

Parkinson's disease

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Parkinson's disease (PD) is a chronic progressive neurodegenerative movement disorder characterized by a profound and selective loss of nigrostriatal dopaminergic neurons. Clinical manifestations of this complex disease include motor impairments involving resting tremor, bradykinesia, postural instability, gait difficulty and rigidity. Current medications only provide symptomatic relief and fail to halt the death of dopaminergic neurons. A major hurdle in development of neuroprotective therapies are due to limited understanding of disease processes leading to death of dopaminergic neurons. While the etiology of dopaminergic neuronal demise is elusive, a combination of genetic susceptibilities and environmental factors seems to play a critical role. The majority of PD cases are sporadic however, the discovery of genes linked to rare familial forms of disease (encoding α -synuclein, parkin, DJ-1, PINK-1 and LRRK2) and studies from experimental animal models has provided crucial insights into molecular mechanisms in disease pathogenesis and identified probable targets for therapeutic intervention. Recent findings implicate mitochondrial dysfunction, oxidative damage, abnormal protein accumulation and protein phosphorylation as key molecular mechanisms compromising dopamine neuronal function and survival as the underlying cause of pathogenesis in both sporadic and familial PD. In this review we provide an overview of the most relevant findings made by the PD research community in the last year and discuss how these significant findings improved our understanding of events leading to nigrostriatal dopaminergic degeneration, and identification of potential cell survival pathways that could serve as targets for neuroprotective therapies in preventing this disabling neurological illness.

INTRODUCTION

Parkinson's disease (PD) was first described in the essay entitled, 'An Essay of the Shaking Palsy' by James Parkinson in 1817. PD is a devastating degenerative neurological illness without cure affecting 1–2% of the 'over 50' population with a current estimation of 1.5 million in the US alone. The neuropathological hallmarks are characterized by progressive and profound loss of neuromelanin containing dopaminergic neurons in the substantia nigra pars compacta (SNpc) with presence of eosinophilic, intracytoplasmic, proteinaceous inclusions termed as Lewy bodies (LB) and dystrophic Lewy neurites in surviving neurons (1). Although, neuronal loss in SNpc is pronounced there is widespread neurodegeneration in the central nervous system (CNS) with the pars compacta being involved in midstages of the disease (2). Clinical features of PD include motor impairments involving resting tremor, bradykinesia, postural instability and rigidity along with non-motoric symptoms like autonomic, cognitive and psychiatric problems. The molecular pathways leading to

this pathological picture and concomitant clinical syndromes are obscure, but it is believed that it may result from an environmental factor, a genetic causation or a combination of the two. Epidemiological studies reveal that <10% of PD has a strict familial etiology while majority of cases are sporadic. The discovery of genes linked to rare familial forms of PD during the last decade have confirmed the role of genetics in development of PD, and provided vital clues in understanding molecular pathogenesis of the common sporadic illness. These genetic breakthroughs provide us with unique avenues to pursue the pathologic mechanisms leading to disease development and help us identify probable targets for developing neuroprotective therapies, which may revolutionize the treatment of this debilitating disorder.

PATHOGENIC MUTATIONS IN PARKINSON'S DISEASE PATHOGENESIS

Numerous attempts have been made to resolve the etiology of PD since its first description in 1817. Until the end of last

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Table 1. Gene loci identified for Parkinson's disease

Locus	Gene	Chromosome	Inheritance	Probable function
PARK1 and PARK4	α -Synuclein	4q21	AD	Presynaptic protein, Lewy body
PARK2	Parkin	6q25.2-27	AR	Ubiquitin E3 ligase
PARK3	Unknown	2p13	AD	Unknown
PARK4	Unknown	4p14	AD	Unknown
PARK5	UCH-L1	4p14	AD	Ubiquitin C-terminal hydrolase
PARK6	PINK1	1p35-36	AR	Mitochondrial kinase
PARK7	DJ-1	1p36	AR	Chaperone, Antioxidant
PARK8	LRRK2	12p11.2	AD	Mixed lineage kinase
PARK9	ATP13A2	1p36	AR	Unknown
PARK10	Unknown	1p32	AD	Unknown
PARK11	Unknown	2q36-37	AD	Unknown
PARK12	Unknown	Xq21-q25	Unknown	Unknown
PARK13	HTRA2	2p12	Unknown	Mitochondrial serine protease

AD, autosomal dominant; AR, autosomal recessive.

century the influence of heredity was controversial however, identification of mutations in several genes responsible for Mendelian forms of PD confirms the role of genetics in disease development. The precise relationship of these familial linked genes to the more common sporadic illness is currently uncertain, however shared pathophysiologies among the two disease entities are parkinsonism with nigrostriatal dopaminergic degeneration suggesting involvement of common pathogenic mechanisms (3). Understanding these common disease-modifying pathways will promote our knowledge of the specific molecular aspects that lead to nigrostriatal degeneration in PD. Several genetic loci are identified for PD (Table 1), however, there are five clearly defined genetic causes of PD. Here, we discuss our current understanding of these gene products linked to monogenic forms of PD (PARK1, 2, 6, 7 and 8) with an emphasis on their normal function and pathogenic dysfunction contributing to disease pathogenesis.

