I deficiency was observed in *substantia nigra* of patients with PD. However, this deficiency in complex I can be mtDNA damage-independent [79]. However, one question arises when considering the role of mitochondrial complex I dysfunction in PD ethiology: why patients with mitochondrial diseases with complex I abnormalities rarely develop a PD phenotype? Currently, no mtDNA mutations were found to be associated with PD (Figure 4), and mitochondrial diseases-associated

PD is restricted to mutations affecting mtDNA maintenance genes, such as DNA polymerase subunit gamma (POLG) and twinkle mtDNA helicase [80,81]. In fact, the exonuclease dysfunctional POLG mutator mouse does not show a PD phenotype itself; however, when crossed with a Parkin knockout mouse, the appearance of a PD phenotype occurs. These observations suggest that other insults are required, besides accumulation of somatic mtDNA mutations to cause PD [82]. It is known that somatic mtDNA mutations are increased in *substantia nigra* of rotenone-treated rats [80].

The mitochondrial respiratory chain in neurons from sPD patients presented defects in complex I and II and several depletions in mtDNA related to decreased mtDNA copy number in *substantia nigra* [75]. Aging is a risk factor for developing sPD, associated with reduced mitochondrial function, particularly an increased number of mtDNA mutations and oxidative stress, and decreasing respiratory chain activity [83]. Thus, mitochondrial dysfunction resulting from mtDNA and respiratory chain abnormalities contributes to PD pathogenesis by decreasing the threshold for the susceptibility to other genetic and environmental insults. Also, alterations in tricarboxylic acid (TCA) cycle fluxes occur in several PD models, including a decrease in α-ketoglutarate dehydrogenase activity. Moreover, complex I inhibitors and paraquat and PINK1 mutations promoted the oxidative inactivation of aconitase [27].

Several mutated genes in familial PD are directly associated with mitochondrial dysfunction, and increased oxidative stress, including *α-synuclein*, *LRRK2*, *Parkin*, *PINK1* and *ATP13A2*. Similarly, several mutated genes in PD, including *DJ-1*, *LRRK2*, *PINK1,* and *Parkin*, encode several regulators of mitochondrial and ROS homeostasis [83]. Alterations in newly found PD-relevant genes lead to mitochondrial dysfunction, including *vacuolar protein sorting-associated protein 35* (*VPS35)* and *coiled-coil-helix-coiled-coil-helix domain containing 2* (*CHCHD2*), which lead to mitochondrial fragmentation and abnormalities in mitochondrial structure, respectively [83]. α-Synuclein has a non-canonical mitochondrial targeting sequence, being also found in mitochondria-associated membranes (MAM), affecting mitochondrial structure and function [84]. Moreover, increased levels of wild-type α-synuclein induce mitochondrial fragmentation and production of ROS both *in vitro* and *in vivo* models [85]. MAM is a structure that forms an interface between the endoplasmic reticulum (ER) and mitochondria, playing important roles in calcium regulation, lipid transfer and apoptosis [86]. Indeed, mutant or overexpressed wild-type α-synuclein leads to dissociation of ER and

mitochondria at MAM, impairing calcium exchange and decreasing mitochondrial energy production [87]. Mutated α-synuclein in human dopaminergic neurons leads to decreased mitochondrial biogenesis through downregulation of peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1-α) [80].

Several models of wild-type mutated *LRRK2* presented higher vulnerability to mitochondrial toxins, increased ROS production and defects in mitochondrial dynamics (Figure 4) [80]. Mutant *VPS35* triggers mitochondrial fragmentation leading to impaired mitochondrial complex I assembly and activity, promoting neurodegeneration [88]. In addition, loss of *ATP12A2* impairs glycolysis impacting cellular bioenergetics and aggravating mitochondrial dysfunction. Furthermore, zinc (Zn^{2+}) homeostasis is deregulated by loss of *ATP13A2* through impairing vesicular sequestration, promoting mitochondrial and lysosomal dysfunction contributing to defective mitophagy (Figure 4) [80,89,90]. While Parkin-deficient models also show several defects in mitochondrial morphology and function [91], defective PINK1 models impact mitochondrial function, especially degradation, morphology, and trafficking [80]. Moreover, *Parkin* mutations are associated with sporadic early-onset parkinsonism and *PINK1* mutations are connected with parkinsonism in pediatric patients [92].

3. Pre-clinical and clinical studies with dietary antioxidants for Parkinson's Disease

3.1. The role of dietary polyphenols as redox modulators

Considerable efforts have been made to search and/or develop compounds capable of reducing ROS levels and/or preventing ROS-induced damage and mitochondrial dysfunction modulating cellular redox circuits.

Exogenous antioxidants or dietary antioxidants can be beneficial for decreasing oxidative stress status as they might compensate the inability of the endogenous defense systems and enhance the overall antioxidant response emerging as attractive drug candidates for PD therapy [93-95].

Dietary polyphenols are bioactive molecules with remarkable antioxidant properties since they can decrease oxidative stress by acting as primary or secondary antioxidants and/or up-regulating endogenous antioxidant defenses [96]. In general, polyphenols can act at the "ROS-regulating level" that include a mechanism of direct ROS- or RNS-scavenging and/or a favorable modulation of removing ROS by redox-regenerating enzyme activity [97]. The direct radical scavenging action of polyphenols is due to its reaction with

$^{\bullet}OH$, $^{\bullet}O_2^-$, $^{\bullet}NO$, alkoxyl ($^{\bullet}RO$) and peroxyl radicals ($^{\bullet}RO_2$), and with non-radical species, including $ONOO^-$ and hypochloride acid (HClO) [98]. The antioxidant activity of polyphenols mediated by ROS-scavenging is attributed to the presence of phenolic groups [98]. Notwithstanding, polyphenols can also be considered secondary antioxidants due to their ability to up-regulate the endogenous antioxidant defense network, particularly the ROS-removing enzymes, such as SOD, catalase, and GPx enzymes [99]. Some redox-regenerating enzymes, including glutathione reductase (GR) and Trx can also be up-regulated by polyphenols [99]. Furthermore, polyphenols can target ROS-formation processes, since they can perform a direct inhibitory action on metal-dependent free radicals formation process, namely $^{\bullet}OH$, $^{\bullet}O_2^-$ and ROS-producing enzymes. Polyphenols are also particularly active in chelating transition metals such as iron and copper ions, rendering them unavailable to participate in free radical-generating reactions [100]. Under physiological conditions, polyphenols' iron-binding properties are related to their adequate capacity to modulate cellular iron homeostasis [96]. Moreover, the polyphenols can inhibit NADPH oxidase, possibly by interfering with the assembly or inhibiting the expression of several subunits, and the mitochondrial-bound monoamine oxidase (MAO) activity [97].

3.2. Dietary polyphenol-based antioxidants in Parkinson's Disease

Dietary polyphenols can overcome the defects of the antioxidant defense system and control ROS levels given their biological potential. Therefore, the neuroprotective potential of several dietary antioxidants, including resveratrol [101], curcumin [102], quercetin [23], epigallocatechin-3-gallate (EGCG) [103], apocynin and diapocynin [104] have been tested.

Resveratrol, a natural polyphenolic non-flavonoid compound, presents several *in vitro* and *in vivo* neuroprotective effects, protecting neurons against oxidative stress damage and toxicity and prevents apoptotic neuronal death. Resveratrol also ameliorates mitochondrial dysfunction observed in some *in vivo* PD models [101]. Moreover, resveratrol crosses the BBB by transmembrane diffusion [101]. Despite the great potential of resveratrol as a therapeutic agent, several limitations were also described. Resveratrol has low bioavailability especially due to its short biological half-life, rapid metabolism, chemical instability, clearance, high

photosensitivity, and poor solubility in water [105]. Low bioavailability of resveratrol hampers its accumulation at necessary concentrations for successful therapy [106].

Curcumin is a polyphenol and an active turmeric component from *Curcuma longa*, a dietary spice used in Indian cuisine and medicine [107]. Neurological benefits of curcumin in PD models are related to its antioxidant function. In the 6-OHDA rat PD model, curcumin protected neurons in substantia nigra against ROS-induced cell death by improving striatal dopamine levels and chelate free Fe^{2+}. The phenolic rings probably mediate its antioxidant mechanism of action, and the diketone groups curcumin serve as an electron trap preventing the generation of H_2O_2, $^{\bullet}OH^-$ and $^{\bullet}O_2^-$. Moreover, curcumin reverses the inhibition of DNA repair enzymes in an *in vivo* PD model and in SH-SY5Y cells [108]. Similarly, curcumin *in vitro* pre-treatment attenuated peroxynitrite ($ONOO^-$)-mediated nitrosative stress and mitochondrial dysfunction [102]. Besides, curcumin can cross the BBB, while it has been regarded as a potential therapeutic factor for many nervous system diseases due to its pleiotropic therapeutic effects [109]. Although beneficial neuroprotective effects of curcumin have demonstrated, its clinical application has been hampered by its low aqueous solubility, reduced absorption in the gastrointestinal tract, high rate of metabolism, poor stability in the body fluids, rapid clearance, and limited bioavailability. Although a large number of clinical trials using curcumin have been demonstrated its safety, the limitations described above and the clinical trials's inconclusive results limit curcumin approval as a drug for clinical application [109-112].

