A Mitocentric View of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disease, yet the underlying causative molecular mechanisms are ill defined. Numerous observations based on drug studies and mutations in genes that cause PD point to a complex set of rather subtle mitochondrial defects that may be causative. Indeed, intensive investigation of these genes in model organisms has revealed roles in the electron transport chain, mitochondrial protein homeostasis, mitophagy, and the fusion and fission of mitochondria. Here, we attempt to synthesize results from experimental studies in diverse systems to define the precise function of these PD genes, as well as their interplay with other genes that affect mitochondrial function. We propose that subtle mitochondrial defects in combination with other insults trigger the onset and progression of disease, in both familial and idiopathic PD.

Keywords

PD genes; reactive oxygen species; mitochondria; electron transport chain; mitochondrial unfolded protein response; mitochondrial dynamics

INTRODUCTION

Parkinson's disease (PD) is a progressive and incurable neurodegenerative disorder affecting 1% of the older adult population. The defining motor characteristics of PD, known collectively as parkinsonism, include tremor, increased muscle tone, slow movements, and impaired gait and balance (Fahn, 2003; Lees et al., 2009). In autopsy studies, PD is characterized by the loss of dopamine (DA) neurons in the midbrain substantia nigra (SN) in

association with α -synuclein protein aggregates, termed Lewy bodies (Goedert et al., 2013; Jellinger, 2009). The SN is the source of DA for other basal ganglia nuclei, which have an essential role in initiating and facilitating movement. In addition, a range of nonmotor manifestations are observed (Chaudhuri and Schapira, 2009), including neuropsychiatric symptoms (e.g., depression, cognitive impairment), autonomic nervous system dysfunction, sleep disturbance, pain, and constipation, which likely relate to more widespread synuclein pathology and neurodegeneration throughout the nervous system.

Although PD pathogenesis has been studied extensively, the primary cause(s) remain(s) elusive. Alterations in numerous cellular processes have been implicated, including oxidative stress (Fariello, 1988), excitotoxicity (Olney et al., 1990), the ubiquitin-proteasome system (Lowe et al., 1988), the endolysosomal compartment, and mitochondrial dysfunction (Schapira et al., 1989). All these may be directly or indirectly related to mechanisms of neurodegeneration in PD. However, mitochondrial toxins potently induce SN degeneration, and genes that have been linked to familial PD and related disorders are increasingly being shown to affect mitochondrial function (Table 1). Based on recent studies, we focus here on a mitocentric view of PD, suggesting that mitochondrial dysfunction is in fact a primary cause of PD.

Mitochondria are double-walled, dynamic, filamentous organelles that constitute the cell's major source of adenosine triphosphate (ATP) production, the chemical energy of the cell. Within mitochondria, ATP is produced by the citric acid cycle (Krebs cycle) in the matrix via the action of four respiratory complexes (CI, CII, CIII, CIV) and ATP synthase in the mitochondrial inner membrane (MIM) (Figure 1). Mitochondrial mechanisms have been implicated in diverse human disorders (Vafai and Mootha, 2012). A severe impairment of mitochondrial function typically has dramatic consequences. For instance, mutations in genes encoding subunits of the respiratory complexes lead to Leigh syndrome, a fatal multisystem disorder of childhood onset. In contrast, more subtle mitochondrial impairment is associated with comparatively insidious onset and heterogeneous clinical manifestations, including cancer, diabetes, cardiomyopathy, anemia, and neurodegeneration (Schapira 2012; Schon and Przedborski, 2011). In fact, numerous studies have documented a mitochondrial complex I (CI) deficiency in the SN of PD patients (Keeney et al., 2006; Mizuno et al., 1989; Parker et al., 1989; Schapira et al., 1989). Moreover, a recent report similarly implicated early dysregulation in the mitochondrial electron transport chain (ETC), on the basis of transcriptional profiling of the SN from PD patients (Zheng et al., 2010).

Below, we first address the environmental and genetic evidence that links the etiology of PD to mitochondria. Next, we address how the many genes implicated in PD affect distinct mitochondrial functions such as the ETC, the mitochondrial unfolded protein response (UPR^{mt}), and mitochondrial dynamics. In the final section, we describe models that explain the relative vulnerability of certain cell types, such as SN DA neurons, despite a global mitochondrial dysfunction, and we further attempt to generalize lessons from rare familial forms of PD to the more common sporadic disease.

MITOCHONDRIAL DYSFUNCTION UNDERLIES PARKINSON'S DISEASE

The Environmental Link

A wave of interest in a potential role for mitochondria in PD came in 1976, when investigators found that drug users who took opioid analogs (MPPP) laced with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) developed PD-like motor symptoms resulting from degeneration of DA neurons in the SN (Langston et al., 1983; Martinez and Greenamyre, 2012). Subsequent investigations showed that MPTP is readily converted into MPP⁺, which crosses the blood brain barrier and is selectively imported in DA neurons (Nicklas et al., 1985). MPP⁺ binds to CI and inhibits electron transport, reducing ATP production and increasing reactive oxygen species (ROS) levels (Nicklas et al., 1985). Together, these observations suggested that SN degeneration and subsequent motor manifestations in PD may similarly result from mitochondrial dysfunction. This hypothesis gained further support when CI defects were documented in postmortem SN tissue from PD patients (Schapira et al., 1989).