PARK1 (α -SYNUCLEIN)

α -Synuclein is a natively unfolded presynaptic protein believed to play a role in synaptic vesicle recycling, storage and compartmentalization of neurotransmitters and associates with vesicular and membranous structures (4–6). Structurally, α -synuclein consists of an N-terminal amphipathic region, a hydrophobic middle region (containing the non-amyloid- β component domain) and an acidic C-terminal region. Three missense mutations in α -synuclein gene (A53T, A30P and E46K) (7–9), and in addition to genomic triplications of a region of α -synuclein gene are associated with autosomal dominant PD (10).

α -Synuclein has an increased propensity to aggregate due to its hydrophobic non-amyloid- β component domain. The presence of fibrillar α -synuclein as a major structural component of LB in PD suggests a role of aggregated α -synuclein in disease pathogenesis (11). Recent studies provide compelling evidence of non-amyloid- β component domain and truncated form of α -synuclein in mediating neurodegeneration *in vivo*. Overexpression of α -synuclein lacking residues 71–82 failed to aggregate and form oligomeric species in flies resulting in an absence of dopaminergic pathology. Contrary to this

expression of a truncated version of α -synuclein, containing the non-amyloid β -component induced increased aggregation into large inclusions bodies, increased accumulation of high molecular weight α -synuclein species and demonstrated enhanced dopaminergic neurotoxicity in flies (12). This was supported by another study where mice expressing C-terminally truncated human α -synuclein (containing residues 1–120) under a rat tyrosine hydroxylase promoter on mouse α -synuclein null background developed progressive loss of nigral dopaminergic neurons with pathological inclusions, and associated behaviors suggesting a critical role of C-terminal truncation of α -synuclein in aggregation and dopaminergic toxicity *in vivo* (13). This suggests that C-terminal of α -synuclein is an important regulator of its aggregation *in vivo* and pathogenic α -synuclein mutations in PD may enhance C-terminal truncation-induced aggregation (14). In addition, a pathological modification involving phosphorylation of Ser129 in α -synuclein promotes aggregation, and that Ser129 phosphorylated α -synuclein is a major component of LB (15,16). Interesting new findings suggests that G-protein-coupled receptor kinase 5 is responsible for catalyzing Ser129 phosphorylation of α -synuclein promoting formation of soluble oligomers and aggregates of α -synuclein (17). Recently, it was demonstrated that insufficiency of Sept4, a pre-synaptic scaffold protein involved in dopaminergic neurotransmission can enhance Ser129 phosphorylated α -synuclein aggregation and toxicity *in vivo*, while a direct association of Sept4 with α -synuclein prevented Ser129 phosphorylation and α -synuclein self aggregation *in vitro* (18). However, the pathological modification of phosphorylated Ser129 of α -synuclein seems to be selective for neurons, and not for platelets from PD and multiple system atrophy patients (19). Presently, it is unclear whether accumulation of misfolded proteins that lead to LB-like inclusions are toxic or protective in PD. Pharmacological compounds known to promote inclusion formation seems to protect against α -synuclein toxicity (20). Using a protein aggregate filtration assay a recent study demonstrates that abundant presynaptic terminal associated α -synuclein aggregates are responsible for synaptic pathology and neurodegeneration, in contrast to α -synuclein aggregates from LBs in postmortem brains from dementia with Lewy body disease (DLBD), supporting a less prominent role of LBs in toxicity (21).

Mechanisms by which abnormal processing and accumulation of α -synuclein disrupt basic cellular functions leading to dopaminergic neurodegeneration are intensely studied. One of the earliest defects following α -synuclein accumulation *in vivo* is blockade of endoplasmic reticulum to golgi vesicular trafficking causing ER stress (22). Furthermore, transgenic mice expressing human A53T α -synuclein develop mitochondrial pathology (23,24) providing a crucial role of α -synuclein in modulating mitochondrial function in neurodegeneration. This may be due to the fact that α -synuclein is a modulator of oxidative damage, since mice lacking α -synuclein are resistant to mitochondrial toxins (25), while nigral dopaminergic neurons are vulnerable to degeneration and mitochondrial dysfunction following parkinsonian neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in human α -synuclein transgenic mice (26,27). In addition, β -synuclein seems to protect α -synuclein-induced toxicity by reducing α -synuclein protein expression (28), by blocking development of pore-like oligomers of α -synuclein (29) and promoting cell survival by activation of *Akt* signaling (30) (Thomas *et al.*, unpublished observation). Furthermore, mutant α -synuclein (A53T and A30P) overexpression increases cytosolic catecholamine concentrations leading to disruption of vesicular pH and normal functioning, and facilitate toxicity of oxidized catechol metabolites implicating selective degeneration in PD (31,32). Biochemical abnormalities in α -synuclein has also been shown to activate stress-signaling protein kinases (33), affect age-related decrease in neurogenesis (34), impair microtubule-dependent trafficking (35), reduce intercellular communications at gap junctions (36) and inhibit histone acetylation in the nucleus to promote toxicity (37). These pathophysiological aspects are detrimental to normal functioning of dopaminergic neurons and provide implications for disease pathogenesis in α -synuclein-induced PD.

