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FORUM REVIEW ARTICLE

Cell Death Pathways in Parkinson's Disease: Role of Mitochondria

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease and is characterized pathologically by selective loss of nigrostriatal dopaminergic neurons and the formation of Lewy bodies. Although in the majority of cases the cause of PD is unknown, mitochondrial dysfunction, environmental toxins, oxidative stress, and abnormal protein accumulation may all be involved in disease pathogenesis. The discovery of genes causing rare familial forms of PD (including α-synuclein, parkin, DJ-1, PINK1, and LRRK2) has shed light on our understanding of the molecular mechanisms of the development of the disease. Further studies from transgenic or toxin-induced experimental models have also provided insights into the etiology of human disease. Recently, accumulating evidence has suggested that mitochondrial dysfunction is one of the key players in molecular cell death pathways of PD. In this review, we provide an overview of the role of mitochondria in the pathogenesis of both sporadic and familial forms of PD. We also discuss the links between different pathways and highlight novel therapeutic opportunities which target mitochondria. *Antioxid. Redox Signal.* 11, 2135–2149.

Introduction

PARKINSON'S DISEASE (PD), first described by James Parkinson in 1817, is one of the most common progressive incurable neurodegenerative diseases. It affects ~1% of the population over 60 and up to 4% of those over 80 years old (31). Clinically, PD is characterized by motor impairments including resting tremor, bradykinesia, postural instability, rigidity, and some nonmotor symptoms such as cognitive and psychiatric problems. The pathological hallmarks of PD include: loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) and appearance of Lewy bodies in the surviving neurons. Substantia nigra DAergic neurons are a part of the interconnecting neuronal circuitry of the basal ganglia, which also involves other brain regions including the striatum, cortex, thalamus, and subthalamic nuclei.

Although the majority of PD cases are sporadic with unknown causes, a small proportion of PD cases are inherited, and mutations in several genes have been identified (Table 1). These genes include: α-synuclein (120), parkin (70), DJ-1 (18), PTEN-induced kinase 1 (PINK1) (157), and leucine-rich repeat kinase 2 (LRRK2) (107, 179). A single mutation has been found in Omi/HtrA2 gene in sporadic PD cases (144). Mutations in UCHL-1 (81), POLG1 (5), and ATP13A2 (124) have also been found in patients with parkinsonism, although there is still debate about whether these genes truly represent PD-causing genes.

The discovery of PD genes, together with the pathological and epidemiological investigation of sporadic PD cases, has provided us with insights into the principal pathways of PD pathogenesis (For reviews, see ref. 169). The identification of α-synuclein-positive Lewy bodies in the brains of PD patients has suggested the importance of protein aggregation. The discovery of parkin mutations further confirmed the importance of Ubiquitin Proteasome System (UPS) dysfunction in PD pathogenesis. The identification of PINK1 and HtrA2, a mitochondrial kinase and protease respectively, has helped reignite interest in the pathophysiology of mitochondria and their potential role in PD. Finally, the identification of mutations in DJ-1, an oxidative stress sensor, and evidence from functional studies suggested the importance of oxidative stress. In this review, we will focus on the role of mitochondrial dysfunction in the pathogenesis of PD.

Abnormal Mitochondria in PD Patients

An important piece of evidence linking mitochondrial dysfunction to PD came from the reports of complex I deficiency in the substantia nigra of patients with idiopathic

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Table 1. Parkinson's Disease-Associated Genes

Locus	*Gene*	*Chromosome*	*Inheritance*	*Phenotype*	*Protein*	*Function*
PARK1/4	SNCA	4q21	AD	Similar to IPD, early, rapid progression	α-synuclein	Implicated in synaptic vesicle formation
PARK2	Parkin	6q25.2-27	AR	Early-onset, slow progression, early dystonia, and dyskinesia	Parkin	Ubiquitin ligase E3
PARK5	UCHL-1	4p14	AD	Similar to IPD	UCHL-1	UPS component
PARK6	PINK1	1p35-36	AR	Early-onset, levodopa-responsive	PTEN-induced kinase 1	A protein kinase, may involved in mitochondira function
PARK7	DJ1	1p36	AR	Early-onset, levodopa-responsive	DJ-1	Protection against oxidative stress
PARK8	LRRK2	12p11.2-q13.1	AD	Similar to IPD	Dardarin	A protein kinase
PARK9	ATP13A2	1p36	–	–	ATPase type 13A2	Lysosomal type 5 P-type ATPase
PARK13	HTRA2	2p13	IPD	IPD	HtrA2/Omi	Mitochondrial serine protease

AD, autosomal dominant; AR, autosomal recessive; IPD, idiopathic Parkinson's disease; UPS, ubiquitin proteasome system.

PD in 1989 (130). Recently Keeney *et al.* suggested that complex I is the only respiratory chain protein complex to be affected by endogenous oxidative damage and reduced structural stability (66), although defects in other complexes have also been reported by Bindoff *et al.* (16). The substantia nigra is not the only affected region in the body: reduced complex I activity has also been found in the frontal cortex, skeletal muscle, and platelets of patients with PD (17, 113, 114). Interestingly, deficiency in the substantia nigra and platelets has been consistently detected by several groups (77, 85, 131), whereas in the skeletal muscle the results are less definitive (85, 115, 152). The complex I deficiency in multiple regions suggests that this is a systemic defect, at least in some cases, but it does not help to differentiate acquired and genetic causes.

Environmental Toxins and PD: Mitochondrial Complex I Inhibition

The link between mitochondrial toxins and the pathogenesis of PD first emerged following the accidental exposure to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), which led to an acute and irreversible parkinsonism (79). MPTP is converted to the active neurotoxic metabolite 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase B in glial cells (139). MPP^+ is a complex I inhibitor and a substrate for the dopamine transporter (Fig. 1). It therefore accumulates in DAergic neurons and causes cell death by complex I inhibition (99). In aged monkeys and baboons treated with MPTP, α-synuclein-positive Lewy body-like intracellular inclusions are seen (39, 74). Paraquat, a complex I inhibitor with structural similarity to MPP+, can also lead to selective DA neuron loss, as well as α-synuclein aggregation and upregulation in mice (37, 86, 155).

Rotenone, another specific complex I inhibitor, is a commonly used pesticide. When infused into rodents at low doses over 1 month, rotenone leads to nigrostriatal neuronal loss, the formation of Lewy body-like inclusions and behavioral phenotypes reminiscent of PD (14). Furthermore, chronic exposure to rotenone can also lead to oxidation of DJ-1, accumulation of α-synuclein, and proteasomal impairment (13, 132, 154). However, the damage induced by rotenone is a multisystem degeneration and more widely spread than that seen in PD (58). This suggested that some of the toxin-induced animal models may not be the true representation of PD and

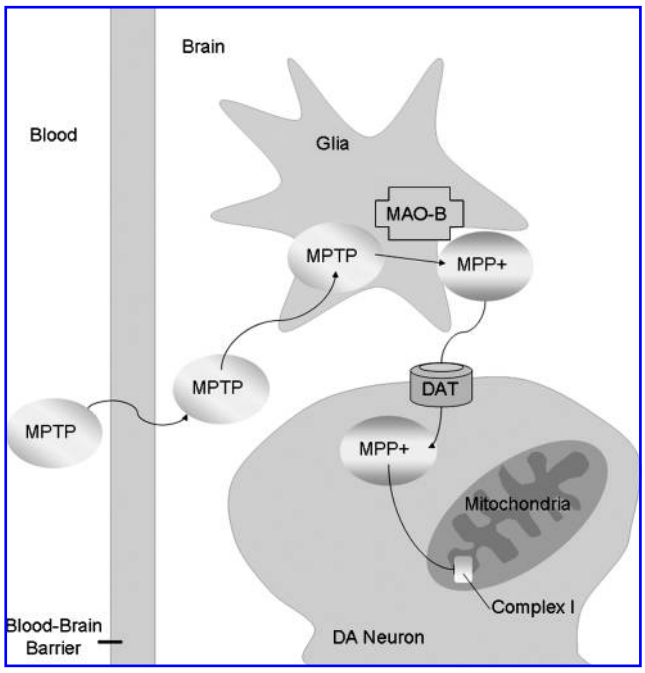

FIG. 1. The MPTP metabolic pathway. After administration, MPTP can freely cross the blood–brain barrier. Once MPTP has entered the brain, it will be metabolized to MPP^+ by the enzyme monoamine oxidase B (MAO-B) in non-DAergic neurons. MPP^+ is the active toxic compound and it will then be taken up by DA transporters. Once inside the DAergic neurons, MPP^+ impairs mitochondrial respiration by inhibiting complex I activity, which will then causes mitochondrial dysfunction, oxidative stress, and cell death.

Table 2. Mitochondria Toxin Animal Models of PD

Toxin	*PD pathology*
MPTP (acute)	20–50% DAergic neuron loss Reduced DA levels in the striatum
MPTP (chronic)	Up to 70–80% DAergic neuron loss Formation of α-synuclein positive inclusions
Paraquat	Small but significant loss of DAergic neurons Up-regulation and aggregation of α-synuclein
Rotenone (chronic)	Progressive loss of DAergic neurons Formation of α-synuclein and ubiquitin positive inclusions Oxidative damage Widespread cell loss were also seen in some cases

the disease is more complicated than just simple mitochondrial complex I inactivation (for a summary, see Table 2).

Genetic Defects and PD: Impaired Mitochondrial DNA and Genomic DNA

Mitochondrial DNA mutation and deletion in PD

In the 1990s, Swerdlow *et al.* (147) and Gu *et al.* (53) reported that the complex I defect seen in PD patients can be transmitted to cybrid cells in which platelets from PD patients were fused with mitochondria-deficient cell lines. These cybrid cell lines have also shown increased reactive oxygen species (ROS) production and increased sensitivity to cell death induced by complex I inhibition (147). These findings suggest that the mitochondrial genome may contribute to the complex I deficiency in PD patients.

Mitochondrial DNA (mtDNA) encodes 13 subunits of the respiratory chain proteins, including seven complex I, one complex III, three complex IV, and two complex V subunits. The majority of the proteins are therefore encoded by the nuclear genome. mtDNA is in a hostile environment and is often the victim of mutations throughout the life of a cell. As well as point mutations, other larger scale abnormalities can be found, including deletion, duplication, and depletion. Increased levels of mtDNA deletion in the striatum of patients with PD were reported by Ikebe *et al.* in the early 1990s (60). Whether such deletions are a cause of PD or an age-related bystander phenomena remains unclear. In two studies using quantitative single cell techniques, high levels of mtDNA deletions were detected in the substantia nigra neurons in both aged controls and PD patients, with a slightly increased trend in PD individuals (12, 75). Interestingly, they have also shown that the mtDNA deletions were significantly higher in neurons with impaired cytochrome oxidase activity (75), suggesting that mtDNA deletions may account for the impairment of cellular respiration. Conversely, despite numerous studies, the role of single base pair mutations/polymorphisms and haplogroups in PD remains controversial (7, 24, 43, 59, 159), although it seems that a single nucleotide polymorphism in the DN3 gene (one of the complex I genes) had a protective effect in PD in certain age and gender groups (24, 43, 59). Overall, these findings suggested that alteration of the mitochondrial genome may be involved in the pathogenesis of PD but further genetic and functional studies are still needed.