Following on these initial findings, a variety of pesticides and toxins have been associated with SN degeneration in animal models (Martinez and Greenamyre, 2012), and evidence from human epidemiologic studies suggests that such exposure may in fact increase PD risk (Tanner et al., 2011). Several of the implicated toxins were shown to inhibit mitochondrial function, and such compounds have facilitated the development of useful animal model systems for PD studies (Martinez and Greenamyre, 2012). For instance, the widely used herbicide paraquat, which is structurally similar to MPTP, is a potent redox cyclor that accepts electrons from CI. This process converts the molecule to a free radical, which interacts with O₂ to generate superoxide anions and other ROS. Similarly, the naturally occurring pesticide rotenone systemically inhibits CI and leads to the degeneration of DA neurons in the SN. Finally, the fungicide maneb contains the mitotoxin manganese ethylene-bisdithiocarbamate, which preferentially inhibits complex III of the ETC and causes proteasomal inhibition, oxidative stress, and cytoplasmic α -synuclein aggregation in DA neuron cell lines (Zhou et al., 2004).

In summary, compelling data from animal models coupled with evidence from epidemiologic studies have led to the hypothesis that environmental exposure to mitochondrial toxins may be an important contributor to PD in the population (Martinez and Greenamyre, 2012). Large meta-analyses suggest an approximately twofold increased risk of PD due to pesticide exposure (Priyadarshi et al., 2000). Thus additional environmental and/or genetic insults, in combination with aging, would likely be required to trigger PD in most affected individuals.

The Genetic Link

Rapid advances in genetics over the past two decades have transformed our thinking about PD from a primarily environmentally influenced disorder to one with a substantial genetic contribution (Shulman et al., 2011; Trinh and Farrer, 2013). In particular, many of the genes responsible for familial forms of PD and related disorders have strong links to mitochondrial function. In 1998, mutations in the *parkin* gene were discovered to cause autosomal

recessive, juvenile parkinsonism (AR-JP) (Kitada et al., 1998), and subsequent work has proven *parkin* to be the most important cause of PD in children and young adults (Lücking et al., 2000; Periquet et al., 2003). As detailed below, studies of *parkin* as well as *pink1* and *DJ-1*, which are less common causes of AR-JP (Bonifati et al., 2003, 2005; Rogaeva et al., 2004; Valente et al., 2004), have strongly implicated these genes in mitochondrial function. At presentation, AR-JP is often clinically indistinguishable from typical, late-onset PD cases. As the disease progresses unusual features emerge, including prominent dystonia, diurnal fluctuations, and an overall benign course without development of characteristic PD nonmotor symptoms, suggesting a more anatomically restricted pathology. In fact, most autopsies of PD patients who carry mutations in *parkin* show SN degeneration in the absence of Lewy body pathology (Poulopoulos et al., 2012), and some have argued that AR-JP and PD should be classified as distinct clinicopathologic entities (Doherty et al., 2013). Nevertheless, AR-JP and other Mendelian disorders with prominent parkinsonism highlight numerous genes with established links to mitochondrial function that appear to be similarly required for DA neurons to survive (Table 1). Therefore, studies of these loci will likely provide important mechanistic and perhaps therapeutic insights relevant to PD.

Although only a minority of idiopathic PD cases report a family history (10–30%), these figures likely underestimate the genetic contributions because of the strong influence of age on disease manifestation. Indeed, late-onset PD is now recognized to be influenced by a large number of genetic susceptibility variants, including both common and rare alleles with a range of potency (Shulman et al., 2011; Trinh and Farrer, 2013). Families with autosomal dominant PD have led to the discovery of mutations or gene multiplication at the α -synuclein (*SNCA*) locus (Polymeropoulos et al., 1997; Singleton et al., 2003), as well as mutations in *LRRK2* (Paisán-Ruiz et al., 2004; Zimprich et al., 2004) and *VPS35* (Vilariño-Güell et al., 2011; Zimprich et al., 2011). Whereas *SNCA* mutations are associated with earlier-onset and more aggressive familial disease, clinical presentations in families with *LRRK2* and *VPS35* mutations closely overlap with idiopathic PD. In addition to rare, dominant mutations with high penetrance, investigators have identified additional common susceptibility variants at both the *LRRK2* and *SNCA* loci (Healy et al., 2008; Maraganore et al., 2006).

Table 1 highlights selected genetic causes of PD and related disorders with established connections to mitochondrial biology. As expanded upon below, many such genes have been directly or indirectly associated with mitochondrial defects including abnormal mitochondria morphology, decreased ETC-activity, and altered mitochondrial membrane potential. Table 1 also highlights a number of genes, including *ATP13A2* and *FBXO7*, that cause recessive disorders characterized by prominent parkinsonism along with additional neurologic features not seen in PD. We have limited our discussion to Mendelian causes of parkinsonism in which the causal genes are clearly established. However, large genome-wide association studies have recently identified numerous common polymorphisms with strong statistical evidence of association with PD susceptibility (Int. Parkinson's Dis. Genomics Consort., 2011). Although these genetic loci individually have modest effects on disease risk, they likely have a major effect on PD at the population level because they are common. In the coming years, it will be of great interest to confirm the genes responsible for

associations at these loci and to explore their potential links to mitochondrial and/or other cellular pathways.

PROTEINS ENCODED BY PD LOCI AFFECT DIVERSE MITOCHONDRIAL FUNCTIONS

As described above, mutations in a plethora of genes cause PD (Table 1). Over the past decade, many of these genes were found to directly or indirectly affect mitochondria. Studies in different model organisms revealed their roles in mitochondrial biogenesis, physiology, structure, and dynamics, as well as in quality control.

Deficits in the Electron Transport Chain

Pink1 and Parkin regulate the activity of different ETC complexes—Mutations in *parkin* and *pink1* are two important genetic causes of AR-JP, and the encoded proteins appear to have pivotal roles in regulating mitochondrial function. Much of our understanding originates from pioneering studies on *parkin* and *pink1* homologs in the fruit fly, *Drosophila melanogaster*, where mutations lead to prominent mitochondrial phenotypes in muscles and sperm (Clark et al., 2006; Greene et al., 2003; Park et al., 2006; Pesah et al., 2004). Subsequent genetic analyses have established convincingly that the proteins function in a linear pathway to regulate mitochondrial morphology and to induce clearance of dysfunctional mitochondria, a process termed mitophagy (Figure 1 and see paragraph below).