PARK2 (PARKIN)

The *parkin* gene encodes a 465 amino acid protein containing an N-terminal ubiquitin like domain, a central linker region and C-terminal RING domain consisting of two RING finger motifs separated by an in between RING domain. *Parkin* functions as an E3 ubiquitin protein ligase similar to other RING finger containing proteins by targeting misfolded proteins to the ubiquitin proteasome pathway for degradation, and the loss of its E3 ligase activity due to mutations lead to autosomal recessive early-onset PD (38–40). Mutations in the *parkin* gene are a major cause of autosomal recessive early onset PD. Several putative substrates of *parkin* have been identified and the accumulation of one or several of these substrates is implicated in neurodegeneration (41).

Parkin functions as a multipurpose neuroprotective protein in a variety of toxic insults crucial for dopamine neuron survival (42). New research has identified neuroprotective mechanisms mediated by *parkin*. Recent studies suggest that *parkin* mediates neuroprotection through activation of IkappaB kinase/nuclear factor-kappaB signaling, whereas *parkin* mutants failed to stimulate this pathway (43). Furthermore, the UBL domain of *parkin* interacts with ubiquitin interacting

motifs (UIM) of Eps15 [an adaptor protein involved in epidermal growth factor receptor (EGFR) endocytosis and trafficking] and ubiquitinates in a proteasome-independent manner. *Parkin* interferes with the ability of Eps15 UIMs to bind ubiquitinated EGFR delaying EGFR internalization and degradation to promote phosphatidylinositol 3-kinase/*Akt* cell survival signaling (44). *Parkin* also seems to modulate key mitochondrial functions which include, a role in mitochondrial morphogenesis during spermiogenesis (45), and enhancing mitochondrial biogenesis in proliferating cells through transcription and replication of mitochondrial DNA (46). *Parkin* also rescues mitochondrial dysfunction, muscle degeneration and dopaminergic loss in flies due to inactivation of a putative mitochondrial serine/threonine kinase (*PINK1*), that cause autosomal recessive PD (47–49). This is consistent with increased susceptibility of mesencephalic dopaminergic neurons in cultures to mitochondrial complex I inhibitor rotenone-induced death (50). In addition, a recent study demonstrates α -synuclein-induced mitochondrial dysfunction is further enhanced due to lack of *parkin* activity *in vivo* implicating crucial role of *parkin* in modulating mitochondrial functions in α -synuclein-induced PD (23). Post-translational modification of *parkin* either due to oxidative or nitrosative stress also compromise its protective function by impairing the E3 ligase activity (51,52). Dopaminergic neurons are especially vulnerable to activation of the Cyclin-dependent kinase 5 (*Cdk5*) (53). *Cdk5* interacts and phosphorylates *parkin* at Ser131 of the linker region. This modification blocks autoubiquitylation leading to *parkin* aggregation both *in vitro* and *in vivo* (54). Both disease-specific mutants of *parkin* and RING-IBR-RING type ubiquitin ligases similar to *parkin* are susceptible to solubility alterations due to oxidative damage (55,56). Recent studies also provide important new insights for the first time on the role of mutant *parkin in vivo*. Age-dependent dopaminergic neurodegeneration and motor impairments are observed due to expression of mutant human *parkin* and not wild-type *parkin* in flies implying a toxic gain of function mechanism (57). This is in contrast to lack of nigral dopaminergic degeneration in mouse models generated by targeted deletion of *parkin* representing loss of function phenomenon (58). Surprisingly, catecholaminergic neurons from *parkin* knockout mice fail to show increased susceptibility to neurodegeneration against neurotoxins (59,60) and human α -synuclein-induced disease (23,61), contrary to this mesencephalic dopaminergic neurons from *parkin* knockout mice show resistance to nitric oxide-induced toxicity by compensatory increase in glutathione levels (62). These findings suggest that although *parkin* is considered as a multipurpose neuroprotective agent, its neuroprotective efficiency is very selective and identification of the specific neuroprotective pathways that are affected due to *parkin* deficiency will help identify its role in PD pathogenesis.

PARK7 (DJ-1)

Loss-of-function mutations in the *DJ-1* locus are associated with rare forms of autosomal recessive early-onset parkinsonism (63). *DJ-1* mutations account for 1–2% of all early-onset PD (64), with a number of different pathogenic mutations,

including exonic deletions, truncations and homozygous and heterozygous point mutations. *DJ-1* is a highly conserved protein of 189 amino acids that belongs to the DJ-1/Thi/PfpI protein super family. It has ubiquitous expression in a variety of mammalian tissues including brain and localized to mitochondria (65,66). *DJ-1* is a homodimeric protein originally identified as an oncogene with proposed roles in sperm maturation and fertilization. Association of DJ-1 through pathogenic mutations in familial PD has identified its novel functions that shed light in disease pathogenesis. These include proposed antioxidant, transcriptional co-activator and chaperone activity.