Nuclear Encoded Proteins and Familial PD

PINK1

Mutations in the PINK1 gene were first identified in three families with an autosomal recessive form of PD in 2004 (157). The PINK1 gene contains 8 exons and encodes a 581 amino acid (63 kDa) protein with an amino-terminal mitochondrial targeting sequence and a serine–threonine kinase catalytic domain (136). To date, PD-causing mutations of PINK1 have been described both within and outside the kinase domain with no obvious clustering within the gene. Some of the mutations have been reported to reduce the kinase activity of PINK1 *in vitro* (10, 137). Western blot analysis of PINK1 overexpressing cells suggested that the full length PINK1 protein could also be cleaved into a shorter form, which normally runs at 54 kDa (41, 122, 136). It has been shown that Rhomboid-7, a mitochondrial protease, is responsible for the cleavage of the precursor forms of PINK1 (168) in *Drosophila*. Further identification of the cleavage enzymes and cleavage sites of PINK1 in mammalian systems will help us gain more understanding of the processing of PINK1. In particular, it will be important to determine whether the active form is the full length or the cleaved form and which molecular steps are involved in its cleavage. PINK1 mRNA is ubiquitously expressed in human tissues and the highest expression is found in heart, muscle, and testes (156). In mammalian brains, PINK1 is uniformly expressed, with highest expression levels found in the cell bodies of neurons and glia (41, 153). Most reports have indicated that PINK1 localizes subcellularly in the inner membrane of mitochondria (41, 122, 136), the mitochondrial intermembrane space (122, 136), and/or the outer mitochondrial membrane (41, 178). However, the cytoplasmic localization of PINK1 has also been reported (10, 55, 166). To date, none of the known mutations affect the localization of PINK1, but some mutations can affect protein stability and cause increased degradation (10, 97). It is possible that some of the mutations may affect the cleavage of PINK1 and therefore affect its stability and functions. Further investigation in this area could increase our understanding the role of PINK1 in PD pathogenesis.

Knockdown of PINK1 using transient RNA interference has shown reduced mitochondrial membrane potential and increased rate of basal and stress-induced apoptosis (55, 118, 157). In contrast, overexpression of wild-type, but not mutant, PINK1 in tumor cell lines was shown to prevent activation of caspase-3 and release of cytochrome c from mitochondria to cytosol under stress conditions (118, 157, 165). More recently, stable knockdown of PINK1 by retrovirus showed reduced long-term neuronal viability, increased sensitivity to staurosporine- induced apoptosis, reduced mitochondria membrane potential, and increased basal free radical production in human DAergic neurons (170).

In *Drosophila* PINK1 knockout models, motor deficits including abnormal wing posture, rigidity, flight impairment, and reduced climbing ability have been reported and some of the motor phenotypes were progressive with age (26, 112, 164). The numbers of DAergic neurons in a certain key cluster of the fly brain also showed a small but significant decrease

(~10%) (26, 164, 173). In addition, cell loss was also found in the thoracic indirect flight muscles (173), and swollen mitochondria were seen in the muscle fibers as well as in surviving DAergic neurons (112). Those flies also display an increased sensitivity to paraquat and rotenone. This phenotype is similar to parkin knockout flies. Interestingly, the PINK1 knockout fly phenotypes can be rescued by overexpressing wild-type parkin (112, 149, 173).

Taken together, these studies suggest a chronic mitochondrial dysfunction in the absence of PINK1 in cell models and non-mammalian models, but further evidence in *in vivo* mammalian models is needed. To date, no motor impairments or DAergic neuron losses have been found in PINK1 loss-of-function mouse models (71, 178). Abnormalities have been observed in the nigrostriatal circuitry in PINK1 knockout mice. Kitada *et al.* reported a reduction in evoked dopamine release in striatal slices and in corticostriatal long-term potentiation and depression (71). These defects can be alleviated by D1 and D2 agonists, suggesting impaired release of synaptic DA. More recently, reduced mitochondrial complex activities, and increased sensitivity to oxidative stress have been detected in the same mice (42).

Parkin

Mutations in the parkin gene were found in autosomal recessive early-onset PD (70), and parkin mutations are relatively common (accounting for ~50% of all autosomal recessive PD cases) (133). Parkin is a 465 amino acid protein with two RING fingers separated by an in-between RING domain at the carboxyl terminus; it functions as an E3 ubiquitin ligase (133). E3 ubiquitin ligases are one component of the UPS, a main pathway for removing misfolded or unwanted proteins from the cells (25). On the amino terminus of parkin, a ubiquitin-like domain binds to the RPN10 subunit of the 26S proteasome (127). Parkin can be phosphorylated by casein kinase-1, protein kinase A, and protein kinase C *in vitro*, and the phosphorylation is regulated by cellular stress, such as proteasomal dysfunction and endoplasmic reticulum stress (172).

Although usually described as an essentially cytosolic protein, parkin is mostly localized into the mitochondria in dividing neuroblastoma cells, and within the mitochondria parkin is mainly localized to the matrix and inner membrane (78). It has been suggested that parkin's mitochondrial translocation can be promoted by the phosphorylation of parkin by PINK1 (69). In neuronally differentiated PC12 cells, parkin was shown to associate with the outer membrane of mitochondria, where it plays an important role in preventing mitochondrial swelling, cytochrome c release, caspase activation, and the apoptosis induced by ceramide (29). This anti-apoptotic effect of parkin is abolished by PD-causing mutations and the proteasome inhibitor epoxomicin (29), suggesting that it is mediated by the E3 ubiquitin ligase activity of parkin. When cells are stressed with the complex I inhibitor rotenone, parkin is released from mitochondria into the cytosol (78).

In transgenic *Drosophila* models, swollen mitochondria with disintegrated cristae and apoptotic cell death were observed in the flight muscles of parkin knockout fly lines and flies overexpressing mutant parkin (51, 163). In mammalian models, parkin knockout mice showed a mild but significant decrease in mitochondrial respiratory capacity in the striatum, as well as reduced expression of specific respiratory chain and antioxidant proteins, although the mitochondria morphology was not affected (108). Intriguingly, although a mild nonprogressive motor deficit, reduced synaptic excitability, and increased striatal extracellular dopamine concentrations were reported in young parkin knockout mice (49, 61); no loss of DAergic neurons or inclusion formations were described. These findings suggested that while parkin plays an important role in maintaining mitochondrial function, parkin deficiency alone may not be sufficient to cause PD, at least in mammalian transgenic models. This raised the hypothesis that neurodegeneration in these models may require further environmental or genetic stresses. For example, primary midbrain neurons from parkin knockout mice showed increased sensitivity to apoptotic cell death induced by mitochondria complex I inhibition induced by rotenone (21). Similar mechanisms might also apply in humans, since people with heterozygous parkin mutations have an increased risk of developing PD and progressive nigrostriatal dysfunction visualized by [^{18}F]dopa positron emission tomography (PET) (67).

DJ-1

A variety of missense, truncating, and deletion mutations have been identified in the DJ-1 gene, which causes an autosomal recessive form of PD (18). Similar to patients with parkin mutations, patients with DJ-1 mutations exhibit slow progressing young-onset PD with a good response to levodopa treatment and may have dystonia. However, unlike parkin, DJ-1 mutations are very rare, only accounting for ~1–2% of early-onset cases (18). The protein encoded by DJ-1 is a 23 kDa protein, which is expressed in both peripheral tissues and central nervous system. In the brain, DJ-1 is highly expressed in the cerebellum, hippocampus, and olfactory bulb, but has also been detected in striatum, substantia nigra pars compacta, and reticulate (8, 64, 125, 177). Subcellularly, the endogenous DJ-1 protein is predominately found in the cytoplasm, but a small proportion is also localized to the mitochondrial intermembrane space and the matrix (177). This mitochondrial localization suggests that DJ-1 might have a role in mitochondrial function, although no mitochondrial morphological changes have been reported in DJ-1 deficient models.

DJ-1 is a multifunctional protein. It can function as a modulator of androgen receptor-dependent transcription, an oncogene, and an oxidative stress sensor (98, 148). In terms of PD pathogenesis, the oxidative stress sensor function of DJ-1 is likely to be most crucial because it acts as an antioxidant protein or a redox sensor (20, 93). In cultured mammalian cells, knockdown of DJ-1 by siRNA leads to increased cell death induced by oxidative stress, ER stress, and proteasome inhibition (175). In terms of transgenic animal models, DJ-1 knockout mice showed motor abnormalities but no DAergic neuron loss was seen in the substantia nigra (50). Furthermore, increased sensitivity to mitochondrial complex I inhibition and oxidative stress has also been reported in DJ-1 knockout mice (68) and a physical interaction has been reported between DJ-1 and PINK1 (150). Interaction between DJ-1 and parkin has also been reported under oxidative conditions (96). However, both of these interactions have yet to be confirmed *in vivo*. In fly models, overexpression of DJ-1 failed to rescue the muscle phenotypes caused by PINK1 knockout

(173), suggesting that DJ-1 is either upstream of PINK1 or acts in a parallel pathway to PINK1.

Omi/HtrA2

Omi/HtrA2 is a mitochondrial serine protease and has also been associated with PD. The Omi/HtrA2 protein is localized to the mitochondrial intermembrane space and is released into the cytosol when cells are stimulated by pro-apoptotic stimuli (146). A G399S mutation in Omi/HtrA2 has been reported in four sporadic PD cases and an A141S polymorphism was also found to increase the risk of developing PD (144). Both mutations are suggested to affect the regulation of the proteolytic activity of Omi/HtrA2 and modulate cell death. However, genetically the role of this gene in PD pathogenesis remains unproven, as other reports have failed to identify a mutation or an association (126, 138). Interestingly, Omi/HtrA2 knockout mice have a strong parkinsonian phenotype, including rigidity, tremor, and striatal damage (89). Finally, HtrA2 phosphorylation has been shown to be PINK1 dependent (119). The phosphorylation of Omi/HtrA2 at Ser142 demonstrates increased protease activity and enhanced protective effect under stressed condition. In brains of patients with PD-associated PINK1 mutations, the phosphorylation of HtrA2 at Ser142 is decreased (119).