In addition, recent studies showed that both proteins directly regulate mitochondrial function. Under basal conditions, low levels of the Pink1 kinase span the mitochondrial outer and inner membranes (MOM and MIM), where it acts on respiratory complexes. Wu et al. (2013) recently suggested that Pink1 regulates CI activity in flies by activating a kinase pathway that involves tricorned and mTORC2 (Figure 1). Loss of Pink1 also leads to a reduction in CI activity in flies, mice, and humans (Hoepken et al., 2007; Morais et al., 2009). Moreover, phenotypes observed in *Drosophila pink1* mutants are rescued by Ndi1p, a yeast protein that bypasses CI by transferring electrons from NADH to ubiquinone (Vilain et al., 2012). Although *pink1* mutants display a mild defect in CI activity, Vincow et al. (2013) recently documented that Pink1 regulates the protein turnover of multiple subunits of all 5 ETC complexes (Figure 1).

The E3 ubiquitin ligase Parkin normally localizes to the cytosol and is recruited only to dysfunctional mitochondria. Similar to *pink1* mutants, *parkin* mutant flies display reduced ATP levels (Vos et al., 2012). However, unlike *pink1* mutants, *parkin* mutants do not display CI defects and hence cannot be rescued by Ndi1p expression (Vilain et al., 2012). This finding suggests that Parkin may differentially regulate the activity of various ETC complexes, compared with Pink1. Indeed, Parkin was recently shown to ubiquitinate and thereby regulate the protein levels of many of the ETC subunits in a human cell line (Sarraf et al., 2013; Vincow et al., 2013). These studies suggest that, in addition to displaying CI deficiency, *parkin* mutant flies may display dysfunction of other subunits of the ETC,

similar to results from studies of leukocytes from patients with *parkin* mutations (Müftüoğlu et al., 2004).

A screen for mutants in flies that enhance the *pink1*-associated phenotypes led to the isolation of a modifier, *heixuedian* (*heix*), which encodes a protein that synthesizes vitamin K₂ (Vos et al., 2012). Vitamin K₂ functions as an electron carrier, downstream of complex II (CII), and can therefore compensate for defects in upstream respiratory complexes. Loss of *heix* enhances the *pink1* defects associated with loss of the mitochondrial membrane potential (MMP), ATP production, and motility. Overexpression of *heix* or food supplementation of vitamin K₂ rescues the energy defects and the mitochondrial morphological defects of both *pink1* and *parkin* mutants. Together, these findings suggest that Pink1 and Parkin are required to support ATP production, although both proteins affect the activity of different complexes within the ETC and their functions may turn out to be more complicated than the simple linear pathway suggested here.

DJ-1 supports ATP production—Mutations in DJ-1, which encodes a protein related to a family of molecular chaperones, are a rare cause of AR-JP (Table 1) (Bonifati et al., 2003; Klein and Lohmann-Hedrich, 2007). DJ-1 is expressed in most cell types and localizes to the cytosol, mitochondrial matrix, and intermembrane space (Moore et al., 2006; Zhang et al., 2005). We previously reviewed DJ-1's role in protection against oxidative stress and in PD pathogenesis (Jaiswal et al., 2012). Unlike vertebrates, who have only one copy of *DJ-1*, the *Drosophila* genome encodes two paralogs (*DJ-1α* and *DJ-1β*). When both are deleted, flies display an age-dependent decline in mitochondrial DNA and respiration, leading to reduced ATP levels (Hao et al., 2010). Fly or human *DJ-1* overexpression rescues the phenotypes of *pink1* mutants, suggesting that the two proteins possess at least partially overlapping functions (Hao et al., 2010). In vertebrates, *DJ-1* null mouse embryonic fibroblasts and DA primary neurons display reduced mitochondrial respiration and MMP (Heo et al., 2012; Krebiehl et al., 2010). These cells also display reduced CI assembly and an increased sensitivity to oxidative stress owing to downregulation of mitochondrial uncoupling proteins (UCPs) (Guzman et al., 2010; Heo et al. 2012). In sum, data from various systems suggest that DJ-1 plays a role in maintaining respiratory complex stability and/or MMP, similar to the roles of other genes implicated in PD and related disorders.

α-Synuclein inhibits complex I—As introduced above, aggregation of α-synuclein comprises the defining neuropathology of PD (Lewy bodies). Furthermore, common polymorphisms and rare mutations in α-synuclein have been genetically linked to PD risk (Table 1). Although most studies have focused on the cytoplasmic role of α-synuclein in PD pathogenesis, recent evidence also links the protein to mitochondria (reviewed in Nakamura, 2013). α-Synuclein contains a cryptic mitochondrial targeting signal and the protein accumulates in the SN and striatal mitochondria of PD patients, where it inhibits CI activity (Butler et al., 2012; Devi et al., 2008). Moreover, several groups have reported that overexpression of wild-type or mutant α-synuclein increases ROS levels in various mammalian cell lines (Junn and Mouradian, 2002; Parihar et al., 2009). Elevated ROS levels may enhance mitochondrial translocation of α-synuclein, which is dependent on the phospholipid cardiolipin. Under basal conditions, cardiolipin only localizes to the MIM, but

the lipid becomes more abundant on the MOM under conditions of high oxidative stress (Cole et al., 2008). One possible interpretation is that aggregated or misfolded α -synuclein can inhibit CI activity, increase ROS, and thereby potentiate its own mitochondrial translocation in a vicious cycle that further disrupts ETC function (Figure 1). In this context, it is intriguing that several mitochondrial toxins have also induced α -synuclein aggregation in animal models (Martinez and Greenamyre, 2012).