Quercetin, a plant flavanol from the flavonoid group, has been used as a PD supplemental therapy. Several reports showed that its neuroprotective effect is related to its anti-inflammatory and antioxidant properties [113]. Treatment with quercetin attenuated motor deficits, biochemical and neurotransmitter alterations against rotenone- and iron- supplemented-induced PD rats [114]. Moreover, quercetin's chronic administration in a 6-OHDA mice model of PD decreased ROS levels and lipid peroxidation [23]. Although quercetin showed beneficial effects on various *in vivo* models of neural disorders, contrasting data also exist, resulting from quercetin-induced neurotoxicity. Thus, quercetin efficacy in neurodegenerative disorders lacks evidence [115]. Also, the low solubility and bioavailability of quercetin limit its clinical use [116].

EGCG, the most abundant green tea polyphenol, harbors free-radical scavenging, iron-chelating, and anti-inflammatory properties. Several studies reported neuroprotective effects of EGCG against MPP^+- or

MPTP-induced neurodegeneration in both *in vitro* and *in vivo* PD models [103]. EGCG inhibited MPP^+-induced oxidative stress in PC12 cells by increasing antioxidant enzymes and through sirtuin 1/ PCG1-α (SIRT1/PCG1-α) signaling pathway. Moreover, EGCG inhibited microglial activation protecting against MPTP-induced neuronal loss. EGCG upregulates ferroportin, an iron-export protein, in *substantia nigra*, reduces oxidative stress, and rescues MPTP-induced functional and neurochemical deficits in mice [103]. However, EGCG bioavailability in the brain is low, limiting its use as a neuroprotective agent [117]. Due to the lack of evidence of EGCG activity in humans and the lack of well-controlled clinical trials, the EGCG effect in preventing oxidative stress-related disorders, namely PD, is still under evaluation.

Apocynin, a plant-derived antioxidant, also protected dopaminergic neurons against MPP^+-induced oxidative stress and cell death. Apocynin is converted into diapocynin, which is an inhibitor of NADPH oxidase. In MPTP-treated mice, the administration of diapocynin protected against nigrostriatal damage and oxidative stress [118]. Apocynin stability is low due to the phenolic hydroxyl group in its structure [119], and apocynin bioavailability is also low since it quickly metabolizes into glucuronic conjugate [104]. While apocynin crosses the BBB at a low rate, this effect was not observed in diapocynin [104].

All the antioxidants described above, despite their demonstrated beneficial effects in several PD models, mostly *in vitro*, also presented common limitations, especially at the level of limited bioavailability, incapacity to cross BBB or unspecificity in tissue/organelle-targeted, which have hampered their clinical applications.

3.3. Controlling ROS production by targeting mitochondrial function

Discovering mitochondrial drug candidates is a great challenge since it requires a target-specific organelle affinity and, at the same time, a restricted drug safety window. Applying a concept pioneered by Skulachev et al. to measure $\Delta\Psi m$ *in vitro* [120], the covalent attachment of lipophilic cations, such as triphenylphosphonium cation (TPP^+), is a golden-standard method to target small bioactive molecules to mitochondria [121]. TPP^+ derivatives are rapidly and extensively accumulated *in vivo* by mitochondria driven by the large $\Delta\Psi m$, negative inside the matrix [122]. These compounds pass through all biological membranes and accumulate in the mitochondrial matrix faster than their non-targeted molecules. The uptake mechanism is by

now well understood. TPP cations can cross the plasma and mitochondrial inner membrane due to their extensive hydrophobic surface area and the cation's large outer ionic radius, effectively lowering the activation energy needed for membrane passage [123,124]. The Nernst equation adequately describes the uptake dependent of membrane potential of the lipophilic cations, increasing 10-fold for every ~60 mV of $\Delta\Psi m$. It is estimated that a hundred- or thousand-fold increase in the antioxidant concentration occurs inside mitochondria. The extent of TPP^+ derivative-uptake depends on plasma $\Delta\Psi$ and $\Delta\Psi m$, cell volume, the external media, and the number of mitochondria within a given cell. Once inside mitochondria, these derivatives can promote beneficial effects through several mechanisms, namely by scavenging reactive radicals and control mitochondrial redox signaling and/or preventing membrane lipid peroxidation [121].

Considering that mitochondria are main sites for the ATP production, it is not surprising that most targets of mitochondrial drug are related and involved in energy metabolism and mostly related with mechanisms that regulate ROS, respiration and mitochondrial biogenesis. Ultimately, potential novel drugs should restore mitochondrial function as well as normalize mitochondrial ROS production [125]. Nowadays, the central focus on finding drugs that target mitochondrial function is related to the discovery and development of antioxidants inhibiting mitochondrial oxidative damage [121].

3.4. Mitochondria-targeted antioxidants used in Parkinson's Disease pre-clinical studies

By using antioxidants that target mitochondrial oxidative stress in order to decrease mitochondrial ROS (mtROS) in pathological processes may be essential to both normal cell function and in disease prevention.

The most studied mitochondria-targeted antioxidants are MitoQ and SkQ1. While MitoQ consists of a TPP^+ unit covalently attached to the endogenous antioxidant ubiquinone [126], SkQ1 consists of a TPP^+ unit covalently attached to plastoquinone, a chloroplast quinone, as core moiety [127]. MitoQ is a promising neuroprotective compound because of its direct antioxidant action. Similar to its parent antioxidant (CoQ10), MitoQ scavenges peroxyl, $ONOO^-$, and $^{\bullet}O_2^-$, protecting mitochondria against lipid peroxidation (Figure 4) [118]. After detoxifying oxidants, MitoQ is recycled by the respiratory chain complex II to the active ubiquinol antioxidant form [118]. MitoQ inhibited 6-OHDA-induced mitochondrial fragmentation in SH-SY5Y cells and

prevented MPP^+-induced loss of neurons in a PD's dopaminergic cell culture model [118]. In a Parkinson's disease mouse model treated for 14 days with MitoQ, the number of dopaminergic neurons increased relative to untreated PD mouse model [128]. The successful of pre-clinical trials obtained with MitoQ led to clinical trials for several diseases, including PD (NCT00329056). However, the results of the trials were disappointing. Some justifications include time elapsed from PD diagnosis, in which 50% of dopaminergic neurons have already been lost or even the insufficient penetration of the BBB by MitoQ [129].

Administration of SkQ1 to a MPTP-model of PD using C57BL/6 mice showed that SkQ1 increased dopamine levels (Figure 4), while the loss of dopaminergic neurons in the *substantia nigra* and signs of sensory-motor deficiency were decreased [130].

Similarly, other mitochondria-targeted antioxidants have been tested with beneficial effects in several PD models, including MitoVitE (combination of the vitamin E with TPP^+), MitoTEMPO (combination of the piperidine nitroxide TEMPO with TPP^+), and MitoApocynin (combination of the apocynin with TPP^+), MitoPBN (combination of the nitrone antioxidant derived from IBTP with TPP^+), MitoSOD (combination of the SOD with TPP^+) [118]. Zhelev et al. [131] demonstrated that MitoTEMPO increased the activity of dopaminergic neurons in the brain of MPTP-treated mice [131]. Also, MitoApocynin prevented microglial activation, oxidative damage, mitochondrial dysfunction, and progressive neurodegeneration in $TFAM^{-/-}$ mice [132]. The beneficial effects of these mitochondria-targeted compounds have been extensively studied using several disease models [133-137]; however their effects in PD models have been poorly studied.

Although attractive, the success of these approaches has been troubled by several challenges and limitations, with none resulting yet in a Food and Drug Administration (FDA)-approved drug for PD therapy. The lack of efficacy may relate with the pre-clinical assessment of these compounds in inadequate PD animal models [138]. Moreover, the pre-clinical data set should define better neuroprotective dose-response relationships, pharmacokinetic-pharmacodynamic correlations, therapeutic windows, optimum doses regimens, and treatment durations [138]. Another possible explanation for the disastrous results of the compounds in clinical trials could be due to PD's multifactorial and heterogeneous nature, which will reduce any single therapeutic agent less beneficial than it seemed in simpler pre-clinical models. An interesting viewpoint would be not only to fine-tune the chemical-biology interactions of these compounds to eliminate

the undesirable effects, but also to identify new bioactive compounds against mitochondrial oxidative damage [138].

Facing the pitfalls of clinical trials, new chemical compounds directed to mitochondria must be developed. Hydroxycinnamic (HCAs) and hydroxybenzoic (HBA) acids are the main subgroup of phenolic acids present in plants. HCAs are potent antioxidant compounds primarily due to several physiological roles in biological systems [93].