α-Synuclein

Mutations and multiplications in the α-synuclein gene were found associated with an autosomal dominant form of PD (103, 120). Although mutations are extremely rare, α-synuclein is regarded as central to disease pathogenesis, as it is a key component of Lewy bodies (143). α-Synuclein protein is usually localized to the cytosol and synaptic terminals. However, it has recently been suggested that α-synuclein may localize in or interact with the mitochondria. This has been observed in cells overexpressing wild-type or A53T mutant α-synuclein and in isolated rat brain mitochondria. Evidence has suggested that this interaction can cause reduced mitochondrial complex I activity, increased ROS production, cytochrome c release, and increased mitochondrial calcium (34, 110). Overexpression of the G209A mutant or wild-type α-synuclein can also increase the sensitivity of neurons to mitochondrial toxins such as rotenone in mammalian cells (105). In terms of transgenic models, mice overexpressing the human α-synuclein gene are also more sensitive to complex I inhibition induced by MPTP and showed abnormal mitochondrial morphology (142), whereas α-synuclein knockout mice are more resistant to the complex I inhibitor MPTP, and the complex II inhibitors malonate and 3-nitropropionic acid (30, 72). It is believed that this protective effect is mediated by the decreased free radical production. Moreover, transgenic mice overexpressing A53T or A30P mutant α-synuclein developed a decreased complex IV activity in the spinal cord, neuronal loss, and accumulation of intraneuronal inclusions, which contain both α-synuclein and degenerating mitochondria (88). Together, these findings suggest an important role of α-synuclein in mitochondrial toxicity and cell death. Interestingly, α-synuclein toxicity has also been shown in aging yeast models and this toxicity can be inhibited by abrogation of mitochondrial DNA (19), suggesting that functional mitochondria are important for α-synuclein toxicity. Therefore the relation between α-synuclein and mitochondrial function may be complicated and the precise molecular pathways have yet to be characterized.

LRRK2

Mutations in the LRRK2 gene were first found in relatively late onset autosomal dominant PD (107, 179). LRRK2 mutations are found to account for 5–6% of familial PD cases and up to 1.6% of patients with sporadic PD (45). The protein encoded by LRRK2 is called dardarin (from the Basque word for tremor) (107). Dardarin is a large protein (2527 amino acids) with several predicted functional domains, including: the N-terminal leucine-rich repeats, the Ras/GTPase domain (Roc), the COR (C-terminal of Roc) domain, the serine/threonine protein kinase domain, and the carboxy terminal WD-40 like domain (52). Mutations have been reported in all the protein domains and there is no obvious correlation between the mutation sites and clinical phenotype. Although some mutations, such as G2019S, I2020T, and R1441G have been shown to increase the kinase activity of LRRK2, others seem to decrease or not affect the kinase activity (48, 52, 63, 83, 84). LRRK2 is widely expressed throughout the brain and peripheral tissues (44). Subcellularly, LRRK2 is mostly associated with the cytoplasmic membrane and has also been reported to be associated with mitochondrial outer membrane fractions (46, 141, 167), suggesting a role for LRRK2 in mitochondrial function. In primary cortical neuron cultures, overexpression of some LRRK2 mutants (R1441C, Y1699C, or G2019S) can lead to neuronal degeneration (52, 140, 141). Moreover, some LRRK2 mutations can also result in progressive reduction in neurite length and branching, both in primary neuronal cultures and in the intact rodent brain, where LRRK2 knockdown has the opposite effects (84). Interestingly, the LRRK2 GTPase domain has a significant sequence similarity with the Rho GTPase domain of Miro (54), which is essential for anterograde transport of mitochondria along microtubules (47).

Taken together, most of the PD-linked genes are found associated with mitochondria (Fig. 2) and mutations in those genes often lead to impaired mitochondrial function. However, the mechanisms for the mitochondrial dysfunction are yet not very clear. Studies of genetic and toxin models have suggested that impaired mitochondrial dynamics may contribute to the mitochondrial dysfunction in PD.

Mitochondrial Dynamics and PD: Possible Mechanisms for Mitochondrial Dysfunction?

Fission and fusion

Mitochondria are extremely dynamic organelles and are undergoing continual fusion and fission (Fig. 3). The balance between fusion and fission not only determines the morphology of mitochondria in cells but also has a significant effect on mitochondrial function (23). Fusion and fission events lead to mixing of mitochondrial membranes and exchange of mitochondria contents. These events also contribute to the control of mitochondrial subcellular localization and dramatic mitochondrial morphology changes that occur during apoptosis. Mutations in some mitochondrial fusion and fission genes have been reported in neurodegenerative disease. For example, mutations in the optic atrophy 1 (OPA1) gene can cause a hereditary optic neuropathy leading to

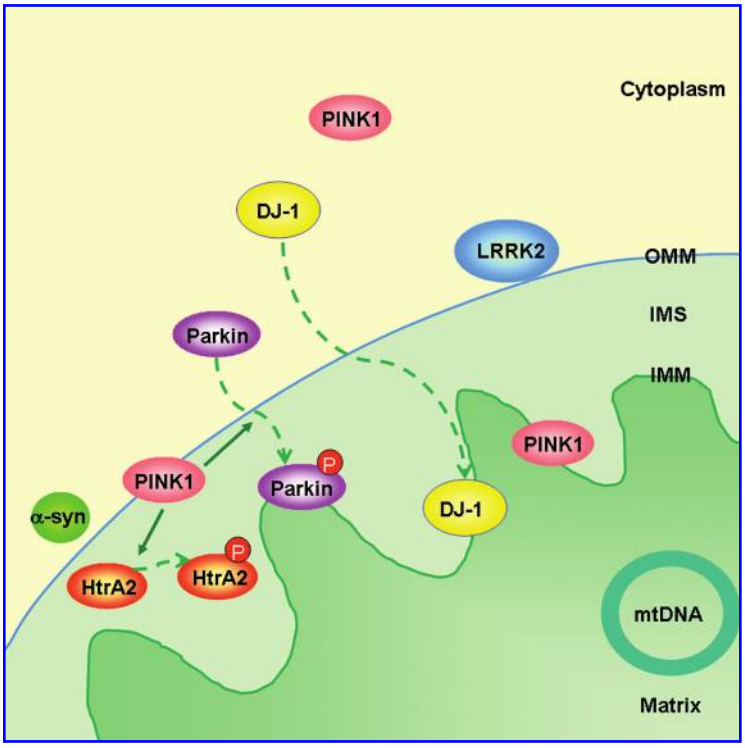

FIG. 2. PD genes associated with mitochondria. Among the PD-associated genes, both LRRK2 and α-synuclein have been found associated with mitochondria outer membrane. Although known as a mitochondria kinase, PINK1 can localize to both the cytoplasm and the mitochondria, where it can affect HtrA2 phosphorylation. Parkin is a cytosolic protein, but can also been translocated to mitochondria, which may be promoted by phosphorylation by PINK1. DJ-1, another cytosolic protein, can also be translocated to mitochondria. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

blindness (32). OPA1, a GTPase, is a pro-fusion protein that is localized to the mitochondrial intermembrane space, and is tightly associated with the inner membrane (104). Another example is human mitofusin-2 (MFN2). Mutations in MFN2 cause Charcot–Marie–Tooth disease, a dominantly inherited neuropathy characterized by degeneration of peripheral sensory and motor axons (180). Mfn2 is a GTPase localized to the mitochondrial outer membrane and can promote mitochondrial fusion by forming complexes and tethering mitochondria together. It has been shown that MFN2 mutations can cause abnormal clustering of small fragmented mitochondria in both neuronal cell bodies and axons, and those clusters were unable to be transported down the axon (6). These

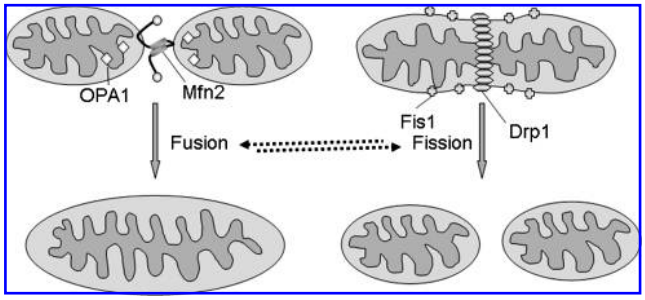

FIG. 3. Mitochondrial fission and fusion. During mitochondria fusion, mitofusin proteins link the outer mitochondrial membrane of two mitochondria, which lead to membrane fusion. Then Opa1, which is anchored on the inner mitochondrial membrane, regulates the fusion of the inner membrane. During fission, Fis1 circumscribes the outer mitochondrial membrane and interacts with Drp1, which leads to separation of the outer and inner membranes.

suggested that mitochondrial dynamics are critical for the integrity of the nervous system.

In terms of PD, no mutations in typical mitochondria fission and fusion genes have yet been found in PD patients. However, increased evidence from PINK1 and parkin studies has suggested potential involvement of mitochondrial dynamics in PD (111, 129, 164). As mentioned earlier, PINK1 or parkin deficiency can lead to abnormal mitochondrial morphology in the flight muscle and DAergic neurons of flies. Interestingly, these phenotypes can be suppressed by overexpressing the Drp1 gene, a mitochondrial fission-promoting component, or by downregulation of mitochondrial fusion promoting genes Opa1 and Marf, the *Drosophila* homologue of human Mfn gene (33, 111, 121, 174), suggesting that lack of PINK1 or Parkin can lead to increased mitochondrial fission in *Drosophila*. In mammalian cells, overexpression of PINK1 can lead to increased mitochondrial fission, where knockdown of PINK1 causes excessive fusion (173). Increased mitochondrial fusion has also been found in primary fibroblast cells from patients with PINK1 mutations (36). It is noteworthy that PINK1 deficiency led to opposite effects in *Drosophila* and mammalian systems. Further investigations are required to explain this discrepancy and also to gain more understanding of the role of mitochondrial fission and fusion in PD pathogenesis.

Maintaining calcium homeostasis

Apart from energy generation, another key function of mitochondria is to maintain the calcium (Ca^{2+}) homeostasis within the cells. Mitochondria have a vast but finite capacity for buffering intracellular Ca^{2+}, by uptake and accumulation of Ca^{2+} into mitochondria. When mitochondrial Ca^{2+} is overloaded, the permeability transition pore (PTP) will be activated and therefore lead to permeablization of mito-

chondrial inner membrane, collapse of bioenergetics, and release of Ca^{2+}. In neurons, calcium homeostasis and calcium signaling can regulate numerous neuronal functions, such as synaptic transmission, neuronal plasticity, and cell survival, and therefore disruption in intracellular calcium homeostasis can have a major impact on neuronal health. Dysregulation of calcium homeostasis has been reported both in the physiological aging process and a range of neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (1, 2, 15, 109, 158). Recently, calcium homeostasis dysregulation has also been suggested to play a role in PD. In neuroblastoma cells expressing mutant α-synuclein (A30P and A53T), Furukawa *et al.* have found higher plasma membrane ion permeability, an increase in both the basal level of intracellular Ca^{2+} concentrations and the Ca^{2+} response to membrane depolarization, as well as increased cell death, which can be inhibited by a Ca^{2+} chelator, BAPTA-AM (40). These findings suggest that mutant α-synuclein can lead to perturbation in the Ca^{2+} homeostasis which contributes to the cell death. Although further investigations about whether mitochondria are involved in this process and its mechanisms are still needed, this is one piece of evidence indicating the involvement of impaired Ca^{2+} homeostasis in PD pathogenesis.