Many lines of evidence suggest that *DJ-1* functions as an antioxidant protein. Oxidative stress leads to an acidic shift in the *DJ-1* isoelectric point by oxidation of Cys106 which can be converted to cysteine sulfinic acid (Cys-SO₂H) (67). Because of its inherent ability to undergo self oxidation to eliminate H₂O₂ it may function as a scavenger of reactive oxygen species (ROS) (68). Overexpression of wild-type *DJ-1* both in cell culture and to dopaminergic neurons *in vivo* protects against wide variety of toxic injury due to oxidative stress (68–70). The apparent antioxidant action appears to be due to the ability of *DJ-1* to stabilize the antioxidant transcriptional master regulator Nrf2 (nuclear factor erythroid 2-related factor) by preventing association with its inhibitor, Keap1 and ubiquitination of Nrf2 (71). This is consistent with the ability of *DJ-1* to increase cellular glutathione levels by activating the glutamate cysteine ligase (72). *DJ-1* also functions like a redox-dependent chaperone to inhibit α -synuclein aggregation and subsequent death (73,74). Furthermore, it associates with *parkin* during oxidative stress suggesting a common role in neuroprotection (75). Familial PD-linked mutations in *DJ-1* are considered to cause nigral degeneration through loss-of-function mechanism consistent with the recessive inheritance. The classic L166P mutation in *DJ-1* prevents its dimerization by unfolding its C-terminal region leading to decreased steady-state levels due to accelerated protein degradation by the proteasome (76). Recently, familial substitutions (M26I and E64D) together with H₂O₂-induced cysteine 106 oxidation and cleavage have been shown to destabilize *DJ-1* (77,78). Furthermore, mass spectrometric identification of methionine oxidized *DJ-1* in sporadic PD brains suggests a role of methionine oxidation in disease pathogenesis (79). Mouse models lacking *DJ-1* develop age-dependent motor deficits, hypokinesia and dopaminergic dysfunction with no neuronal loss (80,81). Nigrostriatal dopaminergic neurons in these mice show increased vulnerability to the parkinsonian neurotoxin MPTP via an unknown mechanism (82). Increased vulnerability in *DJ-1* knockout mice could be due to increased p53 and Bax expression (83), deficits in phase II detoxification enzyme NQO1 (NADPH quinone oxidoreductase 1) (71), irreversible membrane potential changes due to impaired Na⁺/K⁺ ATPase (84), defective phosphatidylinositol 3-kinase/*Akt* signaling (85), and inability of the death protein Daxx to inhibit ASK1- (apoptosis signal regulating kinase 1) induced cell death (86). Of particular significance to dopaminergic neuronal function is the ability of *DJ-1* to transcriptionally upregulate tyrosine hydroxylase expression by suppressing the sumoylation of pyrimidine tract-binding protein-associated splicing factor (87). These

studies conclusively prove that *DJ-1* plays a crucial role in maintenance and survival of dopaminergic neurons. Characterization of the molecular details of *DJ-1*'s role in dopaminergic neuronal function will help provide us with novel insights into its role in disease pathogenesis.

PARK6 (*PINK1*)

Mutations in the *PINK1* [phosphatase and tensin (PTEN) homolog-induced putative kinase 1] gene were identified to cause early-onset familial PD (88). *PINK1* mutation frequency varies between 1 and 9% with considerable variation among different ethnic groups (89). *PINK1* is a 581 amino acid protein that contains an N-terminal mitochondrial targeting sequences and a highly conserved protein kinase domain similar to serine/threonine kinases of the Ca²⁺ calmodulin family. It has a ubiquitous and punctate expression pattern suggesting mitochondrial localization (90). Very little is known about the precise function of *PINK1* although its mitochondrial localization, presence of kinase domain with identification of majority of mutations in the kinase domain and regions close to it suggest a role in mitochondrial dysfunction, protein stability and kinase pathways in pathogenesis of PD (91,92). No putative substrates to the kinase have been identified till date, however *PINK1* has been shown to undergo autophosphorylation and phosphorylate an artificial substrate histone H1. C-terminus truncation of *PINK1* and disease-related mutations downregulate its serine/threonine kinase activity and confer different autophosphorylation patterns suggesting the importance of its kinase activity in mitochondrial function (93,94). Recent study emphasize the role of *PINK1* in mitochondrial biogenesis and demonstrate that human *PINK1* locus is regulated by non-coding naturally occurring antisense RNA *in vivo* implying for the first time, a role of non-coding RNAs in regulating functions of familial PD-linked genes (95).

In vitro studies suggest that overexpression of wild-type *PINK1* can prevent staurosporine-induced, mitochondrial cytochrome *c* release and subsequent apoptosis by caspase 3 activation, which is abrogated by familial PD-linked *PINK1* mutants (96). This is consistent with increased vulnerability to dopaminergic SH-SY5Y cells to the mitochondrial toxins rotenone and MPP⁺ (1-methyl-4-phenyl-pyridinium ion) following suppression of *PINK1* function by siRNA (97), or due to expression of *PINK1* disease mutants (98). Proteasomal stress enables *PINK1* to undergo altered cleavage impairing its function (99), a phenomenon that may enable it to accumulate in LBs, whereas mutations affect protein stability (92,100). *In vivo* *PINK1* loss of function either due to its inactivation by siRNA or due to expression of disease-causing mutations leads to muscle and dopaminergic degeneration as a consequence of mitochondrial dysfunction in flies. Interestingly, this degenerative phenotype was rescued by overexpression of the ubiquitin E3 ligase *parkin*, implicating the importance of both *parkin* and *PINK1* in regulating mitochondrial physiology and survival in flies (47–49). At this juncture, the functional implications of *parkin* and *PINK1* interaction seem to be unclear, however loss of *PINK1* function might impair proteasomal activity due to mitochondrial dysfunction. Consistent