LRRK2 affects ETC activity—Mutations in *LRRK2* are a common cause of familial PD (Healy et al., 2008). The LRRK2 protein is localized primarily to the cytoplasm and membranes, including the mitochondrial membrane. It functions as a kinase, a GTPase, and a scaffolding protein (Papkovskaia et al., 2012; West et al., 2005). Several lines of evidence support a role for LRRK2 in mitochondria. The MMP and total intracellular ATP levels were reduced both in cells derived from skin biopsies of patients with LRRK2 mutations as well as in human cell lines expressing mutant *LRRK2* (Mortiboys et al., 2010). Furthermore, LRRK2's kinase activity regulates UCPs to maintain MMP and ATP production (Figure 1) (Papkovskaia et al., 2012). In *Drosophila*, overexpression of human, PD-associated mutant *LRRK2* reduces life span and increases sensitivity to rotenone, suggesting a potentially conserved mitochondrial role (Ng et al., 2009). Coexpression of *parkin* substantially rescues these *LRRK2*-induced phenotypes. Moreover, activating AMP kinase, a key cellular regulator of energy metabolism, significantly improves mitochondrial function in both *LRRK2* and *parkin* mutant flies (Ng et al., 2012). Together, these results suggest that Parkin and LRRK2 may operate in a common pathway. Wild-type *LRRK2* overexpression enhanced the viability of DA neurons in *Caenorhabditis elegans* exposed to rotenone (Saha et al., 2009), suggesting that LRRK2 protects against mitochondrial damage. In conclusion, numerous studies implicate mitochondrial impairment in LRRK2-associated PD caused by *LRRK2* mutations. However, additional work is needed to better define the relevant mechanisms and their relationships with other known LRRK2 functions (Kett and Dauer, 2012).

Defects in Protein Homeostasis: The Mitochondrial Unfolded Protein Response

When mitochondria receive a toxic insult that impairs protein folding, they induce a mitochondrial-specific unfolded protein response (UPR^{mt}) (Haynes and Ron, 2010). This response involves a signal that is sent from mitochondria to the nucleus, leading to expression of mitochondrially targeted chaperones and proteases. Although the UPR^{mt} signaling pathway is not well understood, recent studies link mitochondrial chaperones to PD pathogenesis.

The chaperones Mortalin and DJ-1 in the response to oxidative stress—An initial clue linking impaired UPR^{mt} to PD came from the finding that levels of Mortalin (HSPA9), a conserved mitochondrial chaperone of the Hsp70 protein family, are reduced in the SN of PD patients (De Mena et al., 2009; Jin et al., 2006). Mortalin is a multifunctional protein that localizes predominantly to mitochondria and interacts with the translocase of the inner membrane (TIM) complex (D'Silva et al., 2004; Kang et al., 1990). Mortalin may function with another interaction partner, Hsp60, to help refold matrix proteins following TIM-mediated import (Wadhwa et al., 2005). In addition to playing a role in protein import,

Mortalin is implicated in the response to oxidative stress. Reducing Mortalin expression in mammalian cells leads to a collapse of the MMP and a spike in ROS production (Burbulla et al., 2010). Furthermore, in DA primary neurons, Jin et al. identified a physical interaction between Mortalin and DJ-1, which is enhanced upon rotenone treatment (Figure 1) (Jin et al., 2007). Although this finding could support a direct role for Mortalin in the oxidative stress response, the enhanced interaction may also be a consequence of increased mitochondrial DJ-1 levels induced by ROS.

Identification of rare variants in PD cohorts produced genetic evidence that potentially links *Mortalin* and PD (Burbulla et al., 2010; De Mena et al., 2009). Although these results require confirmation, it is notable that mitochondrial phenotypes, induced by knockdown of *Mortalin* expression in mammalian cells, were rescued by wild-type *Mortalin* but not by forms of *Mortalin* harboring PD-associated variants (Burbulla et al., 2010). All mitochondrial impairments could also be rescued by overexpressing *Parkin* (Yang et al., 2011). In addition, Pink1 interacts with Mortalin in vitro (Rakovic et al., 2011), suggesting that the Pink1/Parkin pathway may impinge on regulating this chaperone. Further evidence supporting involvement of the UPR^{mt} in PD etiology came from the finding that α -synuclein physically interacts with Mortalin and that *Mortalin* expression is reduced upon overexpression of wild-type and mutant α -synuclein (Jin et al., 2007). In conclusion, although the precise effects of *Mortalin* loss on neuronal function and maintenance remain unclear, changes in the expression or function of this protein will likely promote sensitivity to subsequent mitochondrial insults.

The chaperone Trap-1 functions with Pink1—Another mitochondrial chaperone that has been linked to PD is TNF-receptor associated protein 1 (Trap1), a member of the Hsp90 family that localizes predominantly to mitochondria (Felts et al., 2000). This chaperone was the first in vivo target identified for Pink1, and evidence proposed that Pink1's function in protecting the cell from ROS-induced cell death depends on Trap1 phosphorylation (Figure 1) (Pridgeon et al., 2007). Similar to a loss of *pink1* or *parkin*, *Drosophila trap1* null mutants display dysfunctional mitochondria as well as an age-dependent decline in motor performance (Costa et al., 2013). Overexpression of Trap1 rescues *pink1*, but not *parkin*-mutant phenotypes, suggesting that Trap1 and Parkin may function in parallel to protect the cell from dysfunctional mitochondria (Zhang et al., 2013b).