Their antioxidant-related mechanism of action is suggested to be through its scavenger activity, related to their ability to donate hydrogen or electrons and the stability of the resulting phenoxyl radicals. However, other mechanisms of action have been also proposed, including inhibition of enzymes generating ROS and reactive nitrogen species (RNS), gene expression modulation through the antioxidant responsive element (ARE)/Nrf2 pathway, and metal chelators, such as iron and copper [139,140]. New mitochondriotropic antioxidants based on HCA and HBA core have been developed [141]. $AntiOxBEN_2$ is a hydroxybenzoic acid derivative linked to lipophilic TPP^+ through an alkyl spacer, previously demonstrated to have antioxidant capacity. Treatment of SH-SY5Y cells with maximal non-lethal concentrations of $AntiOxBEN_2$ prevented H_2O_2-induced cytotoxicity [142]. Despite considered an antioxidant, AntiOxBEN2 prevention of cell death was paralleled by slightly increased ROS levels, suggesting that this mitochondriotropic antioxidant can also act as a prooxidant, triggering adaptative responses [142]. $AntiOxCIN_4$ is a hydroxycinnamic acid derivative linked to lipophilic TPP^+ through an alkyl spacer and which was previously shown to have antioxidant properties (Figure 5) [141,143]. $AntiOxCIN_4$ expectedly accumulated within mitochondria in its reduced form [144] driven by the $\Delta\Psi m$ without affecting mitochondrial morphology and polarization [145]. Consequently, $AntiOxCIN_4$ showed potent antioxidant and iron-chelation properties and can inhibit oxidative damage in isolated liver mitochondria or hepatic cells [145]. $AntiOxCIN_4$ can indirectly influence an antioxidant by triggering a ROS-induced adaptive response [146]. After treatment with that compound, Nrf2 translocates to the nucleus inducing the transcription of enzymes related with the cellular antioxidant defense system, which act as homeostatic circuits to balance detoxification and production of ROS and consequently preventing cell death induced by ROS [146]. Furthermore, neuroprotective effects were also demonstrated in SH-SY5Y cells against 6-OHDA-induced oxidative damage [142].

More recently, we demonstrated by using human skin fibroblasts from patients with sPD that AntiOxCIN$_4$ reverted metabolic and mitochondrial defects present in skin fibroblasts from sPD patients [147]. AntiOxCIN$_4$ works as mild pro-oxidant, which leads to the stimulation of the Nrf2 pathway (Figure 5) [146,147], having an important role in the cellular response to oxidants. Nrf2 stimulates the total SOD activity, consequently facilitating the conversion of $O2^{\bullet-}$ into H_2O_2 and inhibiting cell death induced by ROS. Downstream of SOD action, H_2O_2 is converted into H_2O by GPx in a glutathione GSH-dependent manner. AntiOxCIN$_4$ increased the NAD(P)H dehydrogenase (quinone) 1 (NQO1) gene expression, which is a target of NFR2. Since those fibroblasts from sPD patients have low ATP levels after treatment with AntiOxCIN$_4$, AMPK-α induces cell cycle arrest in S phase, in a p53-dependent manner, in order to restore mitochondrial function, avoiding the perpetuation of cells with defective mitochondria (Figure 5). A general enhancement in mitochondrial fitness in fibroblasts from sPD patients is demonstrated by increased maximal respiration, decreased mitochondrial swelling, and increased cellular metabolic activity [147]. These and other findings highlight the successful development of new mitochondria-directed antioxidants based on dietary scaffolds and demonstrate their application as first-class candidates with therapeutic pontential for mitochondrial oxidative stress-related diseases, such as PD.

4. Conclusions

Oxidative stress increased and mitochondrial dysfunction have been largely implicated in PD pathogenesis [118]. However, it is not established whether mitochondrial dysfunction is a cause, a consequence or part of a self-sustaining vicious cycle of cellular damage [129].

PD-associated symptoms usually develop slowly over time and that prodromal phase may start as early 5-10 years before diagnosis [148]. With this in mind, this earlier phase may be an ideal time point for therapeutic interventions, in which dietary mitochondriotropic antioxidants have a huge advantage and could be a justification for the lack of success of several candidate compounds used in pre-clinical studies. The future of therapeutic strategies for PD based on mitochondriotropic antioxidant will depend on the capacity to develop better and more efficient strategies that target mitochondria with bioactive molecules or using multifunctional compounds [118] and consider the multifactorial contribution of PD in pre-clinical assays.

Moreover, it is essential to use sPD models in pre-clinical studies, since the etiology of sPD, the most common PD form, is not completely understood. This limitation results not only from the low number of experimental models of sPD used but also in the experimental difficulty in obtaining appropriate human tissues to investigate the pathogenesis of PD. However, a better consider of sPD models in pre-clinical studies may anticipate promising results when undergoing clinical trials.

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6. Conflict of interest

PJO and FB are co-founders of the start-up MitoTAG. The funding agencies had no role in the decision to publish the manuscript.

7. References

1. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *The Lancet Neurology.* 2016/11/01/ 2016;15(12):1257-1272.

2. Moisan F, Kab S, Mohamed F, et al. Parkinson disease male-to-female ratios increase with age: French nationwide study and meta-analysis. *Journal of neurology, neurosurgery, and psychiatry.* Sep 2016;87(9):952-957.

3. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Human molecular genetics.* Apr 15 2009;18(R1):R48-59.

4. Selvaraj S, Piramanayagam S. Impact of gene mutation in the development of Parkinson's disease. *Genes & diseases.* Jun 2019;6(2):120-128.
5. Zeng XS, Geng WS, Jia JJ, Chen L, Zhang PP. Cellular and Molecular Basis of Neurodegeneration in Parkinson Disease. *Frontiers in aging neuroscience.* 2018;10:109.
6. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. *Journal of neurochemistry.* Oct 2016;139 Suppl 1:318-324.
7. Stoker TB. Stem Cell Treatments for Parkinson's Disease. In: Stoker TB, Greenland JC, eds. *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Brisbane (AU)2018.
8. Gasser T. Genomic and proteomic biomarkers for Parkinson disease. *Neurology.* Feb 17 2009;72(7 Suppl):S27-31.
9. Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, eds. *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Brisbane (AU)2018.
10. Obeso JA, Rodriguez-Oroz MC, Goetz CG, et al. Missing pieces in the Parkinson's disease puzzle. *Nature medicine.* Jun 2010;16(6):653-661.
11. Trist BG, Hare DJ, Double KL. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. *Aging cell.* Dec 2019;18(6):e13031.
12. Lin MT, Flint Beal M. The oxidative damage theory of aging. *Clinical Neuroscience Research.* 2003/01/01/ 2003;2(5):305-315.
13. Kolodkin AN, Sharma RP, Colangelo AM, et al. ROS networks: designs, aging, Parkinson's disease and precision therapies. *NPJ systems biology and applications.* Oct 26 2020;6(1):34.
14. Aoyama K, Watabe M, Nakaki T. Regulation of neuronal glutathione synthesis. *Journal of pharmacological sciences.* Nov 2008;108(3):227-238.
15. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. *Journal of cellular physiology.* Jul 2002;192(1):1-15.
16. Raimondi V, Ciccarese F, Ciminale V. Oncogenic pathways and the electron transport chain: a dangeROS liaison. *British journal of cancer.* 2020/01/01 2020;122(2):168-181.
17. Sedeek M, Nasrallah R, Touyz RM, Hebert RL. NADPH oxidases, reactive oxygen species, and the kidney: friend and foe. *Journal of the American Society of Nephrology : JASN.* Oct 2013;24(10):1512-1518.
18. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature reviews. Molecular cell biology.* Jul 2020;21(7):363-383.
19. Rice ME, Avshalumov MV, Patel JC. Hydrogen Peroxide as a Diffusible Messenger: Evidence from Voltammetric Studies of Dopamine Release in Brain Slices. In: Michael AC, Borland LM, eds. *Electrochemical Methods for Neuroscience*. Boca Raton (FL)2007.

Accepted Article

20. Rahal A, Kumar A, Singh V, et al. Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed research international.* 2014;2014:761264.
21. Szeliga M. Peroxiredoxins in Neurodegenerative Diseases. *Antioxidants.* Nov 30 2020;9(12).
22. Zeevalk GD, Manzino L, Sonsalla PK, Bernard LP. Characterization of intracellular elevation of glutathione (GSH) with glutathione monoethyl ester and GSH in brain and neuronal cultures: relevance to Parkinson's disease. *Experimental neurology.* 2007;203(2):512-520.
23. Ciulla M, Marinelli L, Cacciatore I, Stefano AD. Role of Dietary Supplements in the Management of Parkinson's Disease. *Biomolecules.* Jul 10 2019;9(7).
24. Maiti P, Manna J, Dunbar GL. Current understanding of the molecular mechanisms in Parkinson's disease: Targets for potential treatments. *Translational neurodegeneration.* 2017;6:28.
25. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell communication and signaling : CCS.* May 17 2013;11(1):34.
26. Chaudhry FA, Edwards RH, Fonnum F. Vesicular Neurotransmitter Transporters as Targets for Endogenous and Exogenous Toxic Substances. *Annual review of pharmacology and toxicology.* 2008;48(1):277-301.
27. Anandhan A, Jacome MS, Lei S, et al. Metabolic Dysfunction in Parkinson's Disease: Bioenergetics, Redox Homeostasis and Central Carbon Metabolism. *Brain research bulletin.* Jul 2017;133:12-30.
28. Berman SB, Hastings TG. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. *Journal of neurochemistry.* Sep 1999;73(3):1127-1137.
29. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. *Frontiers in neuroanatomy.* 2015;9:91.
30. Liu X, Yamada N, Maruyama W, Osawa T. Formation of dopamine adducts derived from brain polyunsaturated fatty acids: mechanism for Parkinson disease. *The Journal of biological chemistry.* Dec 12 2008;283(50):34887-34895.
31. Surmeier DJ, Guzman JN, Sanchez-Padilla J, Goldberg JA. What causes the death of dopaminergic neurons in Parkinson's disease? *Progress in brain research.* 2010;183:59-77.
32. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxidants & redox signaling.* Sep 15 2011;15(6):1583-1606.
33. Biosa A, Arduini I, Soriano ME, et al. Dopamine Oxidation Products as Mitochondrial Endotoxins, a Potential Molecular Mechanism for Preferential Neurodegeneration in Parkinson's Disease. *ACS chemical neuroscience.* Nov 21 2018;9(11):2849-2858.
34. Biosa A, De Lazzari F, Masato A, et al. Superoxide Dismutases SOD1 and SOD2 Rescue the Toxic Effect of Dopamine-Derived Products in Human SH-SY5Y Neuroblastoma Cells. *Neurotox Res.* Nov 2019;36(4):746-755.