with mitochondrial dysfunction, immortalized lymphoblasts from patients with G309D-*PINK1* mutations show increased lipid peroxidation and defects in mitochondrial complex I activity, and a compensatory increase in mitochondrial superoxide dismutases and glutathione (101). Furthermore, overexpression of human SOD1 prevented dopaminergic neuronal loss due to *PINK1* inactivation in flies suggesting that mitochondrial dysfunction modulates oxidative damage pathways (102). This phenomenon gains further support from the fact that oxidative damage due to *PINK1* dysfunction recruits the antioxidant *DJ-1* in the pathway for rescue by maintaining steady-state levels of *PINK1* through physical interaction and overexpression of *DJ-1* (98). At this stage its premature to conclude the physiological function of *PINK1* through its direct interaction with both *parkin* and *DJ-1*. However, the interaction suggests involvement of three different gene products causing familial PD in sharing common pathways for PD pathogenesis. Future studies on identification of *PINK1* substrates and detailed characterization of *in vivo* models of *PINK1* knockouts will shed light on its normal physiological function and provide us important clues on how pathogenic mutations mediate disease progression and pathogenesis.

PARK8 (LRRK2)

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) or *dar* cause autosomal dominant PD (103,104). This gene has obtained considerable attention because of the presence of *LRRK2* mutations beyond familial cases of disease with evidence that mutations occur at high frequency in 1–7% of patients from European origin and 20–40% in Ashkenazi Jews and North African Arabs, although the prevalence varies markedly between populations (105). *LRRK2* encodes a 2527 amino acid multidomain, 280 kDa protein belonging to ROCO protein family that includes a Rho/Ras-like GTPase domain, a protein kinase domain of the MAPKKK family, as well as a WD40-repeat and a leucine-rich repeat domains. An additional domain C-terminal to the GTPase domain, termed COR (for carboxy-terminal of Ras), is of unknown function. Point mutations have been found in almost all of the identified domains. The presence of mutations in several different domains, as well as the lack of deletions or truncations, along with dominant inheritance, is consistent with a gain-of-function mechanism. The precise physiological role of this protein is unknown but presence of multiple functional domains suggesting involvement in wide variety of functions.

A series of studies were conducted to identify the intracellular and tissue-specific location of *LRRK2* both in cell culture and *in vivo* to identify a probable function based on its localization. The majority of forebrain structures including nigrostriatal dopaminergic neurons express *LRRK2* and it seems to be predominantly cytoplasmic especially in the golgi apparatus, synaptic vesicles, plasma membrane, lysosomes and associates with the outer mitochondrial membrane (106–110). Deletion mutants of *LRRK2* homolog in *Caenorhabditis elegans* *LRK-1*, led to depletion of synaptic vesicle proteins in dendritic endings of neurons determining its role in polarized sorting of synaptic vesicle proteins to axons (111).

Recent studies also show the ability of *LRRK2* to associate with lipid rafts, localize to LBs and regulate neurite length and branching (112–114). These suggest that *LRRK2* modulates synaptic vesicle recycling, neurite outgrowth and functions inherent to golgi, lysosomes and mitochondria, dysfunctions of which may compromise dopamine neuron survival (115).

The domain structure of *LRRK2* protein suggests a wide variety of functions that could be responsible for the pleomorphic pathology found in the mutation carriers. There are several missense mutations identified to date, of which the G2019S mutation is the most prevalent (116). The G2019S and the nearby I2020T mutation are located at the N-terminal portion of the activation loop in kinase domain. These mutations are associated with increased kinase activity of *LRRK2* assessed by autophosphorylation or phosphorylation of generic substrate myelin basic protein when compared with either wild-type *LRRK2*, a kinase dead mutant, or equivalent mutations in paralogous kinase *LRRK1* (117–123). Several studies suggest that the kinase activity of *LRRK2* is regulated by GTP via the intrinsic GTPase ROC domain of *LRRK2* (120–122). This is supported by the fact that mutations in the GTPase ROC domain (R1441C, T1348N) disrupts GTP binding and hydrolysis abolishing the kinase activity (122,124). The *LRRK2* kinase domain provides sequence homology to mixed lineage kinases with specificity for serine/threonine kinase or tyrosine kinase. In a recent study, *in vitro* autophosphorylation using thin-layer chromatography revealed kinase-dependent serine/threonine phosphorylation but not tyrosine phosphorylation suggesting that *LRRK2* might function as a serine/threonine kinase and may not meet the criteria for mixed lineage kinase (121). It is becoming increasingly evident from multiple studies that kinase activity in *LRRK2* due to disease-causing mutations affects cell viability due to apoptosis providing a direct role of pathological activation of *LRRK2* kinase causing neurodegeneration (119–121,125,126). At this stage it is unclear how increased kinase activity affects signaling leading to disease pathogenesis in PD. Recent findings show significant alterations in phosphorylation of key proteins involved in MAPK signaling in leukocytes from patients with G2019S mutations implicating abnormal protein phosphorylation (127). Identification of physiological *LRRK2* substrates and characterization of *in vivo* models of *LRRK2* will help understand both physiological and pathological functions of *LRRK2* affecting disease pathogenesis.

MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE DAMAGE IN PARKINSON'S DISEASE PATHOGENESIS

Multiple lines of evidence suggest a pathogenic role of oxidative damage and mitochondrial dysfunction in causing PD. Consistent deficits in the subunits and activity of mitochondrial complex I of the electron transport chain in blood platelets and SNpc of PD patients is a prominent phenomenon (128,129). Reduced complex I activity is also seen in cytoplasmic hybrid (cybrid) cell lines containing mitochondrial DNA from PD patients (130). Epidemiological studies reveal

that exposure to pesticides, industrial wastes and environmental toxins are involved in disease pathogenesis in PD (131). A classic example is the accidental discovery of MPTP whose toxic metabolite MPP⁺, by selective uptake in dopaminergic neurons caused parkinsonism in designer-drug abusers due to mitochondrial dysfunction (132). Similar to MPTP, other complex I inhibitors like rotenone and paraquat-induced dopaminergic degeneration in rodents, suggesting central role of mitochondrial dysfunction in PD pathogenesis (133,134). Several studies provide support to the notion of mitochondrial dysfunction in the causation of PD (135). A recent study demonstrated that SNpc neurons have high amount of mitochondrial DNA (mtDNA) deletions in postmortem PD patients when compared with other neuronal populations in brain- and age-matched controls (136). A related study identified that nigral neurons from PD patients contain high levels of clonally expanded somatic mtDNA deletions leading to mitochondrial dysfunction (137). These human findings gain further support by a study where targeted deletion of mitochondrial transcription factor A (TFAM) in midbrain dopaminergic neurons led to progressive PD in mice, due to reduced mtDNA expression and respiratory chain deficiency (138). Furthermore, a surprisingly low mitochondrial mass observed in SNpc of mice might be a contributing factor to selective vulnerability of these neuronal populations to mitochondrial dysfunction (139). This suggests that factors which directly or indirectly modulate normal mitochondrial functioning can significantly compromise neuronal survivability suggesting its detrimental role in PD pathogenesis.

Nigrostriatal dopaminergic neurons in general are under tremendous oxidative stress probably due to redox cycling of catechols, leading to increased generation of detrimental ROS. Decrements in reduced glutathione levels in SNpc of pre-symptomatic PD suggest that oxidative damage occurs much earlier than the actual neuronal loss (140). Interplay between oxidative stress and mitochondrial dysfunction is further suggested by an impairment of mitochondrial complex I due to chronic depletion of antioxidant glutathione (141). Furthermore, PPAR γ coactivator 1 α (PGC-1 α), which is involved in mitochondrial biogenesis and respiration, is a modulator of ROS generation during oxidative stress (142). In a recent study it was demonstrated that PGC-1 α is required for induction of many ROS detoxifying enzymes like glutathione peroxidase-1, catalase and manganese superoxide dismutase upon oxidative stress. Nigrostriatal dopaminergic neurons in mice lacking PGC-1 α were more vulnerable to parkinsonian neurotoxin MPTP. Furthermore, overexpression of PGC-1 α protected neural cells due to oxidative stress-induced death providing compelling evidence to the role of PGC-1 α as a powerful regulator of ROS metabolism. The ability of PGC-1 α to increase activity of mitochondrial electron transport chain while stimulating a broad anti-ROS program makes it an important target to limit the damage that has been associated with defective mitochondrial function and oxidative damage seen in several neurodegenerative diseases including PD (143). A recent study from postmortem brains suggests a prominent role of Nrf2/ARE signaling in PD pathogenesis (144). The leucine-zipper transcription factor Nrf2 regulates coordinated induction of antioxidant

response element-(ARE) driven battery of cytoprotective genes, including a variety of both antioxidant and anti-inflammatory proteins (145). Oxidation of a critical cysteine in Keap 1 allows Nrf2 to translocate into the nucleus, where it then activates transcription of genes encoding phase II detoxification enzymes, such as NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione S-transferases (GST), glutamate-cysteine ligase (GCL), hemeoxygenase 1 (HO-1) and downregulates inflammatory enzymes like cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and many others (146–148). Disruption of Nrf2 renders neuronal tissues more susceptible to death due to oxidative stress and mitochondrial dysfunction in Nrf2 knockout mice (149–151). Recent findings from our laboratory suggest that pharmacological activation of the Nrf2/ARE pathway rescue neurodegeneration in a mouse model of PD due to induction of antioxidant enzymes and downregulation of inflammatory molecules like iNOS and COX-2. (Yang, Thomas *et al.*, unpublished observations). Since both oxidative damage and inflammation are implicated in PD pathogenesis this pathway may serve as an important target for neurotherapeutics (152,153). Another promising pathway that has emerged in dopamine neuronal survival is the phosphatidylinositol 3-kinase/*Akt* pathway. A recent study demonstrates that overexpression of the oncoprotein *Akt*, protected against 6-OHDA-induced dopaminergic toxicity. *Akt* conferred pronounced neurotrophic effects on dopamine neurons of adult and aged mice, including increases in neuron size, and sprouting (154). In addition, our results also support the importance of *Akt* activation in dopaminergic neuronal survival via a selective regulatory mechanism involving modulation of β -synuclein expression by α -synuclein *in vivo* (Thomas *et al.*, unpublished observation). Furthermore, other familial PD-linked proteins, *parkin* (44), *DJ-1* (85) and *PINK1* (88) mediate cell survival through the *Akt* pathway supporting a pathogenic role of *Akt* regulation in PD.