On the other hand, some studies using toxin models have implicated the involvement of Ca^{2+} homeostasis dysregulation, mitochondrial dysfunction, and PD-related cell death. In isolated brain mitochondria, the complex I inhibitors MPP^+ and rotenone can induce Ca^{2+} release into cytosol, membrane depolarization, and mitochondrial swelling, all of which can be inhibited by preventing the PTP opening (22, 106). It has also been shown that PTP inhibitors can reduce the cell death caused by MPP^+ in animal models (27). Whether the MPP^+ induced PTP opening and Ca^{2+} release is due to complex I inhibition has yet to be investigated. The mechanisms of calcium dysregulation in PD and its contributions to the pathogenesis of PD are still unclear and further studies in genetic and toxin PD models are required.

Mitochondrial Dysfunction, Oxidative Stress, and Protein Aggregation: A Network of Pathways

As mentioned earlier, mitochondrial dysfunction, oxidative stress and abnormal protein accumulation have all been implicated in the cell death pathways in PD. Accumulating evidence has suggested that these pathways are not functioning independently but rather as a network (Fig. 4).

Protein aggregation/proteasomal inhibition is another important pathway for PD pathogenesis. In PD patients, decreased 20S proteasomal enzymatic activities and reduced expression of the alpha-subunits of 26/20S proteasomes were detected, suggesting a possible failure of the UPS in PD brains (91). In toxin-induced animal models, proteasomal inhibition can lead to loss of nigral DAergic neurons (91, 92, 176), although there is still a debate as to whether these are good PD models because some reports failed to reproduce these observations (87). The UPS is an ATP-dependent protein degradtion system (161). Therefore, deficiency in mitochondrial respiratory chain activity may lead to impaired ATP production, which can then result in limited proteasomal activity. In addition, mitochondrial dysfunction will also result in increased free radical generation, which can lead to an increase

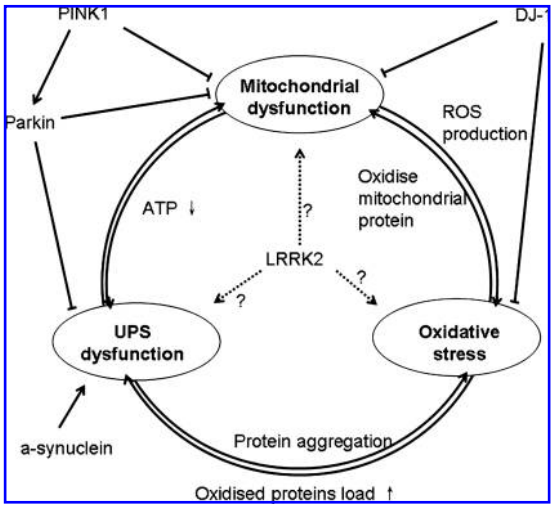

FIG. 4. Network of PD pathways. Interactions between the three major pathways and several PD associated genes.

in substrate load (oxidized proteins) for the proteasome (58). The proteasomal dysfunction will then lead to accumulation of damaged proteins, which have a potentially negative effect on cell function and survival. Conversely, proteasomal stress can also lead to mitochondrial dysfunction. For example, proteasome inhibition can lead to reduced mitochondrial complex I and complex II activities, increased mitochondrial ROS production, and reduced intramitochondrial protein translation (145).

Oxidative stress is another common underlying feature in PD pathogenesis and it is the result of unregulated production of ROS, which could originate from the mitochondria, cytoplasm, and also outside the cells. Mitochondria consume a great amount of oxygen via the electron transport chain and a small proportion of the oxygen is reduced to ROS, even under normal situations. In DAergic neurons, dopamine can generate ROS via monoamine oxidase (MAO) (82). Dopamine is synthesized in the cytosol and pumped into synaptic vesicles by vesicular monoamine transporter 2 (VMAT2). Defects in the secretory pathway can lead to synaptic vesicle shortage and reduced VMAT2 in the synapse, which will impede dopamine loading and cause increased cytosolic dopamine levels. This consequently leads to increased ROS production and oxidative stress. Furthermore, oxidative stress is not only generated in neurons. Glial cells are also a host of ROS when they are activated (76, 171). On PD brains, activation of glial cells has been reported in all affected regions, which may also contribute to the oxidative stress (123). In the other hand, oxidative stress can induce abnormal mitochondrial function, conversely inhibition of complex I by environmental toxins or mutations in complex I subunits leads to increased free-radical generation (128).

Cell Death Mechanisms in PD

There is now substantial evidence that mitochondrial dysfunction plays a crucial role in the pathogenesis of PD. However, the exact mechanism of cell death in PD is not very clear (Fig. 5). Extensive mitochondrial injury may impact the maintenance of sufficient cellular ATP, which can lead to necrotic cell death. Mild mitochondrial dysfunction can cause

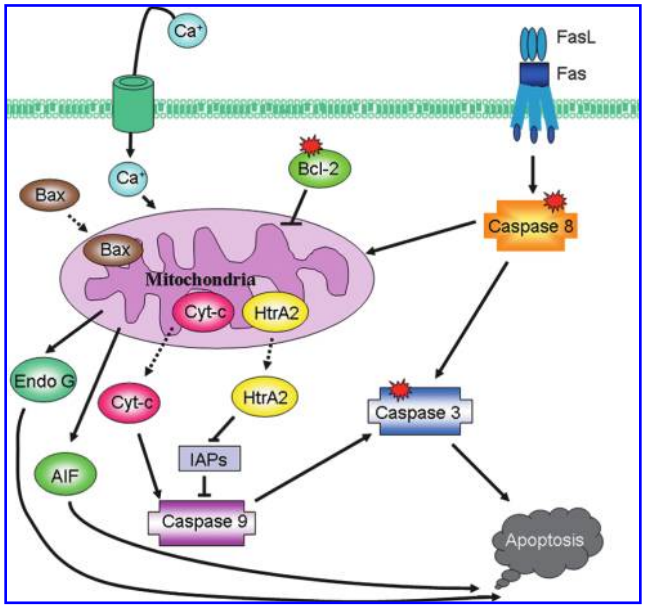

FIG. 5. Role of mitochondria in apoptosis. *Stars* indicate molecules affected in PD brains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

abnormal intracellular Ca^{2+} accumulation in neurons in response to excitotoxicity induced by neurotransmitters such as glutamate (38) and can also lead to excessive production of ROS and trigger apoptotic cell death. Mitochondria are also one of the key players in apoptosis (Fig. 5). Apoptotic stimuli lead to the release of small pro-apoptotic proteins, such as cytochrome c and apoptosis-inducing factor (AIF) that are normally localized in the mitochondrial intermembrane space, into cytosol. Upon entry into the cytosol, those pro-apoptotic proteins will trigger a caspase-dependent mechanism. Cytochrome c can bind to Apaf-1 (apoptotic protease-activating factor-1) and form the apoptosome. The apoptosome then activates caspase-9, which further cleaves and activates caspase-3. Caspase-3 then processes a variety of substrates such as PARP and ICAD (caspase-activated DNase) and leads to DNA fragmentation, cytoskeletal alterations, and cell death.

Apoptotic cell death has been suggested to play an important role in DA neuron loss. In postmortem brain tissue, a positive correlation was detected between the level of DA neuron loss and the percentage of caspase-3-positive neurons in the SN tissue (56). In addition, the percentage of active caspase-3-positive neurons among DA neurons was significantly higher in PD brains compared to controls (56). An increase in level of anti-apoptotic protein bcl-2 has also been reported in SN but not in the cerebral cortex of PD patients (95), although some other studies have shown conflicting results (162). Furthermore, a significantly higher percentage of activated caspase-8 positive DA neurons was detected in SN of PD patients (57). Surprisingly, inhibition of the caspase-8 pathway did not show neuroprotective effects against neurotoxin MPTP in primary DA neuron cultures, but seemed to trigger a switch from apoptotic cell death to necrosis (57). This suggested that necrosis might also play a role in DA neuron loss in PD.

In PD mouse models induced by chronic regimens of MPTP, inhibition of mitochondrial complex I led to transcriptional induction and translocation of a pro-apoptotic protein Bax, which in turn can cause cytochrome c release and activation of mitochondrial-dependent cell death pathways (116, 117). Caspase-3 activation and DNA fragmentation has also been reported in those models (151). Ablation of Bax or inhibition of Apaf-1 or the downstream substrate of caspase-3, poly (ADP-ribose) polymerase (PARP), can protect against MPTP-induced neurotoxicity (28, 94, 160). In cultured cells, MPP+ induces cell death via increased Bax expression, release of cytochrome c and caspase-9/-3 activation (65, 80). More recently, it has been suggested that deficiency of a PD-associated gene (PINK1) can lead to cell death via the mitochondrial mediated apoptosis (Bax translocation to mitochondria, activation of caspase-9/-3 and PARP) in long-term culture of human DAergic neurons (170). All these findings suggest that although necrosis might also play a role in neurodegeneration in PD, the mitochondrial-mediated apoptosis pathway is the key player of neuronal loss in PD. Of course, other cell death pathways may also contribute to the neuronal loss in PD. For example, acute high doses of MPTP can lead to nonapoptotic cell death (62, 73, 100).

Therapeutic Opportunities

It is hypothesized that drugs which can improve mitochondrial function might slow the progression of PD. Co-enzyme Q10 was one of the first to be investigated. Co-enzyme Q10 is a component of the respiratory chain that shuttles electrons between complexes I/II and III and it also functions as an antioxidant (35). In aged mice, administration of co-enzyme Q10 attenuates the reduction of striatal dopamine concentration and dopaminergic axons induced by MPTP (9). A pilot study in PD patients showed that oral administration of co-enzyme Q10 produced a trend towards increased complex I activity (134). A later study from Shults and colleagues showed that co-enzyme Q10 supplementation could slow the motor functional decline in patients with early PD (135). In 2007, a randomized, double blind, futility clinical trial run by the NINDS NET-PD Investigators could not reject co-enzyme Q10 as futile and suggested that this compound is worth future studies (101).

Creatine, another potential treatment, is a substrate for cytosolic and mitochondrial creatine kinases. It can be converted to phosphocreatine, which will then transfer its phosphoryl group to ADP to synthesise ATP and produce energy (4). Creatine showed neuroprotective effects against MPP^+ in primary neuronal cultures (3). In MPTP mouse models, oral supplementation with creatine reduced dopaminergic neuron loss (90). In a double-blind placebo-controlled study, oral creatine supplementation failed to show a significant treatment effect on UPDRS scores (11). However, in patients with creatine treatment there was a lower requirement for dopamine therapy, which suggested a potential neuroprotective effect of creatine. Another randomized, double blind, futility clinical trial in early PD patients also could not reject creatine as futile (102).

Despite these relatively disappointing results, the recent strengthening evidence of the role of mitochondria in PD does motivate the search for more molecules that can address the deficiencies in the pathways.

Conclusions

The important question for understanding the cell death pathways of PD and for finding potential therapies is to determine the common pathogenesis pathway of not only Mendelian genetic forms of PD but also sporadic PD. Mitochondrial dysfunction may be the key player in the PD pathogenesis pathway. Mitochondrial dysfunction has been detected in both sporadic PD patients and patients with familial forms of PD. Abnormal mitochondrial function has also been reported in various transgenic animal models with either overexpression or knockout of PD associated genes. Moreover, mitochondrial toxin-induced animal models have displayed some typical PD symptoms, including loss of DA neurons, aggresome formation, and movement problems. However, many questions still need to be addressed in this field: how does mitochondria dysfunction occur in PD; is it the primary event or a secondary consequence; is restoring the mitochondria function sufficient to rescue the neuronal cell death in PD? Further investigation into the mechanism of mitochondria dysfunction in PD may provide answers to some of these questions.