Accepted Article

35. Zhou C, Huang Y, Przedborski S. Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance. *Annals of the New York Academy of Sciences.* Dec 2008;1147:93-104.
36. Filograna R, Godena VK, Sanchez-Martinez A, et al. Superoxide Dismutase (SOD)-mimetic M40403 Is Protective in Cell and Fly Models of Paraquat Toxicity: IMPLICATIONS FOR PARKINSON DISEASE. *The Journal of biological chemistry.* Apr 22 2016;291(17):9257-9267.
37. Bostantjopoulou S, Kyriazis G, Katsarou Z, Kiosseoglou G, Kazis A, Mentenopoulos G. Superoxide dismutase activity in early and advanced Parkinson's disease. *Funct Neurol.* 1997 Mar-Apr 1997;12(2):63-68.
38. Silva-Adaya D, Gonsebatt ME, Guevara J. Thioredoxin system regulation in the central nervous system: experimental models and clinical evidence. *Oxidative medicine and cellular longevity.* 2014;2014:590808.
39. Chae HZ, Kim HJ, Kang SW, Rhee SG. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. *Diabetes research and clinical practice.* Sep 1999;45(2-3):101-112.
40. Felix L, Mylonakis E, Fuchs BB. Thioredoxin Reductase Is a Valid Target for Antimicrobial Therapeutic Development Against Gram-Positive Bacteria. *Front Microbiol.* 2021;12:663481.
41. Bindoli A, Rigobello MP. Principles in redox signaling: from chemistry to functional significance. *Antioxidants & redox signaling.* May 1 2013;18(13):1557-1593.
42. Jastrzab A, Skrzydlewska E. Thioredoxin-dependent system. Application of inhibitors. *Journal of enzyme inhibition and medicinal chemistry.* Dec 2021;36(1):362-371.
43. Rhee SG, Chae HZ, Kim K. Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radical Biology and Medicine.* 2005/06/15/ 2005;38(12):1543-1552.
44. Im J-Y, Lee K-W, Woo J-M, Junn E, Mouradian MM. DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway. *Human molecular genetics.* 2012;21(13):3013-3024.
45. Lopert P, Day BJ, Patel M. Thioredoxin reductase deficiency potentiates oxidative stress, mitochondrial dysfunction and cell death in dopaminergic cells. *PloS one.* 2012;7(11):e50683.
46. Zeng XS, Jia JJ, Kwon Y, Wang SD, Bai J. The role of thioredoxin-1 in suppression of endoplasmic reticulum stress in Parkinson disease. *Free radical biology & medicine.* Feb 2014;67:10-18.
47. Lee YM, Park SH, Shin DI, et al. Oxidative modification of peroxiredoxin is associated with drug-induced apoptotic signaling in experimental models of Parkinson disease. *The Journal of biological chemistry.* Apr 11 2008;283(15):9986-9998.
48. Basso M, Giraudo S, Corpillo D, Bergamasco B, Lopiano L, Fasano M. Proteome analysis of human substantia nigra in Parkinson's disease. *Proteomics.* Dec 2004;4(12):3943-3952.

Accepted Article

49. Angeles DC, Gan BH, Onstead L, et al. Mutations in LRRK2 increase phosphorylation of peroxiredoxin 3 exacerbating oxidative stress-induced neuronal death. *Hum Mutat*. Dec 2011;32(12):1390-1397.

50. Hu X, Weng Z, Chu CT, et al. Peroxiredoxin-2 protects against 6-hydroxydopamine-induced dopaminergic neurodegeneration via attenuation of the apoptosis signal-regulating kinase (ASK1) signaling cascade. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Jan 5 2011;31(1):247-261.

51. Silva C, Pinto M, Fernandes C, Benfeito S, Borges F. Antioxidant Therapy and Neurodegenerative Disorders: Lessons From Clinical Trials. In: Wolkenhauer O, ed. *Systems Medicine*. Oxford: Academic Press; 2021:97-110.

52. Sedlak TW, Paul BD, Parker GM, et al. The glutathione cycle shapes synaptic glutamate activity. *Proceedings of the National Academy of Sciences of the United States of America.* Feb 12 2019;116(7):2701-2706.

53. Espinosa-Diez C, Miguel V, Mennerich D, et al. Antioxidant responses and cellular adjustments to oxidative stress. *Redox biology.* Dec 2015;6:183-197.

54. Kang Y, Viswanath V, Jha N, Qiao X, Mo JQ, Andersen JK. Brain gamma-glutamyl cysteine synthetase (GCS) mRNA expression patterns correlate with regional-specific enzyme activities and glutathione levels. *Journal of neuroscience research.* Nov 1 1999;58(3):436-441.

55. Liang LP, Kavanagh TJ, Patel M. Glutathione deficiency in Gclm null mice results in complex I inhibition and dopamine depletion following paraquat administration. *Toxicological sciences : an official journal of the Society of Toxicology.* Aug 2013;134(2):366-373.

56. Pileblad E, Magnusson T, Fornstedt B. Reduction of brain glutathione by L-buthionine sulfoximine potentiates the dopamine-depleting action of 6-hydroxydopamine in rat striatum. *Journal of neurochemistry.* Mar 1989;52(3):978-980.

57. Wullner U, Loschmann PA, Schulz JB, et al. Glutathione depletion potentiates MPTP and MPP+ toxicity in nigral dopaminergic neurones. *Neuroreport.* Mar 22 1996;7(4):921-923.

58. Pradhan P, Majhi O, Biswas A, Joshi VK, Sinha D. Enhanced accumulation of reduced glutathione by Scopoletin improves survivability of dopaminergic neurons in Parkinson's model. *Cell death & disease.* 2020/09/10 2020;11(9):739.

59. Fernandez-Mosquera L, Yambire KF, Couto R, et al. Mitochondrial respiratory chain deficiency inhibits lysosomal hydrolysis. *Autophagy.* 2019/09/02 2019;15(9):1572-1591.

60. Bruni F, Lightowlers RN, Chrzanowska-Lightowlers ZM. Human mitochondrial nucleases. *The FEBS journal.* Dec 07 2016.

61. Raimundo N. Mitochondrial pathology: stress signals from the energy factory. *Trends in molecular medicine.* May 2014;20(5):282-292.

Accepted Article

62. Baixauli F, Acin-Perez R, Villarroya-Beltri C, et al. Mitochondrial Respiration Controls Lysosomal Function during Inflammatory T Cell Responses. *Cell metabolism.* Sep 1 2015;22(3):485-498.

63. Boveris A, Navarro A. Brain mitochondrial dysfunction in aging. *IUBMB life.* May 2008;60(5):308-314.

64. Puspita L, Chung SY, Shim JW. Oxidative stress and cellular pathologies in Parkinson's disease. *Molecular brain.* Nov 28 2017;10(1):53.

65. Guo JD, Zhao X, Li Y, Li GR, Liu XL. Damage to dopaminergic neurons by oxidative stress in Parkinson's disease (Review). *International journal of molecular medicine.* Apr 2018;41(4):1817-1825.

66. Subramaniam SR, Chesselet MF. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. *Progress in neurobiology.* Jul-Aug 2013;106-107:17-32.

67. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science.* Feb 25 1983;219(4587):979-980.

68. Khaldy H, Escames G, Leon J, Bikjdaouene L, Acuna-Castroviejo D. Synergistic effects of melatonin and deprenyl against MPTP-induced mitochondrial damage and DA depletion. *Neurobiol Aging.* May-Jun 2003;24(3):491-500.

69. Zeng XS, Geng WS, Jia JJ. Neurotoxin-Induced Animal Models of Parkinson Disease: Pathogenic Mechanism and Assessment. *ASN neuro.* Jan-Dec 2018;10:1759091418777438.

70. Levitt P, Pintar JE, Breakefield XO. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proceedings of the National Academy of Sciences of the United States of America.* Oct 1982;79(20):6385-6389.

71. Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet.* Jun 3 1989;1(8649):1269.

72. Martinez TN, Greenamyre JT. Toxin models of mitochondrial dysfunction in Parkinson's disease. *Antioxidants & redox signaling.* May 1 2012;16(9):920-934.

73. Johnson ME, Bobrovskaya L. An update on the rotenone models of Parkinson's disease: Their ability to reproduce the features of clinical disease and model gene–environment interactions. *Neurotoxicology.* 2015/01/01/ 2015;46:101-116.

74 Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. *Trends in pharmacological sciences.* Apr 2010;31(4):141-142; author reply 142-143.

75. Grunewald A, Rygiel KA, Hepplewhite PD, Morris CM, Picard M, Turnbull DM. Mitochondrial DNA Depletion in Respiratory Chain-Deficient Parkinson Disease Neurons. *Annals of neurology.* Mar 2016;79(3):366-378.

76. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *Journal of neurochemistry.* Mar 1990;54(3):823-827.

Accepted Article

77. Stoker TB, Greenland JC. Parkinson's Disease: Pathogenesis and Clinical Aspects. Brisbane (AU)2018.

78. Chen C, Turnbull DM, Reeve AK. Mitochondrial Dysfunction in Parkinson's Disease-Cause or Consequence? *Biology.* May 11 2019;8(2).

79. Flones IH, Fernandez-Vizarra E, Lykouri M, et al. Neuronal complex I deficiency occurs throughout the Parkinson's disease brain, but is not associated with neurodegeneration or mitochondrial DNA damage. *Acta neuropathologica.* Mar 2018;135(3):409-425.

80. Park JS, Davis RL, Sue CM. Mitochondrial Dysfunction in Parkinson's Disease: New Mechanistic Insights and Therapeutic Perspectives. *Current neurology and neuroscience reports.* Apr 3 2018;18(5):21.

81. Reeve A, Meagher M, Lax N, et al. The impact of pathogenic mitochondrial DNA mutations on substantia nigra neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Jun 26 2013;33(26):10790-10801.

82. Pickrell AM, Huang CH, Kennedy SR, et al. Endogenous Parkin Preserves Dopaminergic Substantia Nigral Neurons following Mitochondrial DNA Mutagenic Stress. *Neuron.* Jul 15 2015;87(2):371-381.

83. Park J-S, Davis RL, Sue CM. Mitochondrial Dysfunction in Parkinson’s Disease: New Mechanistic Insights and Therapeutic Perspectives. *Current neurology and neuroscience reports.* 2018/04/03 2018;18(5):21.

84. Mullin S, Schapira A. alpha-Synuclein and mitochondrial dysfunction in Parkinson's disease. *Molecular neurobiology.* Apr 2013;47(2):587-597.

85. Ryan BJ, Hoek S, Fon EA, Wade-Martins R. Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends in biochemical sciences.* Apr 2015;40(4):200-210.

86. Guardia-Laguarta C, Area-Gomez E, Rub C, et al. alpha-Synuclein is localized to mitochondria-associated ER membranes. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Jan 1 2014;34(1):249-259.

87. Paillusson S, Gomez-Suaga P, Stoica R, et al. alpha-Synuclein binds to the ER-mitochondria tethering protein VAPB to disrupt Ca(2+) homeostasis and mitochondrial ATP production. *Acta neuropathologica.* Jul 2017;134(1):129-149.

88 Zhou L, Wang W, Hoppel C, Liu J, Zhu X. Parkinson's disease-associated pathogenic VPS35 mutation causes complex I deficits. *Biochimica et biophysica acta. Molecular basis of disease.* Nov 2017;1863(11):2791-2795.

89. Abeliovich A, Gitler AD. Defects in trafficking bridge Parkinson's disease pathology and genetics. *Nature.* 11/09/online 2016;539:207.

90. Tsunemi T, Krainc D. Zn(2)(+) dyshomeostasis caused by loss of ATP13A2/PARK9 leads to lysosomal dysfunction and alpha-synuclein accumulation. *Human molecular genetics.* Jun 1 2014;23(11):2791-2801.

Accepted Article

91. Sarraf SA, Raman M, Guarani-Pereira V, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature.* Apr 18 2013;496(7445):372-376.

92. Al-Rumayyan A, Klein C, Alfadhel M. Early-Onset Parkinsonism: Case Report and Review of the Literature. *Pediatric neurology.* Feb 2017;67:102-106 e101.

93. Benfeito S, Oliveira C, Soares P, et al. Antioxidant therapy: still in search of the 'magic bullet'. *Mitochondrion.* Sep 2013;13(5):427-435.

94. Calabrese V, Cornelius C, Cuzzocrea S, Iavicoli I, Rizzarelli E, Calabrese EJ. Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. *Molecular aspects of medicine.* Aug 2011;32(4-6):279-304.

95. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry.* Jun 5 2015;97:55-74.

96. Obrenovich ME, Nair NG, Beyaz A, Aliev G, Reddy VP. The role of polyphenolic antioxidants in health, disease, and aging. *Rejuvenation research.* Dec 2010;13(6):631-643.

97. Sandoval-Acuna C, Ferreira J, Speisky H. Polyphenols and mitochondria: an update on their increasingly emerging ROS-scavenging independent actions. *Archives of biochemistry and biophysics.* Oct 1 2014;559:75-90.

98. Upadhyay S, Dixit M. Role of Polyphenols and Other Phytochemicals on Molecular Signaling. *Oxidative medicine and cellular longevity.* 2015;2015:504253.

99. Tsuji PA, Stephenson KK, Wade KL, Liu H, Fahey JW. Structure-activity analysis of flavonoids: direct and indirect antioxidant, and antiinflammatory potencies and toxicities. *Nutrition and cancer.* 2013;65(7):1014-1025.

100. Mladenka P, Macakova K, Filipsky T, et al. In vitro analysis of iron chelating activity of flavonoids. *Journal of inorganic biochemistry.* May 2011;105(5):693-701.

101. Andrade S, Ramalho MJ, Pereira MDC, Loureiro JA. Resveratrol Brain Delivery for Neurological Disorders Prevention and Treatment. *Frontiers in pharmacology.* 2018;9:1261.

102. Mythri RB, Bharath MM. Curcumin: a potential neuroprotective agent in Parkinson's disease. *Current pharmaceutical design.* 2012;18(1):91-99.

103. Xu Q, Langley M, Kanthasamy AG, Reddy MB. Epigallocatechin Gallate Has a Neurorescue Effect in a Mouse Model of Parkinson Disease. *The Journal of nutrition.* Oct 2017;147(10):1926-1931.

104. Simonyi A, Serfozo P, Lehmidi TM, et al. The neuroprotective effects of apocynin. *Frontiers in bioscience.* Jan 1 2012;4:2183-2193.

105. Wang S, Wang Z, Yang S, et al. Tissue Distribution of trans-Resveratrol and Its Metabolites after Oral Administration in Human Eyes. *Journal of ophthalmology.* 2017;2017:4052094.

106. Berman AY, Motechin RA, Wiesenfeld MY, Holz MK. The therapeutic potential of resveratrol: a review of clinical trials. *NPJ precision oncology.* 2017;1.

Accepted Article

107. Pinto M, Benfeito S, Fernandes C, Borges F. Chapter 9 - Antioxidant therapy, oxidative stress, and blood-brain barrier: The road of dietary antioxidants. In: Martin CR, Preedy VR, eds. *Oxidative Stress and Dietary Antioxidants in Neurological Diseases*: Academic Press; 2020:125-141.

108. Hegde ML, Hegde PM, Holthauzen LM, Hazra TK, Rao KS, Mitra S. Specific Inhibition of NEIL-initiated repair of oxidized base damage in human genome by copper and iron: potential etiological linkage to neurodegenerative diseases. *The Journal of biological chemistry.* Sep 10 2010;285(37):28812-28825.

109. Maiti P, Dunbar GL. Use of Curcumin, a Natural Polyphenol for Targeting Molecular Pathways in Treating Age-Related Neurodegenerative Diseases. *International journal of molecular sciences.* May 31 2018;19(6).

110. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm.* Nov-Dec 2007;4(6):807-818.

111. Yavarpour-Bali H, Ghasemi-Kasman M, Pirzadeh M. Curcumin-loaded nanoparticles: a novel therapeutic strategy in treatment of central nervous system disorders. *International journal of nanomedicine.* 2019;14:4449-4460.

112. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. *Journal of medicinal chemistry.* Mar 9 2017;60(5):1620-1637.

113. Tamtaji OR, Hadinezhad T, Fallah M, et al. The Therapeutic Potential of Quercetin in Parkinson's Disease: Insights into its Molecular and Cellular Regulation. *Current drug targets.* Nov 12 2019.

114. Sharma S, Raj K, Singh S. Neuroprotective Effect of Quercetin in Combination with Piperine Against Rotenone- and Iron Supplement–Induced Parkinson's Disease in Experimental Rats. *Neurotoxicity Research.* 2020/01/01 2020;37(1):198-209.

115. Ossola B, Kaariainen TM, Mannisto PT. The multiple faces of quercetin in neuroprotection. *Expert opinion on drug safety.* Jul 2009;8(4):397-409.

116. Ghaffari F, Hajizadeh Moghaddam A, Zare M. Neuroprotective Effect of Quercetin Nanocrystal in a 6-Hydroxydopamine Model of Parkinson Disease: Biochemical and Behavioral Evidence. *Basic and clinical neuroscience.* Sep-Oct 2018;9(5):317-324.

117. Nakagawa K, Miyazawa T. Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. *Journal of nutritional science and vitaminology.* Dec 1997;43(6):679-684.

118. Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. Mitochondria-targeted antioxidants for treatment of Parkinson's disease: preclinical and clinical outcomes. *Biochimica et biophysica acta.* Aug 2014;1842(8):1282-1294.

119. Wang Q, Smith RE, Luchtefeld R, et al. Bioavailability of apocynin through its conversion to glycoconjugate but not to diapocynin. *Phytomedicine : international journal of phytotherapy and phytopharmacology.* Jun 2008;15(6-7):496-503.