Several genes associated with PD also link mitochondria and oxidative damage in disease pathogenesis. These include α -synuclein, *parkin*, *DJ-1*, *PINK1* and *LRRK2*. Several direct or indirect pathogenic mechanisms enable familial PD-associated genes link to mitochondria. α -Synuclein, a major component of LB, seems to link abnormal protein degradation to oxidative stress and mitochondrial dysfunction. Mice overexpressing human A53T α -synuclein induce mitochondrial damage due to aberrant α -synuclein accumulation (24). We have shown that mice lacking α -synuclein are resistant to mitochondrial toxins like MPTP, 3-nitropropionic acid and malonate while overexpression of human α -synuclein in mice enhances vulnerability to mitochondrial toxin MPTP (25,26) (Thomas *et al.*, unpublished observations). *Parkin*, an E3 ligase, seems to link the ubiquitin proteasome system, oxidative stress and mitochondrial dysfunction. Gene knockouts of *parkin* mouse and flies show increased oxidative stress and mitochondrial dysfunction (155,156). *Parkin* also prevents mitochondrial swelling, cytochrome *c* release and caspase activation which is abrogated due to *parkin* mutations and proteasome inhibitors (157). In proliferating cells, *parkin* localizes to mitochondria to associate with TFAM and enhances mitochondrial biogenesis (46). Oxidative and nitrosative modification of *parkin* either due to a dopamine