In addition to rescuing *pink1* mutant phenotypes in *Drosophila*, *Trap1* expression in rat primary cortical neurons and a human DA cell culture protected DA neurons from α -synuclein-induced toxicity (Butler et al., 2012). Although investigators initially interpreted these data as linking α -synuclein overexpression and mitochondrial dysfunction, further studies showed that Trap1 can also induce a more general ER-mediated UPR. Thus, the observed protective effect of Trap1 may, in fact, be mediated by an increased ability of the cell to cope with unfolded cytoplasmic proteins (Takemoto et al., 2011).

Mitochondrial Hsp60 may play a role in the Pink1/Parkin pathway—The third mitochondrial chaperone that may be associated with PD is mitochondrial Hsp60. This key player of the UPR^{mt} interacts in vitro with both Pink1 and Parkin (Davison et al., 2009; Rakovic et al., 2011). In addition, mitochondrial Hsp60 is downregulated by about 40%

upon loss of Pink1 in a DA neuronal cell culture (Kim et al., 2012). Although this observation requires confirmation, Parkin and/or Pink1 may interact with Hsp60 to shut down the UPR^{mt} when a mitochondrion is deemed too damaged to save. Alternatively, if the UPR^{mt} is extended, Hsp60 might interact with both proteins to induce mitophagy.

Taken together, because PD has been associated with mitochondrial dysfunction and a concomitant increase in ROS, one might expect to find the UPR^{mt} to be activated in PD model systems. However, several components of the UPR^{mt} have instead been found to be either reduced or dysfunctional in such models, and consistent observations have been made in PD patients. Furthermore, experiments in mammalian cell cultures showed that the three chaperones (mitochondrial Hsp60, Mortalin, and Trap1) control the gating of the mitochondrial permeability transition pore (Ghosh et al., 2010; Qu et al., 2012). Hence, their loss may sensitize a cell for apoptotic death, owing to a reduced threshold for pore opening and release of cytochrome c. *Pink1*-mutant mice similarly show a lower threshold for opening of the mitochondrial transition pore and are indeed more susceptible to toxic insults (Morais et al., 2009). Whether this observation is due to a change in the activity or levels of any of the aforementioned chaperones remains to be determined.

Defects in Mitochondrial Dynamics

As mitochondria age or are exposed to environmental toxins, they accumulate numerous mitochondrial DNA mutations and protein insults that impair their function and may lead to cell death if repair does not occur. It is therefore important for a cell to maintain a healthy pool of mitochondria. Several protective measures exist to restore or eliminate dysfunctional mitochondria. These processes include mitochondrial fusion and fission, mitochondrial trafficking, and the clearance of dysfunctional mitochondria via mitophagy. These three processes play a critical role in mitochondrial quality control, especially in cells that consume much energy and do not divide, such as neuronal cells (Itoh et al., 2012; Schon and Przedborski, 2011). Several genes linked to PD and related parkinsonian disorders have been implicated in the regulation of mitochondrial dynamics, suggesting that failure of these mechanisms may promote disease pathogenesis.

Tipping the balance toward demise: most PD loci alter fusion/fission—In response to mitochondrial dysfunction, fusion mixes key constituents, potentially complementing deficiencies from damaged proteins, DNA, and/or membrane lipids. Among the required cellular machinery, mitofusin (Mfn) and optic atrophy 1 (Opa 1) mediate fusion of the outer and inner mitochondrial membranes, respectively. Conversely, mitochondrial fission requires dynamin related protein 1 (Drp1). Loss of Mfn or Opa1 leads to small, fragmented mitochondria, whereas the loss of Drp1 causes a network of large interconnected mitochondria (Chan, 2012; Youle and van der Bliek, 2012). Hence, a constant balance of fission and fusion is required to maintain a healthy pool of mitochondria. Mutations in Mfn2 lead to Charcot-Marie-Tooth type 2A (Züchner et al., 2004), an inherited peripheral neuropathy, and loss of Opa1 leads to autosomal dominant optic atrophy (Eiberg et al., 1994). Loss of Drp1 causes a rare infantile mitochondrial encephalopathy, consisting of early neurologic failure and death (Waterham et al., 2007). Although these genes are not

known to be genetically linked with PD themselves, they do show convincing interactions with established PD genes in model organisms.

In *Drosophila*, mutations in *pink1* or *parkin* lead to aberrant mitochondria that are swollen and lose their typical cristae structure (Clark et al., 2006; Greene et al., 2003; Park et al., 2006). Reducing mitochondrial fusion or increasing fission in either *pink1* or *parkin* mutant flies restores the morphological defects (Deng et al., 2008; Poole et al., 2008). However, although adding one copy of *drp1* partially restores energy levels in a *pink1* mutant, it fails to restore CI-activity (Liu et al., 2011; Vilain et al., 2012). Finally, whereas *pink1* or *parkin* mutant flies are viable, removing only a single copy of *drp1* in a *pink1* or *parkin* mutant is fatal (Poole et al., 2008). These genetic interactions strongly suggest that the functions of Parkin and Pink1 and mediators of mitochondrial fusion/fission may be linked.

Several groups have reported that round, fragmented mitochondria appear upon overexpression of either wild-type or mutant forms of α -synuclein, both in vitro as well as in vivo (Kamp et al., 2010; Nakamura et al., 2011). The ability of α -synuclein to induce membrane fragmentation is specific for mitochondrial membranes and appears to be independent of the fission protein Drp1 (Nakamura et al., 2011). Indeed, an age-dependent decrease in both Mfn1 and Mfn2 was observed in neurons of mice overexpressing mutant α -synuclein (Xie and Chung, 2012), suggesting a possible pathological role for α -synuclein in reducing mitochondrial fusion.