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References

1. Abramov AY, Canevari L, and Duchen MR. Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. *J Neurosci* 23: 5088–5095, 2003.
2. Abramov AY, Canevari L, and Duchen MR. Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J Neurosci* 24: 565–575, 2004.
3. Andres RH, Ducray AD, Perez–Bouza A, Schlattner U, Huber AW, Krebs SH, Seiler RW, Wallimann T, and Widmer HR. Creatine supplementation improves dopaminergic cell survival and protects against MPP+ toxicity in an organotypic tissue culture system. *Cell Transplant* 14: 537–550, 2005.
4. Andres RH, Ducray AD, Schlattner U, Wallimann T, and Widmer HR. Functions and effects of creatine in the central nervous system. *Brain Res Bull* 76: 329–343, 2008.
5. Baloh RH, Salavaggione E, Milbrandt J, and Pestronk A. Familial parkinsonism and ophthalmoplegia from a mutation in the mitochondrial DNA helicase twinkle. *Arch Neurol* 64: 998–1000, 2007.
6. Baloh RH, Schmidt RE, Pestronk A, and Milbrandt J. Altered axonal mitochondrial transport in the pathogenesis of Charcot–Marie–Tooth disease from mitofusin 2 mutations. *J Neurosci* 27: 422–430, 2007.
7. Bandmann O, Sweeney MG, Daniel SE, Marsden CD, and Wood NW. Mitochondrial DNA polymorphisms in pathologically proven Parkinson's disease. *J Neurol* 244: 262–265, 1997.
8. Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, Pittman AM, Lashley T, Canet–Aviles R, Miller DW, MCLendon C, Strand C, Leonard AJ, bou–Sleiman PM, Healy DG, Ariga H, Wood NW, de SR, Revesz T, Hardy JA, and Lees AJ. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* 127: 420–430, 2004.
9. Beal MF, Matthews RT, Tieleman A, and Shults CW. Coenzyme Q10 attenuates the 1-methyl-4-phenyl-1,2,3,tetrahydropyridine (MPTP) induced loss of striatal dopamine and dopaminergic axons in aged mice. *Brain Res* 783: 109–114, 1998.
10. Beilina A, Van Der BM, Ahmad R, Kesavapany S, Miller DW, Petsko GA, and Cookson MR. Mutations in PTEN-induced putative kinase 1 associated with recessive parkinsonism have differential effects on protein stability. *Proc Natl Acad Sci USA* 102: 5703–5708, 2005.
11. Bender A, Koch W, Elstner M, Schombacher Y, Bender J, Moeschl M, Gekeler F, Muller–Myhsok B, Gasser T, Tatsch K, and Klopstock T. Creatine supplementation in Parkinson disease: A placebo-controlled randomized pilot trial. *Neurology* 67: 1262–1264, 2006.
12. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betty J, Klopstock T, Taylor RW, and Turnbull DM. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* 38: 515–517, 2006.
13. Betarbet R, Canet–Aviles RM, Sherer TB, Mastroberardino PG, McLendon C, Kim JH, Lund S, Na HM, Taylor G, Bence NF, Kopito R, Seo BB, Yagi T, Yagi A, Klinefelter G, Cookson MR, and Greenamyre JT. Intersecting pathways to neurodegeneration in Parkinson's disease: Effects of the pesticide rotenone on DJ-1, alpha-synuclein, and the ubiquitin-proteasome system. *Neurobiol Dis* 22: 404–420, 2006.
14. Betarbet R, Sherer TB, MacKenzie G, Garcia–Osuna M, Panov AV, and Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3: 1301–1306, 2000.
15. Bezprozvanny I and Hayden MR. Deranged neuronal calcium signaling and Huntington disease. *Biochem Biophys Res Commun* 322: 1310–1317, 2004.
16. Bindoff LA, Birch–Machin M, Cartlidge NE, Parker WD, Jr., and Turnbull DM. Mitochondrial function in Parkinson's disease. *Lancet* 2: 49, 1989.
17. Bindoff LA, Birch–Machin MA, Cartlidge NE, Parker WD, Jr., and Turnbull DM. Respiratory chain abnormalities in skeletal muscle from patients with Parkinson's disease. *J Neurol Sci* 104: 203–208, 1991.
18. Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G, and Heutink P. DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurol Sci* 24: 159–160, 2003.
19. Buttner S, Bitto A, Ring J, Augsten M, Zabrocki P, Eisenberg T, Jungwirth H, Hutter S, Carmona–Gutierrez D, Kroemer G, Windericks J, and Madeo F. Functional mitochondria are required for alpha-synuclein toxicity in aging yeast. *J Biol Chem* 283: 7554–7560, 2008.
20. Canet–Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, and Cookson MR. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci USA* 101: 9103–9108, 2004.
21. Casarejos MJ, Menendez J, Solano RM, Rodriguez–Navarro JA, Garcia de YJ, and Mena MA. Susceptibility to rotenone is increased in neurons from parkin null mice and is reduced by minocycline. *J Neurochem* 97: 934–946, 2006.

22. Cassarino DS, Parks JK, Parker WD, Jr., and Bennett JP, Jr. The parkinsonian neurotoxin MPP+ opens the mitochondrial permeability transition pore and releases cytochrome c in isolated mitochondria via an oxidative mechanism. *Biochim Biophys Acta* 1453: 49–62, 1999.
23. Chan DC. Mitochondria: Dynamic organelles in disease, aging, and development. *Cell* 125: 1241–1252, 2006.
24. Chen CM, Kuan CC, Lee–Chen GJ, and Wu YR. Mitochondrial DNA polymorphisms and the risk of Parkinson's disease in Taiwan. *J Neural Transm* 114: 1017–1021, 2007.
25. Ciechanover A and Schwartz AL. The ubiquitin-protea some pathway: The complexity and myriad functions of proteins death. *Proc Natl Acad Sci USA* 95: 2727–2730, 1998.
26. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Soel JH, Yoo SJ, Hay BA, and Guo M. *Drosophila* pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 441: 1162–1166, 2006.
27. Cleren C, Starkov AA, Calingasan NY, Lorenzo BJ, Chen J, and Beal MF. Promethazine protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Neurobiol Dis* 20: 701–708, 2005.
28. Cosi C, Colpaert F, Koek W, Degryse A, and Marien M. Poly(ADP-ribose) polymerase inhibitors protect against MPTP-induced depletions of striatal dopamine and cortical noradrenaline in C57B1/6 mice. *Brain Res* 729: 264–269, 1996.
29. Darios F, Corti O, Lucking CB, Hampe C, Muriel MP, Abbas N, Gu WJ, Hirsch EC, Rooney T, Ruberg M, and Brice A. Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum Mol Genet* 12: 517–526, 2003.
30. Dauer W, Kholodilov N, Vila M, Trillat AC, Goodchild R, Larsen KE, Staal R, TIeu K, Schmitz Y, Yuan CA, Rocha M, Jackson–Lewis V, Hersch S, Sulzer D, Przedborski S, Burke R, and Hen R. Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. *Proc Natl Acad Sci USA* 99: 14524–14529, 2002.
31. de Lau LM and Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol* 5: 525–535, 2006.
32. Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc–Carel C, Perret E, Starie–Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, and Hamel CP. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26: 207–210, 2000.
33. Deng H, Dodson MW, Huang H, and Guo M. The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in *Drosophila. Proc Natl Acad Sci USA* 105: 14503–14508, 2008.
34. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, and Anandatheerthavarada HK. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* 283: 9089–9100, 2008.
35. Ernster L and Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1271: 195–204, 1995.
36. Exner N, Treske B, Paquet D, Holmstrom K, Schiesling C, Gispert S, Carballo–Carbajal I, Berg D, Hoepken HH, Gasser R, Kruger R, Winklehofer KF, Vogel F, Reichert AS, Augurger G, Kahle PH, Schmid B, and Haass C. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J Neurosci* 27: 12413–12418, 2007.
37. Fernagut PO, Hutson CB, Fleming SM, Tetreaut NA, Salcedo J, Masliah F, and Chesselet MF. Behavioral and histopathological consequences of paraquat intoxication in mice: Effects of alpha-synuclein over-expression. *Synapse* 61: 991–1001, 2007.
38. Fiskum G. Mitochondrial participation in ischemic and traumatic neural cell death. *J Neurotrauma* 17: 843–855, 2000.
39. Forno LS, Langston JW, DeLanney LE, and Irwin I. An electron microscopic study of MPTP-induced inclusion bodies in an old monkey. *Brain Res* 448: 150–157, 1988.
40. Furukawa K, Matsuzaki–Kobayashi M, Hasegawa T, Kikuchi A, Sugeno N, Itoyama Y, Wang Y, Yao PJ, Bushlin I, and Takeda A. Plasma membrane ion permeability induced by mutant alpha-synuclein contributes to the degeneration of neural cells. *J Neurochem* 97: 1071–1077, 2006.
41. Gandhi S, Muqit MM, Stanyer L, Healy DG, bou-Sleiman PM, Hargreaves J, Heales S, Ganguly M, Parsons L, Lees AJ, Latchman DS, Holton JL, Wood NW, and Revesz T. PINK1 protein in normal human brain and Parkinson's disease. *Brain* 129: 1720–1731, 2006.
42. Gautier CA, Kitada T, and Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proc Natl Acad Sci USA* 105: 11364–11369, 2008.
43. Ghezzi D, Marelli C, Achilli A, Goldwurm S, Pezzoli G, Barone P, Pellecchia MT, Stanzione P, Brusa I, Bentivoglio AR, Bonuccelli U, Petrozzi I, Abbruzzese G, Marchese R, Cortelli P, Grimaldi D, Martinelli P, Ferrarese C, Garvaglia B, Sangiogi S, Carelli V, Torroni A, Albanese A, and Zeviani M. Mitochondrial DNA haplogroup K is associated with a lower risk of Parkinson's disease in Italians. *Eur J Hum Genet* 13: 748–752, 2005.
44. Giasson BI, Covy JP, Bonini NM, Hurtig HI, Farrer MJ, Torjanowski JQ, and Van DV. Biochemical and pathological characterization of Lrrk2. *Ann Neurol* 59: 315–322, 2006.
45. Gilks WP, bou–Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, Shaw K, Bhatia KP, Bonifati V, Quinn NP, Lynch J, Healy DG, Holton JL, Revesz T, and Wood NW. A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* 365: 415–416, 2005.
46. Giordana MT, D'Agostino C, Albani G, Mauro A, Di FA, Antonini A, and Bonifati V. Neuropathology of Parkinson's disease associated with the LRRK2 Ile1371Val mutation. *Mov Disord* 22: 275–278, 2007.
47. Glater EE, Megeath LJ, Stowers RS, and Schwarz TL. Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. *J Cell Biol* 173: 545–557, 2006.
48. Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, Holch W, Prokisch H, and Ueffing M. The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum Mol Genet* 15: 223–232, 2006.
49. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson L, Klapstein GJ, Gajendiran M, Roth BL, Chesselet MF, Maidment NT, Levine MS, and Shen J. Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* 278: 43628–43635, 2003.
50. Goldberg MS, Pisani A, Haburcak M, Vortherms TA, Kitada T, Costa C, Tong Y, Martella G, Tscherter A, Martins