Accepted Article

120. Liberman EA, Topaly VP, Tsofina LM, Jasaitis AA, Skulachev VP. Mechanism of coupling of oxidative phosphorylation and the membrane potential of mitochondria. *Nature.* Jun 14 1969;222(5198):1076-1078.

121. Smith RA, Hartley RC, Cocheme HM, Murphy MP. Mitochondrial pharmacology. *Trends in pharmacological sciences.* Jun 2012;33(6):341-352.

122. Smith RA, Hartley RC, Murphy MP. Mitochondria-targeted small molecule therapeutics and probes. *Antioxidants & redox signaling.* Dec 15 2011;15(12):3021-3038.

123. Porteous CM, Logan A, Evans C, et al. Rapid uptake of lipophilic triphenylphosphonium cations by mitochondria in vivo following intravenous injection: implications for mitochondria-specific therapies and probes. *Biochimica et biophysica acta.* Sep 2010;1800(9):1009-1017.

124. Rodriguez-Cuenca S, Cocheme HM, Logan A, et al. Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. *Free radical biology & medicine.* Jan 1 2010;48(1):161-172.

125. Sorriento D, Pascale AV, Finelli R, et al. Targeting mitochondria as therapeutic strategy for metabolic disorders. *TheScientificWorldJournal.* 2014;2014:604685.

126. Kelso GF, Porteous CM, Coulter CV, et al. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *The Journal of biological chemistry.* Feb 16 2001;276(7):4588-4596.

127. Antonenko YN, Roginsky VA, Pashkovskaya AA, et al. Protective effects of mitochondria-targeted antioxidant SkQ in aqueous and lipid membrane environments. *The Journal of membrane biology.* Apr 2008;222(3):141-149.

128. Xi Y, Feng D, Tao K, et al. MitoQ protects dopaminergic neurons in a 6-OHDA induced PD model by enhancing Mfn2-dependent mitochondrial fusion via activation of PGC-1α. *Biochimica et biophysica acta. Molecular basis of disease.* Sep 2018;1864(9 Pt B):2859-2870.

129. Murphy MP, Hartley RC. Mitochondria as a therapeutic target for common pathologies. *Nature reviews. Drug discovery.* Dec 2018;17(12):865-886.

130. Pavshintsev VV, Podshivalova LS, Frolova OY, et al. Effects of Mitochondrial Antioxidant SkQ1 on Biochemical and Behavioral Parameters in a Parkinsonism Model in Mice. *Biochemistry. Biokhimiia.* Dec 2017;82(12):1513-1520.

131. Zhelev Z, Aoki I, Gadjeva V, Nikolova B, Bakalova R, Saga T. Tissue redox activity as a sensing platform for imaging of cancer based on nitroxide redox cycle. *European Journal of Cancer.* 2013/04/01/ 2013;49(6):1467-1478.

132. Langley M, Ghosh A, Charli A, et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxidants & redox signaling.* Nov 10 2017;27(14):1048-1066.

Accepted Article

133. Chacko BK, Srivastava A, Johnson MS, et al. Mitochondria-targeted ubiquinone (MitoQ) decreases ethanol-dependent micro and macro hepatosteatosis. *Hepatology.* Jul 2011;54(1):153-163.

134. Graham D, Huynh NN, Hamilton CA, et al. Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. *Hypertension.* Aug 2009;54(2):322-328.

135. McManus MJ, Murphy MP, Franklin JL. The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Nov 2 2011;31(44):15703-15715.

136. Mercer JR, Yu E, Figg N, et al. The mitochondria-targeted antioxidant MitoQ decreases features of the metabolic syndrome in ATM+/-/ApoE-/- mice. *Free radical biology & medicine.* Mar 1 2012;52(5):841-849.

137. Neuzil J, Widen C, Gellert N, et al. Mitochondria transmit apoptosis signalling in cardiomyocyte-like cells and isolated hearts exposed to experimental ischemia-reperfusion injury. *Redox report : communications in free radical research.* 2007;12(3):148-162.

138. Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. Mitochondria-targeted antioxidants for treatment of Parkinson's disease: Preclinical and clinical outcomes. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2014/08/01/ 2014;1842(8):1282-1294.

139. Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annual review of pharmacology and toxicology.* 2003;43:233-260.

140. Rodrigo R, Miranda A, Vergara L. Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clinica chimica acta; international journal of clinical chemistry.* Feb 20 2011;412(5-6):410-424.

141. Teixeira J, Oliveira C, Amorim R, et al. Development of hydroxybenzoic-based platforms as a solution to deliver dietary antioxidants to mitochondria. *Scientific reports.* Jul 28 2017;7(1):6842.

142. Benfeito S, Oliveira C, Fernandes C, et al. Fine-tuning the neuroprotective and blood-brain barrier permeability profile of multi-target agents designed to prevent progressive mitochondrial dysfunction. *European journal of medicinal chemistry.* Apr 1 2019;167:525-545.

143. Teixeira J, Soares P, Benfeito S, et al. Rational discovery and development of a mitochondria-targeted antioxidant based on cinnamic acid scaffold. *Free radical research.* May 2012;46(5):600-611.

144. Ribeiro JA, Benfeito S, Cagide F, et al. Electrochemical Behavior of a Mitochondria-Targeted Antioxidant at an Interface between Two Immiscible Electrolyte Solutions: An Alternative Approach to Study Lipophilicity. *Anal Chem.* Jul 3 2018;90(13):7989-7996.

145. Teixeira J, Cagide F, Benfeito S, et al. Development of a Mitochondriotropic Antioxidant Based on Caffeic Acid: Proof of Concept on Cellular and Mitochondrial Oxidative Stress Models. *Journal of medicinal chemistry.* Aug 24 2017;60(16):7084-7098.

Accepted Article

146. Teixeira J, Basit F, Willems P, et al. Mitochondria-targeted phenolic antioxidants induce ROS-protective pathways in primary human skin fibroblasts. *Free radical biology & medicine.* Feb 1 2021;163:314-324.

147. Deus CM, Pereira SP, Cunha-Oliveira T, et al. A mitochondria-targeted caffeic acid derivative reverts cellular and mitochondrial defects in human skin fibroblasts from male sporadic Parkinson's disease patients. *Redox biology.* 2021/09/01/ 2021;45:102037.

148. Postuma RB, Aarsland D, Barone P, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society.* Apr 15 2012;27(5):617-626.

Accepted Article

Table 1: List of mutated genes involved in familial PD

Gene	Locus name	Protein Product	Chromossome localization	Type of mutation	Mode of inheritance
SNCA	PARK1	Alpha-synuclein	4q21.3–22	Missense, Point	Autosomal dominant
LRRK2	PARK8	Leucine-rich repeat kinase 2	12q12	Missense	Autosomal dominant
Parkin	PARK2	Parkin	6q25.2–q27	Missense, Frameshift, Splice site, Point, Nonsense	Autosomal recessive
PINK1	PARK6	PTEN-induced putative kinase 1	1p36.12	Missense, Frameshift, Splice site, Point, Truncating	Autosomal recessive
DJ-1	PARK7	Protein DJ-1	1p36.23	Point, Missense, Frameshift, Exon deletion and Splice site	Autosomal recessive
ATP13A2	PARK9	ATPase 13A2	1p36	Frameshift	Autosomal recessive
PLA2G6	PARK14	Phospholipase A2 Group VI	22q13.1	Missense	Autosomal recessive
FBXO7	PARK15	F-Box protein 7	*22q12-q13*	Missense, splice site	Autosomal recessive
GIGYF2	PARK11	GRB10 interacting GYF protein 2	*2q36-37*	Missense	Autosomal dominant
UCH-L1	PARK5	Ubiquitin C-Terminal Hydrolase L1	4p14	Missense	Autosomal dominant

Figure Legends

Figure 1: Mechanisms suggested contributing to oxidative stress in Parkinson's Disease pathophysiology. Mechanisms suggested contributing to oxidative stress in Parkinson's Disease pathophysiology. Mitochondrial dysfunction, alterations in dopamine metabolism, quality control mechanism impairment, neuroinflammation, and protein misfolding might underlie the pathogenesis of Parkinson's Disease. Oxidative stress plays an irrefutable role in a progressive and complex cascade of neurodegeneration culminating in dopaminergic neurodegeneration. These different pathways and their alterations, resulting either from genetic mutations or environmental factors contribute to disrupted redox balance in cells. All these cellular mechanisms that threaten dopaminergic cell's function are identical, but not linked in an orderly cascade of cause and effect.

Figure 2: The **multifaceted role of ROS.** Under normal conditions, ROS can act as intracellular messengers activating protective stress-response pathways, e.g. through up-regulating antioxidant defense system. These protective mechanisms seem to be incapable of avoiding effectively the gradual accumulation of damage independent of ROS. With aging, the generation of ROS can overwelm antioxidant systems and their toxicity will contribute in causing the very damage by stress pathways dependent of ROS. This triggers a toxic run-away process that might originate the basis of ROS involvement in Parkinson's Disease.

Figure 3: Schematic representation of mitochondrial involvement in Parkinson's Disease pathogenesis. Multifaceted links between changes in mitochondrial function in PD are represented. These mitochondrial alterations are related with the process of aging. The ubiquitin-proteasome system (UPS) dysfunctional is attributed to Lewy Bodies (LBs) pathology.