Studies recently demonstrated that LRRK2 plays a role in regulating mitochondrial dynamics in *Drosophila* DA neurons. Overexpression of mutant, but not wild-type, human LRRK2 resulted in enlarged mitochondria resembling those seen in *pink1* or *parkin* mutants (Ng et al., 2012). These defects were significantly rescued by *parkin* coexpression, suggesting that these proteins may function in a common pathway (Ng et al., 2009). Skin biopsies from patients with *LRRK2* mutations displayed increased mitochondrial length and interconnectivity, decreased ATP levels, and MMP (Mortiboys et al., 2010). In mouse cortical primary neurons, LRRK2 also interacts with and phosphorylates the fission protein Drp1 (Niu et al., 2012). Overexpressing wild-type and mutant *LRRK2* in this system, as well as in primary DA neurons, results in recruitment of Drp1 to the mitochondrial membrane leading to mitochondrial fragmentation and clearance (Niu et al., 2012; Wang et al., 2012b). Similar to *LRRK2*, mutations in *DJ-1* result in increased Drp1 levels and increased mitochondrial fragmentation, although the underlying mechanisms remain elusive (Wang et al., 2012a). These studies suggest that LRRK2 and DJ-1 play important roles in regulating mitochondrial dynamics and quality control, although additional work is needed to understand the mechanisms (Figure 1).

In sum, altered mitochondrial fusion/fission is a feature observed in many experimental systems relevant to PD. However, because enhanced mitochondrial fusion and/or fission can be a response to injury, many of the observations described here could be secondary in nature.

Mitochondrial trafficking is controlled by Pink1 and Parkin—Mitochondrial trafficking delivers a mobile source of ATP to subcellular locations that have high energy

demands, such as the neuronal synapse (Saxton and Hollenbeck, 2012; Sheng and Cai, 2012). Newly formed mitochondria travel along microtubules using kinesins and adaptor proteins that link mitochondria to the motor proteins (Saxton and Hollenbeck, 2012; Sheng and Cai, 2012). Specifically, in flies, Pink1 phosphorylates Miro, a rho-like GTPase that resides on the MOM, where it functions as an adaptor to microtubule motor proteins (Figure 1) (Liu et al., 2012; Wang et al., 2011). Phosphorylation of Miro triggers its degradation via a Parkin-dependent ubiquitination pathway and causes mitochondria to detach from cognate kinesin motors. Because Pink1 is activated upon mitochondrial dysfunction (see next section), this action may serve as a mechanism to block dysfunctional mitochondria from moving along the axon.

Miro and the adaptor protein Milton, which links mitochondria to the heavy chain of kinesin, form a complex with the fusion protein Mfn, which is also targeted for degradation by Parkin in both flies and mammals (Glater et al., 2006; Glauser et al., 2011; Górska-Andrzejak et al., 2003; Ziviani et al., 2010). In addition, genetically altering the mitochondrial fission/fusion balance in a wild-type background impedes mitochondrial trafficking, leading to synapses and dendrites that are devoid of mitochondria (Liu et al., 2012; Sheng and Cai, 2012). Together, these findings suggest a tight link between regulators of mitochondrial dynamics and intracellular trafficking. Pink1 and Parkin affect both processes, possibly to ensure the presence of sufficient functional mitochondria at subcellular locations with energy-intensive demands. Because LRRK2 and DJ-1 each regulate Drp1 levels, they may indirectly regulate mitochondrial trafficking as well (Niu et al., 2012; Wang et al., 2012a).

Impaired clearance of dysfunctional mitochondria in PD—When mitochondria can no longer be repaired, they are cleared through a form of autophagy known as mitophagy (Narendra et al., 2010). For degradation, mitochondria initially undergo fragmentation, allowing autophagosomes to engulf them. The protein machinery that mediates fission and fusion is essential for this process, and genetic manipulations that inhibit fission or enhance fusion interfere with mitophagy (Gomes et al., 2011).

Under normal conditions, the ubiquitously expressed Pink1 spans both the MOM and the MIM (Jin et al., 2010; Weihofen et al., 2009). Within the MIM, it is cleaved by two proteins [mitochondrial processing peptidase (Greene et al., 2012) and presenilin-associated rhomboid-like protease (Jin et al., 2010)], after which it is thought to be degraded (Matsuda et al., 2013). Under basal conditions, Pink1 protein levels are therefore kept low. Once a mitochondrion becomes dysfunctional and loses its MMP, the import of Pink1 into the MIM is blocked, and it is no longer cleaved. Pink1 therefore quickly accumulates on the MOM, where it initiates mitophagy (Jin and Youle, 2012; Youle and Narendra, 2011). It does so by recruiting and activating the E3 ubiquitin ligase Parkin, a process that requires Pink1's kinase activity (Kim et al., 2008). However, whether Pink1 acts directly on Parkin or whether it indirectly recruits Parkin to the MOM is still under debate (Iguchi et al., 2013; Kim et al., 2008; Narendra et al., 2010). In our opinion, the data are most consistent with a model where Pink1 phosphorylates several proteins, one of which would subsequently recruit Parkin. One potential candidate would be the Pink1-target MFN2, which is required to recruit Parkin to damaged mitochondria (Chen and Dorn, 2013).

Upon activation, Parkin functions with E2 ubiquitin-conjugating enzymes, such as the recently identified Rad6, to ubiquitinate its targets (Haddad et al., 2013). Over the past few years, investigators have identified many substrates for parkin-dependent ubiquitination, including numerous mitochondrial proteins (Figure 1) (Sarraf et al., 2013). Overall, activation of the Pink/Parkin pathway halts the trafficking of a dysfunctional mitochondrion, initiates its fragmentation, and leads to its subsequent degradation. In addition, the Pink1/Parkin pathway appears to support mitochondrial biogenesis. Shin et al. recently found that Parkin-activation leads to the degradation of Paris, which represses the transcription of a key regulator of mitochondrial biogenesis, PGC1- α (Shin et al., 2011).