A, Bernardi G, Roth BL, Pothos EN, Calabresi P, and Shen J. Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. *Neuron* 45: 489–496, 2005.
51. Greene JC, Whitworth AJ, Andrews LA, Parker TJ, and Pallanck LJ. Genetic and genomic studies of *Drosophila* parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. *Hum Mol Genet* 14: 799–811, 2005.
52. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, van der Brug MP, Beilina A, Blackinton J, Thomas KJ, Ahmad R, Miller DW, Kesavapany S, Singleton A, Lees A, Harvey RJ, Harvey K, and Cookson MR. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis* 23: 329–341, 2006.
53. Gu M, Cooper JM, Taanman JW, and Schapira AH. Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Ann Neurol* 44: 177–186, 1998.
54. Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood NL, and Zinsmaier KE. The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron* 47: 379–393, 2005.
55. Haque ME, Thomas KJ, D'Souza C, Callaghan S, Kitada T, Slack RS, Fraser P, Cookson MR, Tandon A, and Park DS. Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. Proc Natl Acad Sci USA 105: 1716–1721, 2008.
56. Hartmann A, Hunot S, Michel PP, Muriel MP, Vyas S, Faucheux BA, Mouatt–Prigent A, Turmel H, Srinivasan A, Ruberg M, Evan GI, Agid Y, and Hirsch EC. Caspase-3: A vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc Natl Acad Sci USA* 97: 2875–2880, 2000.
57. Hartmann A, Troadec JD, Hunot S, Kikly K, Faucheux BA, Mouatt–Prigent A, Ruberg M, Agid Y, and Hirsch EC. Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *J Neurosci* 21: 2247–2255, 2001.
58. Hoglinger GU, Carrard G, Michel PP, Medja F, Lombes A, Ruberg M, Friguet B, and Hirsch EC. Dysfunction of mitochondrial complex I and the proteasome: interactions between two biochemical deficits in a cellular model of Parkinson's disease. *J Neurochem* 86: 1297–1307, 2003.
59. Huerta C, Castro MG, Coto E, Blazquez M, Ribacoba R, Guisasola LM, Salvador C, Martinez C, Lahoz CH, and Alvarez V. Mitochondrial DNA polymorphisms and risk of Parkinson's disease in Spanish population. *J Neurol Sci* 236: 4954, 2005.
60. Ikebe S, Tanaka M, Ohno K, Sato W, and Hattori K. Increase of deleted mitochondrial DNA in the striatum in Parkinson's disease and senescence. *Biochem Biophys Res Commun* 170: 1044–1048, 1990.
61. Itier JM, Ibanez P, Mena MA, Abbas N, Cohen–Salmon C, Bohme GA, Laville M, Pratt J, Corti O, Pradier L, Ret G, Joubert C, Periquet M, Araujo F, Negroni J, Casarejos ML, Canals S, Solano R, Serrano A, Gallego E, Sanchez M, Denefle P, Benavides J, Tremp G, Rooney TA, Brice A, and Garcia de YJ. Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum Mol Genet* 12: 2277–2291, 2003.
62. Jackson–Lewis V, Jakowec M, Burke RE, and Przedborski S. Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration* 4: 257–269, 1995.
63. Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, and Alessi DR. LRRK2 phosphorylates moesin at threonine-558: Characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J* 405: 307–317, 2007.
64. Junn E, Taniguchi H, Jeong BS, Zhao X, Ichijo H, and Mouradian MM. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. *Proc Natl Acad Sci USA* 102: 9691–9696, 2005.
65. Kaul S, Kanthasamy A, Kitazawa M, Anantharam V, and Kanthasamy AG. Caspase-3 dependent proteolytic activation of protein kinase C delta mediates and regulates 1-methyl-4-phenylpyridinium (MPP+)-induced apoptotic cell death in dopaminergic cells: Relevance to oxidative stress in dopaminergic degeneration. *Eur J Neurosci* 18: 1387–1401, 2003.
66. Keeney PM, Xie J, Capaldi RA, and Bennett JP, Jr. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. *J Neurosci* 26: 5256–5264, 2006.
67. Khan NL, Brooke DJ, Pavase N, Sweeney NG, Wood NW, Lees AJ, and Pircini P. Progression of nigrostriatal dysfunction in a parkin kindred: an [18F] dopa PET and clinical study. Brain 125: 2248–2256, 2002.
68. Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, Wakeham. A, You–Ten AH, Kalia SK, Horne P, Westaway D, Lozano AM, Anisman H, Park DS, and Mak TW. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. *Proc Natl Acad Sci USA* 102: 5215–5220, 2005.
69. Kim Y, Park J, Kim S, Song S, Kwon SK, Lee SH, Kitada T, Kim JM, and Chung J. PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. *Biochem Biophys Res Commun* 377: 975–980, 2008.
70. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Tokochi M, Mizuno Y, and Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605–608, 1998.
71. Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonzi P, Zhang C, Pothos EN, and Shen J. Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. *Proc Natl Acad Sci USA* 104: 11441–11446, 2007.
72. Klivenyi P, Siwek D, Gardian G, Yang L, Starkov A, Cleren C, Ferrante RJ, Kowall NW, Abeliovich A, and Beal MF. Mice lacking alpha-synuclein are resistant to mitochondrial toxins. *Neurobiol Dis* 21: 541–548, 2006.
73. Kopin IJ. MPTP: An industrial chemical and contaminant of illicit narcotics stimulates a new era in research on Parkinson's disease. *Environ Health Perspect* 75: 45–51, 1987.
74. Kowall NW, Hantraye P, Brouillet E, Beal MF, McKee AC, and Ferrante RJ. MPTP induces alpha-synuclein aggregation in the substantia nigra of baboons. *Neuroreport* 11: 211–1213, 2000.
75. Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, and Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet* 38: 518–520, 2006.
76. Kreutzberg GW. Microglia: A sensor for pathological events in the CNS. *Trends Neurosci* 19: 312–318, 1996.

77. Krige D, Carroll MT, Cooper JM, Marsden CD, and Schapira AH. Platelet mitochondrial function in Parkinson's disease. The Royal Kings and Queens Parkinson Disease Research Group. *Ann Neurol* 32: 782–788, 1992.
78. Kuroda Y, Mitsui T, Kunishige M, Shono M, Akaike M, Azuma H, and Matsumoto T. Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum Mol Genet* 15: 883–895, 2006.
79. Langston JW, Ballard P, Tetrud JW, and Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219: 979–980, 1983.
80. Lee dY, Lee KS, Lee HJ, Noh YH, Kim DH, Lee JY, Cho SH, TOon OJ, Lee WB, Kim KY, Chung YH, and Kim 22. Kynurenic acid attenuates MPP(+)-induced dopaminergic neuronal cell death via a Bax-mediated mitochondrial pathway. *Eur J Cell Biol* 87: 389–397, 2008.
81. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey F, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehujia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, and Polymeropoulos MH. The ubiquitin pathway in Parkinson's disease. *Nature* 395: 451–452, 1998.
82. Lotharius J, and Brundin P. Pathogenesis of Parkinson's disease: Dopamine, vesicles and alpha-synuclein. *Nat Rev Neurosci* 3: 932–942, 2002.
83. Luzon–Toro B, Rubio de la TE, Delgado A, Perez–Tur J, and Hilfiker S. Mechanistic insight into the dominant mode of the Parkinson's disease-associated G2019S LRRK2 mutation. *Hum Mol Genet* 16: 2031–2039, 2007.
84. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, and Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron* 52: 587–593, 2006.
85. Mann VM, Cooper JM, Krige D, Daniel SE, Schapira AH, and Marsden CD. Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. *Brain* 115: 333–342, 1992.
86. Manning–Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, and Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: Paraquat and alpha-synuclein. *J Biol Chem* 277: 1641–1644, 2002.
87. Manning–Bog AB, Reaney SH, Chou VP, Johnston LC, McCormack AL, Johnston J, Langston JW, and Di Monte DA. Lack of nigrostriatal pathology in a rat model of proteasome inhibition. *Ann Neurol* 60: 256–260, 2006.
88. Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, Price DL, and Lee MK. Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J Neurosci* 26: 41–50, 2006.
89. Martins LM, Morrison A, Klupsch K, Fedele V, Moisoi N, Teismann P, Abiun A, Grau E, Geppert M, Livi GP, Creasy CL, Martin A, Hargreaves I, Heales SJ, Okada H, Brandner S, Schulz JB, Mak T, and Downward J. Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* 24: 9848–9862, 2004.
90. Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah–Daouk R, and Beal MF. Creatine and cyclocreatine attenuate MPTP neurotoxicity. *Exp Neurol* 157: 142–149, 1999.
91. McNaught KS, Belizaire R, Isacson O, Jenner P, and Olanow CW. Altered proteasomal function in sporadic Parkinson's disease. *Exp Neurol* 179: 38–46, 2003.
92. McNaught KS, Perl DP, Brownell AL, and Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann Neurol* 56: 149–162, 2004.
93. Mitsumoto A and Nakagawa Y. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radic Res* 35: 885–893, 2001.
94. Mochizuki H, Hayakawa H, Migita M, Shibata M, Tanaka R, Suzuki A, Shimo–Nakanishi Y, Urabe T, Tamada M, Tamayose K, Shimada T, Miura M, and Mizuno Y. An AAV-derived Apaf-1 dominant negative inhibitor prevents MPTP toxicity as antiapoptotic gene therapy for Parkinson's disease. *Proc Natl Acad Sci USA* 98: 10918–10923, 2001.
95. Mogi M, Harada M, Kondo T, Mizuno Y, Narabayashi H, Riederer P, and Nagatsu T. bcl-2 protein is increased in the brain from parkinsonian patients. *Neurosci Lett* 215: 137–139, 1996.
96. Moore DJ, Zhang L, Troncoso J, Lee MK, Hattori N. Mizumo Y, Dawson TM, and Dawson VL. Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. *Hum Mol Genet* 14: 71–84, 2005.
97. Muqit MM, bou–Sleiman PM, Saurin AT, Harvey K, Gandhi S, Deas E, Eaton S, Payne S, Venner K, Matilla A, Healy DG, Gilks WP, Lees AJ, Holton J, Revesz T, Parker PJ, Harvey RJ, Wood NW, and Latchman DS. Altered cleavage and localization of PINK1 to aggresomes in the presence of proteasomal stress. *J Neurochem* 98: 156–169, 2006.
98. Nagakubo D, Taira T, Kitaura H, Ikeda M, Tamai K, Iguchi–Ariga SM, and Ariga H. DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. *Biochem Biophys Res Commun* 231: 509–513, 1997.
99. Nicklas WJ, Vyas I, and Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci* 36: 2503–2508, 1985.
100. Nicotra A and Parvez SH. Cell death induced by MPTP, a substrate for monoamine oxidase B. *Toxicology* 153: 157–166, 2000.
101. NINDS NET-PD Investigators. A randomized clinical trial of coenzyme Q10 and GPI-1485 in early Parkinson disease. *Neurology* 68: 20–28, 2007.
102. NINDS NET-PD Investigators. A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. *Neurology* 66: 664–671, 2006.
103. Nishioka K, Hayashi S, Farrer MJ, Singleton AB, Yoshino H, Imai H, Kitami T< Sato K, Kuroda R, Tomiyama H, Mizoguchi K, Murata M, Toda T, Imoto I, Inazawa J, Mizumo Y, and Hattori N. Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. *Ann Neurol* 59: 298–309, 2006.
104. Olichon A, Emorine LJ, Descoins E, Pelloquin L, Brichese L, Gas N, Guillou E, Delettre C, Valette A, Hamel CP, Ducommun B, Lenaers G, and Belenguer P. The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett* 523: 171–176, 2002.
105. Orth M, Tabrizi SJ, Schapira AH, and Cooper JM. Alpha-synuclein expression in HEK293 cells enhances the mitochondrial sensitivity to rotenone. *Neurosci Lett* 351: 29–32, 2003.
106. Packer MA, Miesel R, and Murphy MP. Exposure to the parkinsonian neurotoxin 1-methyl-4-phenylpyridinium (MPP+) and nitric oxide simultaneously causes cyclosporin