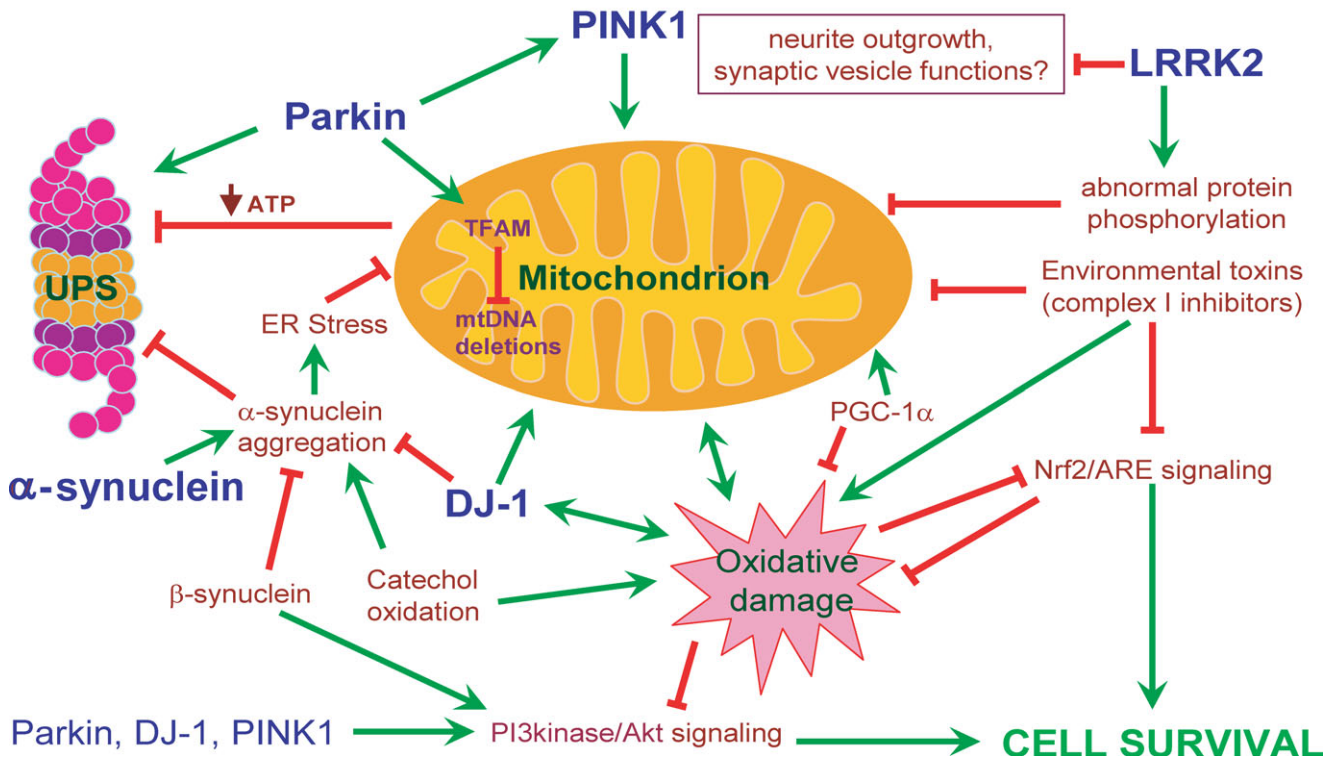


Figure 1. Common intersecting pathways underlying PD pathogenesis. Both environmental factors and mutations in familial PD-linked genes encoding α -synuclein, parkin, DJ-1, PINK1 and LRRK2 are associated with PD pathogenesis. These pathogenic mutations and environmental factors are known to cause disease due to mitochondrial dysfunction, oxidative damage, abnormal protein aggregation and protein phosphorylation compromising key roles of dopaminergic neuronal function and survival. Environmental factors similar to pesticides and toxins directly induce both oxidative damage and mitochondrial dysfunctions. α -Synuclein undergoes aggregation either due to pathogenic mutations or catechol oxidation which in turn compromise ubiquitin proteasome function (UPS), induce ER stress and cause mitochondrial dysfunction. Mitochondrial dysfunction and oxidative damage lead to deficits in ATP which may compromise UPS function promoting abnormal protein aggregation. β -Synuclein is known to prevent α -synuclein aggregation through activation of Akt signaling. Parkin, an ubiquitin E3 ligase, promotes proteasomal degradation, increases mitochondrial biogenesis by activating mitochondrial transcription factor A (TFAM) and block PINK1-induced mitochondrial dysfunction, while pathogenic mutations, oxidative and nitrosative damage severely compromise its protective function. DJ-1 protects against oxidative stress, functions as a chaperone to block α -synuclein aggregation and protects against mitochondrial dysfunction. PINK1 seems to protect against mitochondrial dysfunction which is compromised due to pathogenic mutations, although the precise function of PINK1 in mitochondria still needs to be determined. LRRK2 seems to play a role in synaptic vesicle functions, neurite outgrowth, etc. Pathogenic mutations in LRRK2 cause abnormal protein phosphorylation which induce mitochondria-dependent cell death. In addition, a neuroprotective role of PGC-1 α in preventing oxidative damage and mitochondrial dysfunction is suggested, whereas a pathogenic role of PI3kinase-Akt (phosphatidylinositol 3-kinase/Akt) and Nrf2/ARE signaling is implicated in PD pathogenesis. Familial PD-linked genes namely parkin, DJ-1 and PINK1 activate PI3 kinase-Akt signaling, while activation of Nrf2/ARE pathway prevents against oxidative damage and mitochondrial dysfunction promoting cell survival. Both PI3 kinase-Akt and Nrf2/ARE signaling could be explored as potential targets of therapeutic intervention in dopaminergic neuronal demise. Green arrows indicate promoting or activating effects while red lines with blunt ends indicate inhibitory effects.

quinone modification or S-nitrosylation impairs its ubiquitin E3 ligase activity to compromise its protective function (51,52). In addition, DJ-1 mutations link familial early-onset PD with mitochondrial dysfunction and oxidative stress. The inherent ability of DJ-1 to function as a sensor of oxidative stress and as an antioxidant supports this notion. Furthermore, mitochondrial localization of DJ-1 (66) and hypersensitivity to mitochondrial toxin like MPTP in DJ-1 knockout mice (82) provides substantial evidence on its role in mediating mitochondrial and oxidative stress-mediated neurodegeneration. A possible link to age-dependence in sporadic PD is further supported by increased oxidative inactivation of DJ-1 due to ageing and enhanced susceptibility to oxidative damage in flies (158). Discovery of PINK1, a mitochondrial kinase and the newly identified cytosolic kinase LRRK2 modulate a pathogenic role in mediating mitochondria-dependent death.

Detailed description of these pathways of PINK1 and LRRK2 are described in PARK6 and PARK8 section of this review. Thus, it is becoming increasingly clear from multiple lines of studies that both oxidative damage and mitochondrial dysfunction takes a center stage in disease pathogenesis leading to sporadic and familial PD.

CONCLUSION

PD is a complex disease with multiple etiological factors involved in disease pathogenesis. Studies from familial PD-linked genes have enormously improved our understanding of disease development of the more common sporadic form of the disease. At this juncture there are several different pathways that are important in modulating pathogenic events leading to death of dopaminergic neurons in PD (Fig. 1).

Interestingly, these pathways seem to converge on aspects that affect dopamine neuronal function and survival due to mitochondrial dysfunction, oxidative damage, abnormal protein accumulation and protein phosphorylation. Many familial-linked PD genes and experimental animal models provide an emerging role of Nrf2/ARE and phosphatidylinositol 3-kinase/Akt signaling pathway as a potential target for therapeutic interventions since, these two pathways seem to modify several common pathophysiological aspects. Future research will enable us to further dissect molecular details of various disease-modifying pathways and potential convergence if any, to establish a common pathogenic theme for the two different forms of disease entities. These will further enable us to better understand the etiology of disease and develop novel neuroprotective therapies targeting these common pathways.

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