Because most of the mitophagy-related research has focused on Pink1 and Parkin, the question arises whether impaired mitophagy is a general theme in the pathogenesis of PD. A recent study in mice found that overexpressing wild-type or mutant α -synuclein induces mitophagy (Sampaio-Marques et al., 2012). Similarly, overexpressing mutant LRRK2 in mouse cortical neurons results in autophagic degradation of mitochondria (Cherra et al., 2013). In conclusion, multiple aspects of mitochondrial dynamics appear to be affected to various extents in mutants for PD-related loci (Table 1).

MITOCHONDRIA AND PD: AN INTEGRATED MODEL

As summarized in this review, numerous genes associated with familial forms of PD and related disorders with prominent parkinsonism can be linked to mitochondrial mechanisms. In addition, mitochondrial toxins cause SN degeneration in both humans and various other model systems. These data support models in which mitochondria play a central role in DA neuronal loss in PD. It is remarkable that even in cases of AR-JP caused by mutations in *parkin* or *pink1*, where mitochondrial dysfunction seems to be the primary insult, the overall effect on mitochondrial function appears subtle. This conclusion is based in part on studies of model organisms, where loss of homologous genes causes a mild reduction of CI activity and does not significantly impact viability (Morais et al., 2009; Vilain et al., 2012). A similar partial CI deficiency has been documented in postmortem SN tissue from PD patients (Bindoff et al., 1991; Parker et al., 1989, 2008; Schapira et al., 1989). In humans, mutations that more severely affect ETC function are associated with correspondingly more aggressive disease. For instance, Leigh syndrome, a mitochondrial encephalopathy, has an infantile onset, affects multiple organ systems, and is often fatal (Vafai and Mootha, 2012). Consistent with these observations, mutations in Leigh syndrome associated gene homologs in mice or flies are lethal [e.g., Sco2 (Porcelli et al., 2010; Yang et al., 2010), LRPPRC (Ruzzenente et al., 2012), C8ORF38 (Zhang et al., 2013a), SURF1 (Agostino et al., 2003, Zordan et al., 2006), among others].

Although the overall extent of mitochondrial dysfunction may be modest, one important lesson from studies of Mendelian forms of PD and related parkinsonian disorders is that the responsible genetic lesions appear to have distributed effects on several core features of mitochondrial biology, including (a) the ETC, (b) the UPR^{mt}, and (c) mitochondrial dynamics. As shown in Figure 2, we propose that simultaneous disruptions in these core systems interact and overwhelm the capacity of the mitochondria to compensate for additional insults, promoting a vicious cycle that may ultimately result in cell death.

However, given the near-universal requirement of mitochondria in all eukaryotic cells, this model does not explain the selective vulnerability of certain neuronal subtypes in PD, such as DA neurons in the SN. Therefore, apart from affecting these core mitochondrial processes (a, b, and c), an additional insult (“second hit”) is likely required, tipping the balance toward severe dysfunction in distinct cellular contexts. In the case of DA neurons, elevated endogenous ROS levels may fulfill the second-hit requirement (see sidebar, *Why Are DA Neurons More Susceptible To Stress Than Are Other Neurons?*). For example, in an individual with juvenile parkinsonism due to *parkin* mutations (hit 1), endogenous ROS levels (hit 2) may be augmented to toxic levels owing to baseline deficiencies in the ETC. This action may cause oxidized, misfolded, and dysfunctional proteins to accumulate, which then overwhelms the already-strained UPR^{mt}. As mitochondrial function further deteriorates, clearance mechanisms fail to respond properly, owing to impaired mitochondrial dynamics. Ultimately, injured mitochondria accumulate within neurons, causing progressive functional derangements and eventually cell death.

Compared with the genetic causes of AR-JP, our understanding of the potential mitochondrial impact of dominant causes of PD, such as that due to *SNCA* and *LRRK2* mutations, is less well developed. However, accumulating evidence (detailed above) indicates that α -synuclein aggregates and/or *LRRK2* dysfunction may cause similar, distributed effects on mitochondria, in which case the two-hit model may also apply.

A key remaining question is whether in the absence of known Mendelian mutations, as is true in the vast majority of idiopathic PD cases, primary mitochondrial failure is also a primary cause of neurodegeneration. Alternatively, mitochondrial dysfunction may secondarily follow from other upstream, cellular insults. A third possibility is that neuronal injury and death result from a more complex, bidirectional interplay between mitochondria and other cellular processes, including oxidative metabolism, lysosomal degradation, and vesicle trafficking. Nevertheless, our mitocentric view favors a central, causal role in all PD cases, and we further suggest that the multi-hit model provides an excellent, generalizable framework (Figure 2). Idiopathic PD is currently best understood as a complex genetic disorder that is likely due to the interaction between numerous genetic susceptibility factors and that is modified by the environment and aging. These varied risk factors may therefore constitute multiple hits, and we speculate that as the genetic and nongenetic factors are better defined, many will be found to impinge on the core mitochondrial impairments (ETC, UPR^{mt}, and dynamics) that we have come to understand from the investigation of familial PD.

Thus, we speculate that, collectively, many inherited or de novo PD risk variants are analogous to the single genetic lesion owing to loss of *parkin* or *pink1* in AR-JP, resulting in broad effects on mitochondrial function. Such PD risk variants are predicted to cumulatively degrade mitochondrial reserve mechanisms, establishing a baseline increased susceptibility profile. Although it is necessary, this context alone is likely insufficient. Additional hits, including environmental factors such as mitochondrial toxins and aging, are likely needed to set the disease process in motion. α -Synuclein is likely a key player, given the evidence of a potential feedback loop between mitochondrial toxicity and enhanced aggregation (see above and Figure 2). Furthermore, because α -synuclein pathology is now recognized to have

widespread effects in many cell types beyond the SN, key clinical and pathologic differences may be explained between idiopathic PD and AR-JP, where α -synuclein pathology is not typically seen and neurodegeneration does not appear to extend outside the SN. A more thorough investigation of mitochondrial mechanisms may not only help us identify additional risk factors, but ultimately help explain how such factors interact to trigger the onset and progression of disease, providing important clues to new and successful therapeutic strategies for the future.