A-sensitive mitochondrial calcium efflux and depolarisation. *Biochem Pharmacol* 51: 267–273, 1996.
107. Paisan–Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munian A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de SR, Lees A, Marti–Masso JF, Perez–Tur J, Wood NW, and Singleton AB. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 44: 595–600, 2004.
108. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, and Shen J. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* 279: 18614–18622, 2004.
109. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strettmatter WJ, and Greenamyre JT. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 5: 731–736, 2002.
110. Parihar MS, Parihar A, Fujita M, Hashimoto M, and Ghafourifar P. Mitochondrial association of alpha-synuclein causes oxidative stress. *Cell Mol Life Sci* 65: 1272–1284, 2008.
111. Park J, Lee G and Chung J. The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process. *Biochem Biophys Res Commun* 378: 518–523, 2009.
112. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, and Chung J. Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441: 1157–1161, 2006.
113. Parker WD, Jr., Boyson SJ, and Parks JK. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol* 26: 719–723, 1989.
114. Parker WD, Jr., Parks JK, and Swerdlow RH. Complex I deficiency in Parkinson's disease frontal cortex. *Brain Res* 1189: 215–218, 2008.
115. Penn AM, Roberts T, Hodder J, Allen PS, Zhu G, and Martin WR. Generalized mitochondrial dysfunction in Parkinson's disease detected by magnetic resonance spectroscopy of muscle. *Neurology* 45: 2097–2099, 1995.
116. Perier C, Bove J, Wu DC, Dehay B, Choi DK, Jackson–Lewis V, Rathke–Hartlieb S, Bouillet P, Strasser A, Schulz JB, Przwedborski S, and Vila M. Two molecular pathways initiate mitochondria-dependent dopaminergic neurodegeneration in experimental Parkinson's disease. *Proc Natl Acad Sci USA* 104: 8161–8166, 2007.
117. Perier C, Tieu K, Guegan C, Caspersen C, Jackson–Lewis V, Carelli V, Martinuzzi A, Hirano M, Przedborski S, and Vila M. Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. *Proc Natl Acad Sci USA* 102: 19126–19131, 2005.
118. Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, Chen F, Gu Y, Hasegawa H, Salehi–Rad S, Wang L, Rogaeva E, Fraser P, Robinson B, St George–Hyslop P, and Tandon A. Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* 280: 34025–34032, 2005.
119. Plun–Favreau H, Klupsch K, Moisoi N, Gandhi S, Kjaer S, Frith D, Harvey K, Deas E, Harvey RJ, McDonald N, Wood NW, Martins M, and Downward J. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat Cell Biol* 9: 1243–1252, 2007.
120. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Bowyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lassarini AM, DUvoisin RC, Di JG, Golbe LI, and Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276: 2045–2047, 1997.
121. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, and Pallanck LJ. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA* 105: 1638–1643, 2008.
122. Pridgeon JW, Olzmann JA, Chin LS, and Li L. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol* 5: e172, 2007.
123. Przedborski S. Neuroinflammation and Parkinson's disease. *Handb Clin Neurol* 83: 535–551, 2007.
124. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, Al–Din A, Hillmer AM, Karsak M, Liss B, Woods CG, Behrens ML, and Kubisch C. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* 38: 1184–1191, 2006.
125. Rizzu P, Hinkle DA, Zhukareva V, Bonifati V, Severijnen LA, Martinez D, Ravid R, Kamphorst W, Eberwine JH, Lee VM, Trojanowski JQ, and Heutink P. DJ-1 colocalizes with tau inclusions: A link between parkinsonism and dementia. *Ann Neurol* 55: 113–118, 2004.
126. Ross OA, Soto AI, Vilarino–Guell C, Heckman MG, Diehl NN, Hulihan MM, Aasly JO, Sando S, Gibson JM, Lynch T, Krygowska–Wajs A, Opala G, Bareikowska M, Czyzewski K, Uitti RJ, Wszolek ZK, and Farrer MJ. Genetic variation of Omi/HtrA2 and Parkinson's disease. *Parkinsonism Relat Disord* 14: 539–543, 2008.
127. Sakata E, Yamaguchi Y, Kurimoto E, Kikuchi J, Yokoyama S, Yamada S, Kawahara H, Yokosawa H, Hattori N, Mizuno Y, Tanaka K, and Kato K. Parkin binds the Rpn10 subunit of 26S proteasomes through its ubiquitin-like domain. *EMBO Rep* 4: 301–306, 2003.
128. Schapira AH. Oxidative stress in Parkinson's disease. *Neuropathol Appl Neurobiol* 21: 3–9, 1995.
129. Schapira AH. Etiology of Parkinson's disease. *Neurology* 66: S10–S23, 2006.
130. Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, and Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1: 1269, 1989.
131. Schapira AH, Mann VM, Cooper JM, Dexter D, Daniel SE, Jenner P, Clark JB, and Marsden CD. Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *J Neurochem* 55: 2142–2145, 1990.
132. Sherer TB, Kim JH, Betarbet R, and Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol* 179: 9–16, 2003.
133. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, and Suzuki T. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 25: 302–305, 2000.
134. Shults CW, Beal MF, Fontaine D, Nakano K, and Haas RH. Absorption, tolerability, and effects on mitochondrial activity of oral coenzyme Q10 in parkinsonian patients. *Neurology* 50: 793–795, 1998.
135. Shults CW, Oakes D, Kieburtz K, Beal MF, Haas R, Plumb S, Juncos JL, Nutt J, Shoulson I, Carter J, Kompoliti K, Permutter JS, Reich S, Stern M, Watts RL, Kurlan R, Molho E, Harrison M, and Lew M. Effects of coenzyme Q10 in early Parkinson disease: Evidence of slowing of the functional decline. *Arch Neurol* 59: 1541–1550, 2002.

136. Silvestri L, Caputo V, Bellacchio E, Atorino L, Dallapiccola B, Valente EM, and Casari G. Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum Mol Genet* 14: 3477–3492, 2005.
137. Sim CH, Lio DS, Mok SS, Masters CL, Hill AF, Culvenor JG, and Cheng HC. C-terminal truncation and Parkinson's disease-associated mutations down-regulate the protein serine/threonine kinase activity of PTEN-induced kinase-1. *Hum Mol Genet* 15: 3251–3262, 2006.
138. Simon–Sanchez J, and Singleton AB. Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls. *Hum Mol Genet* 17: 1988–1993, 2008.
139. Singer TP, Salach JI, Castagnoli N, Jr., and Trevor A. Interactions of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidases. *Biochem J* 235: 785–789, 1986.
140. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, and Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci* 9: 1231–1233, 2006.
141. Smith WW, Pei Z, Jiang H, Moore DJ, Liang Y, West AB, Dawson VL, Dawson TM, and Ross CA. Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci USA* 102: 18676–18681, 2005.
142. Song DD, Shults CW, Sisk A, Rockenstein E, and Masliah E. Enhanced substantia nigra mitochondrial pathology in human alpha-synuclein transgenic mice after treatment with MPTP. *Exp Neurol* 186: 158–172, 2004.
143. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, and Goedert M. Alpha-synuclein in Lewy bodies. *Nature* 388: 839–840, 1997.
144. Strauss KM, Martins LM, Plun–Favreau H, Marx FP, Kautzmann S, Berg D, Gasser T, Wszolek Z, Muller T, Bornemann A, Wolburg H, Downward J, Riess O, Schulz JB, and Kruger R. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum Mol Genet* 14: 2099–2111, 2005.
145. Sullivan PG, Dragicevic NB, Deng JH, Bai Y, Dimayuga E, DIng Q, Chen Q, Bruce–Keller AJ, and Keller JN. Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. *J Biol Chem* 279: 20699–20707, 2004.
146. Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, and Takahashi R. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol Cell* 8: 613–621, 2001.
147. Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, Bennett JP Jr, Davis RE, and Parker WD Jr. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol* 40: 663–671, 1996.
148. Taira T, Saito Y, Niki T, Iguchi–Ariga SM, Takahashi K, and Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep* 5: 213–218, 2004.
149. Tan JM and Dawson TM. Parkin blushed by PINK1. *Neuron* 50: 527–529, 2006.
150. Tang B, Xiong H, Sun P, Zhang Y, Wang D, Hu Z, Zhu Z, Ma H, Pan Q, Xia JH, Xia K, and Zhang Z. Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. *Hum Mol Genet* 15: 1816–1825, 2006.
151. Tatton NA and Kish SJ. In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience* 77: 1037–1048, 1997.
152. Taylor DJ, Krige D, Barnes PR, Kemp GJ, Carroll MT, Mann VM, Cooper JM, Marsden CD, and Schapira AH. A 31P magnetic resonance spectroscopy study of mitochondrial function in skeletal muscle of patients with Parkinson's disease. *J Neurol Sci* 125: 77–81, 1994.
153. Taymans JM, Van den HC, and Baekelandt V. Distribution of PINK1 and LRRK2 in rat and mouse brain. *J Neurochem* 98: 951–961, 2006.
154. Testa CM, Sherer TB, and Greenamyre JT. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. *Brain Res Mol Brain Res* 134: 109–118, 2005.
155. Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, and Cory–Slechta DA. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: Implications for Parkinson's disease. J Neurosci 20: 9207–9214, 2000.
156. Unoki M and Nakamura Y. Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. *Oncogene* 20: 4457–4465, 2001.
157. Valente EM, bou–Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del TD, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez–Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Augurger G, and Wood NW. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304: 1158–1160, 2004.
158. Van Den BL, Van DP, Bogaert E, and Robberecht W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim Biophys Acta* 1762: 1068–1082, 2006.
159. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Haines JL, Koller WC, Lyons K, Pahwa R, Stern MB, Colcher A, Hiner BC, Jankovic J, Ondo WG, Allen FH Jr, Goetz CG, Small GW, Mastaglia F, Stajuch JM, McLaurin AC, Middleton JT, Scott BL, Schmechel DE, Pericak–Vance MA, and Vance JM. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am J Hum Genet* 72: 804–811, 2003.
160. Vila M, Jackson–Lewis V, Vukosavic S, Djaldetti R, Liberatore G, Offen D, Korsmeyer SJ, and Przedborski S. Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proc Natl Acad Sci USA* 98: 2837–2842, 2001.
161. Voges D, Zwickl P, and Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 68: 1015–1068, 1999.
162. Vyas S, Javoy-Agid F, Herrero MT, Strada O, Boissiere F, Hibner U, and Agid Y. Expression of Bcl-2 in adult human brain regions with special reference to neurodegenerative disorders. *J Neurochem* 69: 223–231, 1997.
163. Wang C, Lu R, Ouyang X, Ho MW, Chia W, Yu F, and Lim KL. *Drosophila* overexpressing parkin R275W mutant exhibits dopaminergic neuron degeneration and mitochondrial abnormalities. *J Neurosci* 27: 8563–8570, 2007.
164. Wang D, Qian L, Xiong H, Liu J, Neckameyer WS, Oldham S, Zia K, wang J, Bodmer R, and Zhang Z. Antioxidants protect PINK1-dependent dopaminergic neurons in *Drosophila*. *Proc Natl Acad Sci USA* 103: 13520–13525, 2006.
165. Wang HL, Chou AH, Yeh TH, Li AH, Chen YL, Kuo YL, Tsai SR, and Yu ST. PINK1 mutants associated with recessive Parkinson's disease are defective in inhibiting mitochondrial release of cytochrome c. *Neurobiol Dis* 28: 216–226, 2007.