Figure 4: Main pathways for the formation of reactive species in Parkinson's Disease pathogenesis. Oxidative distress can result from increased dopamine levels and its metabolites, mitochondrial dysfunction, and alterations in the antioxidant defense system. Neurotoxicity induced by dopamine is due to ROS produced during dopamine metabolism by monoamine oxidase (MAO) or due to dopamine auto-oxidation. Abbreviations: DA-quinone: dopamine-quinone; DOPAC: dihydroxyphenylacetic acid; ETC: electron transport chain; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: reduced glutathione; GSSG: oxidized glutathione; L-DOPA: levodopa; MAO: monoamine oxidase; NO: nitric oxide; NOX: NADPH oxidase; SOD: superoxide dismutase; PPP: pentose phosphate pathway; Trx: thioredoxin; $TrxS_2$: oxidized thioredoxin; $Trx(SH)_2$: reduced thioredoxin; XO: xanthine oxidase

Figure 5: Effects of mitochondria-directed compounds in PD. The scheme represents the main molecular targets of mitochondria-directed antioxidants tested in PD models, as discussed in the main text.

Abbreviations: DOPAC - 3,4-dihydroxyphenylacetic acid; GPx - glutathione peroxidase; GSH – reduced glutathione; GSSG – oxidized glutathione; IM – inner membrane; IMS – intermembrane space; MAO – monoamine oxidase; mtDNA – mitochondrial deoxyribonucleic acid; NQO1 – NAD(P)H quinone oxidoreductase; Nrf2 - nuclear factor erythroid 2–related factor 2; OM – outer membrane; PGC1-α - peroxisome proliferator-activated receptor gamma coactivator 1 alpha; SOD - superoxide dismutase

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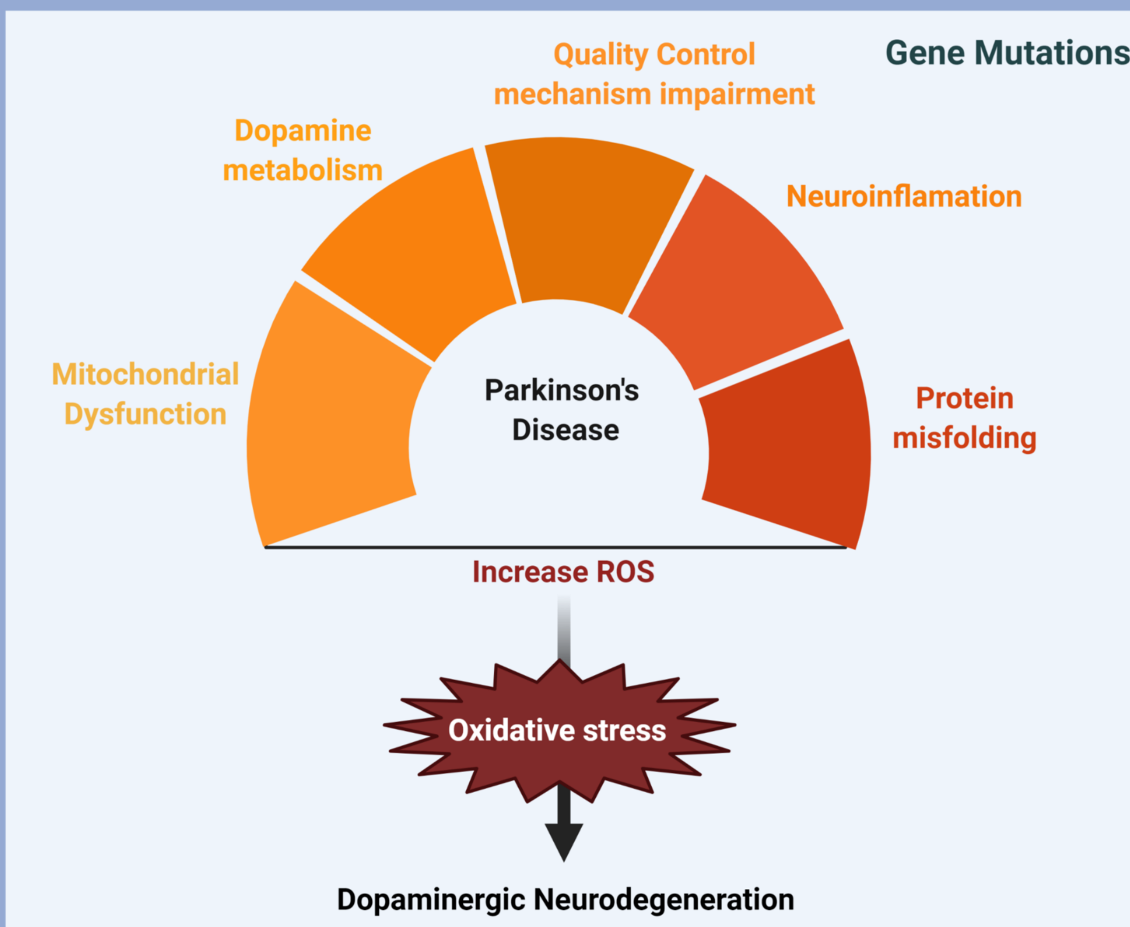

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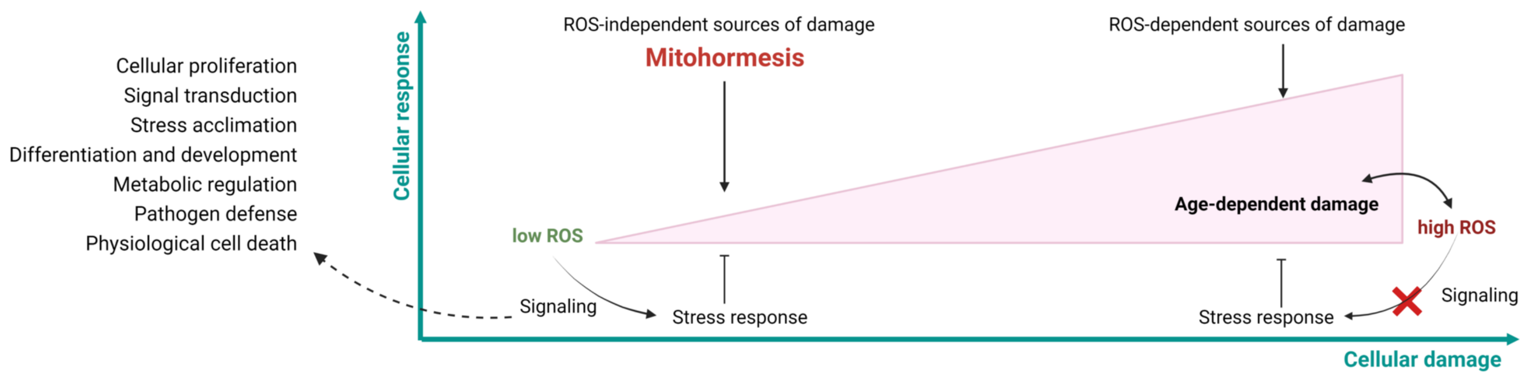

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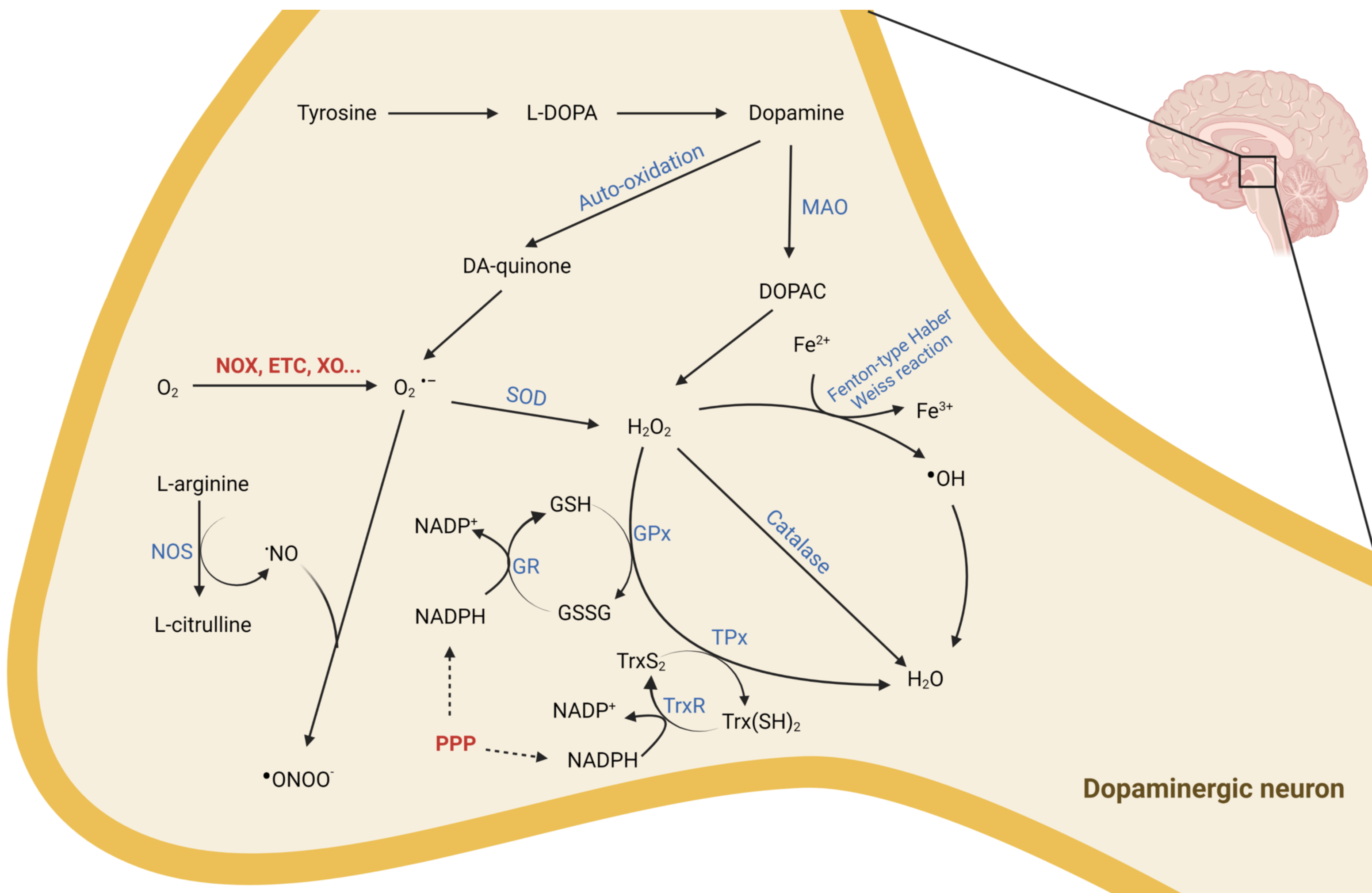