166. Weihofen A, Ostaszewski B, Minami Y, and Selkoe DJ. Pink1 Parkinson mutations, the Cdc37/Hsp90 chaperones and Parkin all influence the maturation or subcellular distribution of Pink1. *Hum Mol Genet* 17: 602–616, 2008.
167. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, Dawson VL, and Dawson TM. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci USA 102: 16842–16847, 2005.
168. Whitworth AJ, Lee JR, Ho VM, Flick R, Chowdhury R, and McQuibban GA. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis Model Mech* 1: 168–174, 2008.
169. Wood–Kaczmar A, Gandhi S, and Wood NW. Understanding the molecular causes of Parkinson's disease. *Trends Mol Med* 12: 521–528, 2006.
170. Wood–Kaczmar A, Gandhi S, Yao Z, Abramov AS, Miljan EA, Keen G, Stanyer L, Hargreaves I, Klupsch K, Deas F, Downward J, Mansfield I, Jat P, Taylor J, Heales S, Duchen MR, Latchman D, Tabrizi SH, and Wood NW. PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS ONE* 3: e2455, 2008.
171. Wyss–Coray T and Mucke L. Inflammation in neurodegenerative disease—A double-edged sword. *Neuron* 35: 419–432, 2002.
172. Yamamoto A, Friedlein A, Imai Y, Takahashi R, Kahle PJ, and Haass C. Parkin phosphorylation and modulation of its E3 ubiquitin ligase activity. *J Biol Chem* 280: 3390–3399, 2005.
173. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang I, Beal MF, Voel H, and Lu B. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. Proc Natl Acad Sci USA 103: 10793–10798, 2006.
174. Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel J, and Lu B. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci USA* 105: 7070–7075, 2008.
175. Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, and Mizusawa H. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. *Biochem Biophys Res Commun* 312: 1342–1348, 2003.
176. Zeng BY, Bukhatwa S, Hikima A, Rose S, and Jenner P. Reproducible nigral cell loss after systemic proteasomal inhibitor administration to rats. *Ann Neurol* 60: 248–252, 2006.
177. Zhang L, Shimoji M, Thomas B, Moore DJ, Yu SW, Marupudi NI, Torp R, Torgner IA, Ottersen OP, Dawson TM, and Dawson VL. Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. *Hum Mol Genet* 14: 2063–2073, 2005.
178. Zhou H, Falkenburger BH, Schulz JB, Tieu K, Xu Z, and Xia XG. Silencing of the Pink1 gene expression by conditional RNAi does not induce dopaminergic neuron death in mice. *Int J Biol Sci* 3: 242–250, 2007.
179. Zimprich A, Muller–Myhsok B, Farrer M, Leitner P, Sharma M, Hulihan M, Lockhart P, Strongosky A, Kacergus J, Calne DB, Stoessl J, Uitti RJ, Pfeiffer RF, Trenwakder C, Homann N, Ott E, Wenzel K, Asmus F, Hardy J, Wszolek Z, and Gasser T. The PARK8 locus in autosomal dominant parkinsonism: Confirmation of linkage and further delineation of the disease-containing interval. *Am J Hum Genet* 74: 11–19, 2004.
180. Zuchner S, Mersiyanova IV, Muglia M, Bissar–Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evfrafoc O, Jonghe PD, Takahasi Y, Tsuji S, Pericak–Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timemrman V, Schroder JM, and Vance JM. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot–Marie–Tooth neuropathy type 2A. *Nat Genet* 36: 449–451, 2004.

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Abbreviations Used

AIF = apoptosis-inducing factor
Apaf-1 = apoptotic protease-activating factor-1
DAergic = dopaminergic
LRRK2 = leucine-rich repeat kinase 2
MAQ = monoamine oxidase MFN2, human mitofusion-2
MPP^+ = 1-methly-4-phenylpyridinium ion mtDNA, mitochondrial DNA
MPTP = 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine
OPA1 = optic atrophy 1
PARP = poly (ADP-ribose) polymerase
PD = Parkinson's disease
PINK1 = PTEN-induced kinase 1
PTP = permeability transition pore
ROS = reactive oxygen species
SNpc = substantia nigra pars compacta
UPS = Ubiquitin Proteasome System
VMAT2 = vesicular monoamine transporter 2

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2. Humberto Rodriguez-Rocha , Aracely Garcia Garcia , Laura Zavala-Flores , Sumin Li , Nandakumar Madayiputhiya , Rodrigo Franco . Glutaredoxin 1 Protects Dopaminergic Cells by Increased Protein Glutathionylation in Experimental Parkinson's Disease. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
3. Min Kong, Maowen Ba, Hui Liang, Lili Ma, Quntao Yu, Tianxia Yu, Ying Wang. 2012. 5#-Aza-dC sensitizes paraquat toxic effects on PC12 cell. *Neuroscience Letters* **524**:1, 35-39. [CrossRef]
4. Laura González-Lafuente, Javier Egea, Rafael León, Francisco J. Martínez-Sanz, Leticia Monjas, Concepción Perez, Cristina Merino, Antonio M. García-De Diego, María I. Rodríguez-Franco, Antonio G. García, Mercedes Villarroya, Manuela G. López, Cristóbal de los Ríos. 2012. Benzothiazepine CGP37157 and Its Isosteric 2#-Methyl Analogue Provide Neuroprotection and Block Cell Calcium Entry. *ACS Chemical Neuroscience* **3**:7, 519-529. [CrossRef]
5. Aracely Garcia-Garcia , Laura Zavala-Flores , Humberto Rodriguez-Rocha , Rodrigo Franco . Thiol-Redox Signaling, Dopaminergic Cell Death, and Parkinson's Disease. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Terina N. Martinez , J. Timothy Greenamyre . 2012. Toxin Models of Mitochondrial Dysfunction in Parkinson's Disease. *Antioxidants & Redox Signaling* **16**:9, 920-934. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Alzbeta Trancikova , Elpida Tsika , Darren J. Moore . 2012. Mitochondrial Dysfunction in Genetic Animal Models of Parkinson's Disease. *Antioxidants & Redox Signaling* **16**:9, 896-919. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Martin Stöckl, Mireille M. A. E. Claessens, Vinod Subramaniam. 2012. Kinetic measurements give new insights into lipid membrane permeabilization by #-synuclein oligomers. *Molecular BioSystems* . [CrossRef]
9. Franziska Kriegenburg , Esben G. Poulsen , Annett Koch , Elke Krüger , Rasmus Hartmann-Petersen . 2011. Redox Control of the Ubiquitin-Proteasome System: From Molecular Mechanisms to Functional Significance. *Antioxidants & Redox Signaling* **15**:8, 2265-2299. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Maria Xilouri, Leonidas Stefanis. 2011. Autophagic pathways in Parkinson disease and related disorders. *Expert Reviews in Molecular Medicine* **13**. . [CrossRef]
11. Donald M. Kuhn, Catherine E. Sykes, Timothy J. Geddes, Karen L. Eskow Jaunarajs, Christopher Bishop. 2011. Tryptophan hydroxylase 2 aggregates through disulfide cross-linking upon oxidation: possible link to serotonin deficits and non-motor symptoms in Parkinson's disease. *Journal of Neurochemistry* **116**:3, 426-437. [CrossRef]
12. Kemal Ugur Tufekci, Sermin Genc, Kursad Genc. 2011. The Endotoxin-Induced Neuroinflammation Model of Parkinson's Disease. *Parkinson's Disease* **2011**, 1-25. [CrossRef]
13. Santos M. Nicolau, Javier Egea, Manuela G. López, Antonio G. García. 2010. Mitochondrial Na+/Ca2+ exchanger, a new target for neuroprotection in rat hippocampal slices. *Biochemical and Biophysical Research Communications* **400**:1, 140-144. [CrossRef]
14. V Franssens, E Boelen, J Anandhakumar, T Vanhelmont, S Büttner, J Winderickx. 2010. Yeast unfolds the road map toward #-synuclein-induced cell death. *Cell Death and Differentiation* **17**:5, 746-753. [CrossRef]
15. Mali Jiang, Yair Porat-Shliom, Zhong Pei, Yong Cheng, Lan Xiang, Katherine Sommers, Qing Li, Frank Gillardon, Bastian Hengerer, Cynthia Berlinicke, Wanli W. Smith, Donald J. Zack, Michelle A. Poirier, Christopher A. Ross, Wenzhen Duan. 2010. Baicalein reduces E46K #-synuclein aggregation in vitro and protects cells against E46K #-synuclein toxicity in cell models of familiar Parkinsonism. *Journal of Neurochemistry* no-no. [CrossRef]
16. Michelle Barbi de Moura, Lucas Santana dos Santos, Bennett Van Houten. 2010. Mitochondrial dysfunction in neurodegenerative diseases and cancer. *Environmental and Molecular Mutagenesis* NA-NA. [CrossRef]
17. Rina Bandopadhyay, Jacqueline de Belleroche. 2010. Pathogenesis of Parkinson's disease: emerging role of molecular chaperones. *Trends in Molecular Medicine* **16**:1, 27-36. [CrossRef]
18. Bobby Thomas . 2009. Parkinson's Disease: From Molecular Pathways in Disease to Therapeutic Approaches. *Antioxidants & Redox Signaling* **11**:9, 2077-2082. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]