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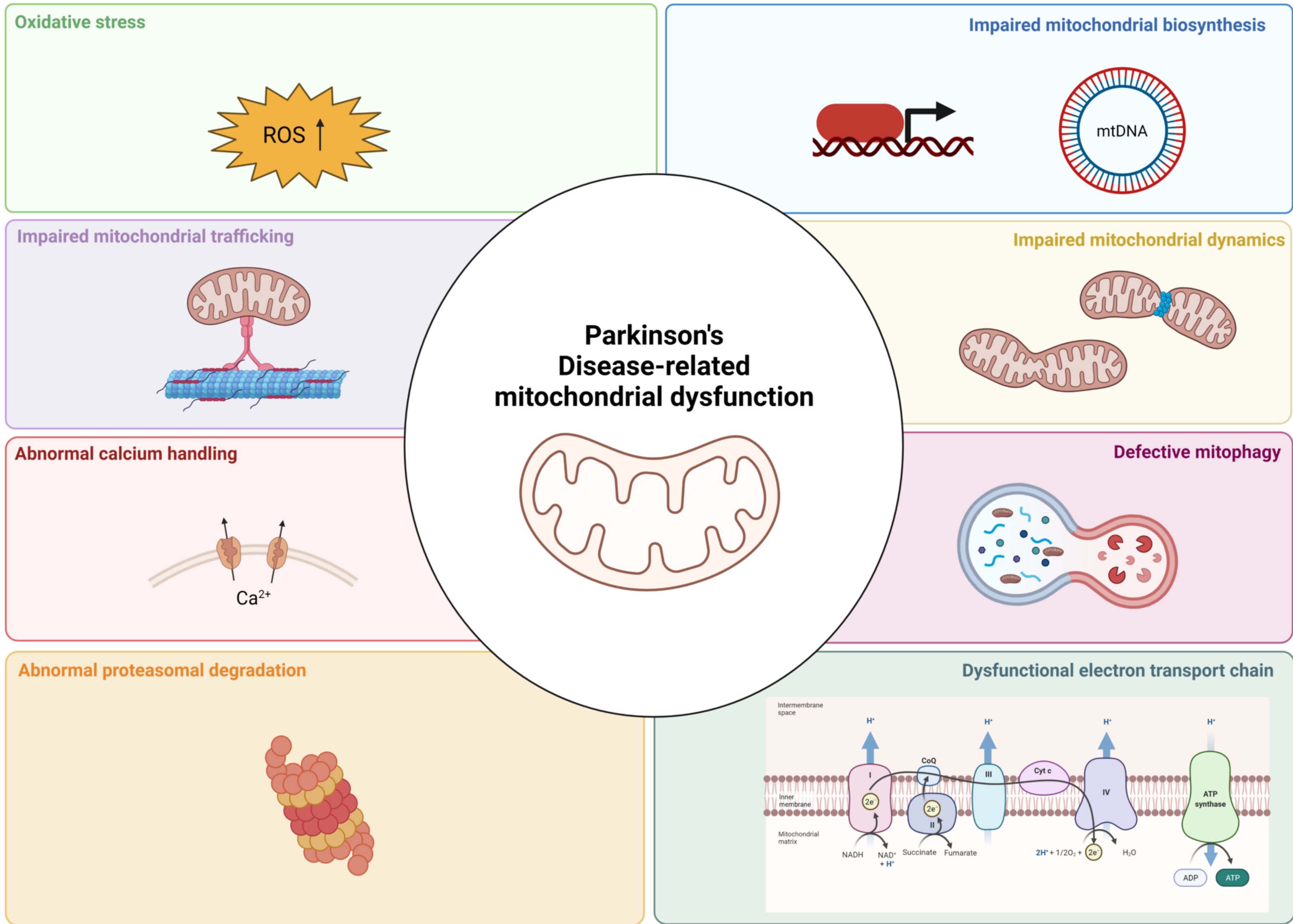

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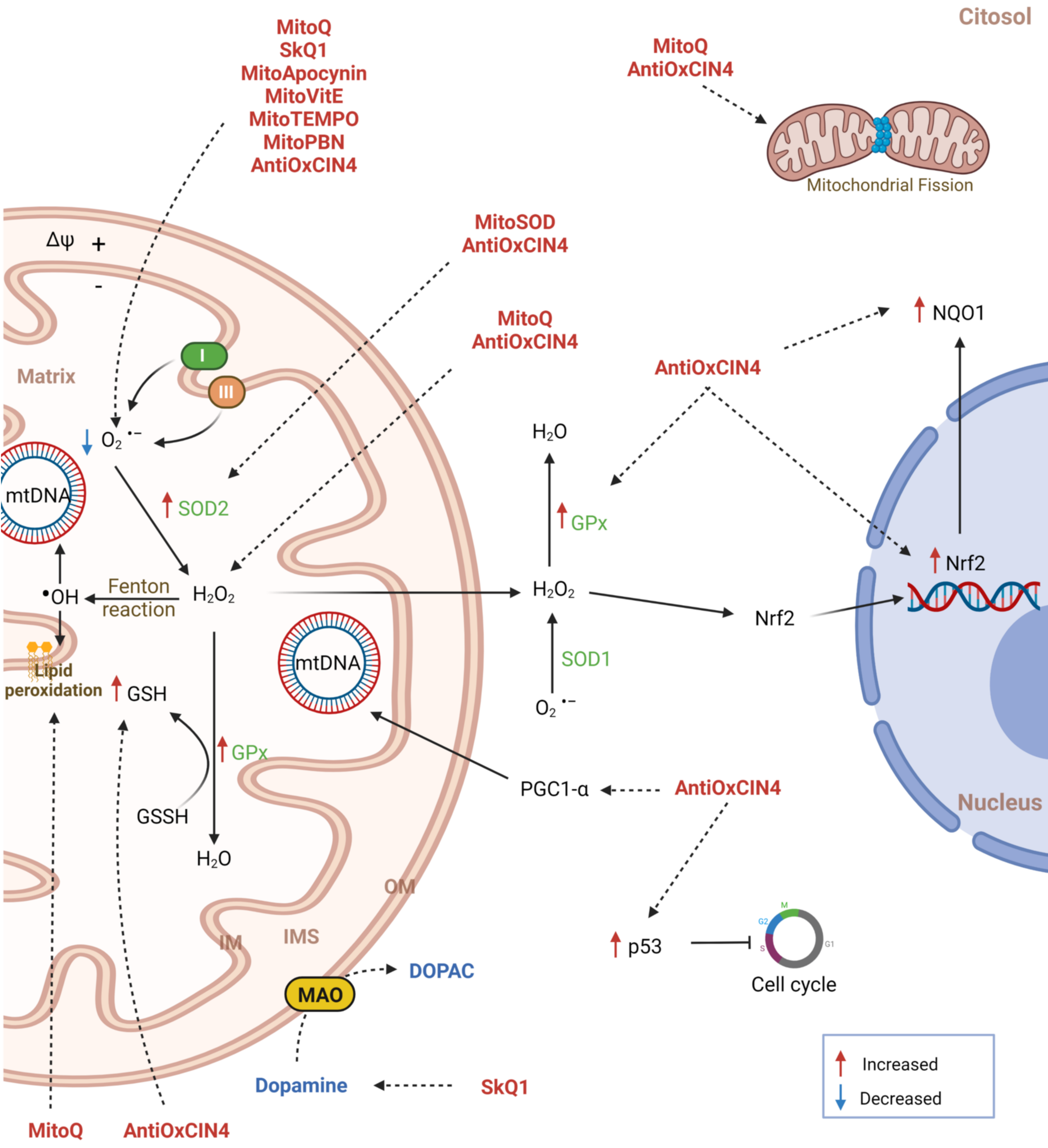

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