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Definitions

PD

Parkinson's disease

Substantia nigra (SN)	pigmented region of the midbrain (due to melanin) containing dopaminergic neurons participates with other basal ganglia nuclei in movement control
Ubiquitin proteasome system	the system that tags misfolded or damaged proteins with ubiquitin and degrades them in the proteasome
Complex I (CI)	large protein complex, located in the inner mitochondrial membrane, that oxidizes nicotinamide adenine dinucleotide (NADH) to transfer electrons from NADH to ubiquinone
Electron transport chain (ETC)	5 protein complexes that produce ATP via an electrochemical proton gradient across the inner mitochondrial membrane by coupling electron transfer between electron donors and acceptors
Mitochondrial unfolded protein response (UPR^{mt})	mitochondrial stress response to an accumulation of unfolded or misfolded proteins in the matrix of mitochondria
Autosomal recessive juvenile parkinsonism (AR-JP)	familial form of early-onset parkinsonism, usually characterized at autopsy by substantia nigra degeneration in the absence of Lewy bodies
Idiopathic PD	most common form of PD, characterized by late-onset, non-Mendelian inheritance and pathologically defined by nigral degeneration and Lewy bodies
Genome-wide association study	unbiased analysis of polymorphisms throughout the human genome to identify variants associated with traits at the population level
Mitophagy	form of autophagy that degrades mitochondria
Ndi1p	<i>S. cerevisiae</i> protein that catalyzes the oxidation of NADH and the reduction of ubiquinone and can hence bypass CI
E3 ubiquitin ligase	enzyme that transfers ubiquitin from E2 ubiquitin-conjugating enzymes to target proteins
Mitochondrial membrane potential (MMP)	electrochemical gradient across the inner mitochondrial membrane that is essential to generate ATP
Chaperone	protein that interacts with unfolded or partially folded proteins to facilitate correct folding or unfolding
Uncoupling proteins (UCPs)	members of the family of mitochondrial anion carrier proteins that dissipate the proton gradient across the inner mitochondrial membrane

Cardiolipin

mitochondria-specific phospholipid that regulates many processes such as mitochondrial dynamics, in addition to various signaling pathways

Translocase of the inner membrane (TIM) complex

protein complex that facilitates the translocation of nuclear-encoded mitochondrial proteins from the intermembrane space into the matrix

WHY ARE DA NEURONS MORE SUSCEPTIBLE TO STRESS THAN ARE OTHER NEURONS?

What makes DA neurons more vulnerable to oxidative stress than other neurons has been a conundrum for PD. One view is that oxidation of cytosolic DA and its metabolites leads to the generation of cytotoxic free radicals. Oxidized DA forms covalent bonds with several proteins, including mitochondrial Trap1, a CI subunit, DJ-1, and even α -synuclein, rendering the latter more prone to aggregation (Van Laar et al., 2009). Potentially inconsistent with this model, however, levodopa (a form of DA) is among the safest and most effective treatments for PD, and large clinical trials do not support enhanced disease progression (Fahn et al., 2004). Alternatively, a cell's activity pattern may render it more susceptible to subsequent toxic insults. Indeed, DA neurons contain pacemaker properties (Guzman et al., 2010). This continuous activity requires high amounts of ATP and therefore imparts a demand on the cell to function properly. Pacemaking activity is found in DA neurons of the SN but not in those of the adjacent ventral tegmental area, which also use DA but are relatively spared in PD. Thus, both ROS production via DA oxidation and pacemaker firing properties may converge to explain the susceptibility of SN DA neurons in PD.

Figure 1.

Proteins implicated in Parkinson's disease (PD) maintain a healthy pool of mitochondria. The effects of PD-linked proteins (*thick green line*) on mitochondrial function can be divided into three different groups. Bioenergetics (*yellow*): Pink1 and Parkin regulate the turnover of several subunits of ETC complexes by the ubiquitin proteasome system (UPS). In addition, Pink1 supports complex I activity through a kinase cascade involving *tricornered* (*trc*) and mTORC2. Mitochondrially localized α -synuclein (α -syn), however, inhibits complex I. Oxidation of the mitochondrial phospholipid cardiolipin translocates this lipid from the inner to the outer mitochondrial membrane where it serves as a receptor for α -synuclein. Hence, in the presence of elevated levels of reactive oxygen species (ROS), mitochondrial α -synuclein import is increased. LRRK2 and DJ-1 regulate the activity of uncoupling proteins (UCPs), which preserve the mitochondrial membrane potential (MMP). Both PD proteins therefore indirectly maintain ATP production. Mitochondrial unfolded protein response (UPR^{mt}, *blue*): When activated, Pink1 phosphorylates the chaperone Trap1, a protein that protects mitochondrial proteins from ROS. In addition, Trap1 works with Hsp60 to refold imported proteins, sustaining mitochondrial protein homeostasis. In the presence of ROS, DJ-1 is translocated into mitochondria, where it interacts with the chaperone Mortalin to salvage oxidized proteins. Mitochondrial dynamics (*orange*): LRRK2 directs mitochondrial fission as it interacts with and recruits the mitochondrial fission protein Drp1. DJ-1 increases mitochondrial fission by regulating Drp1 levels, although the

exact mechanism through which the chaperone executes this function is not clear. The E3 ubiquitin ligase Parkin inhibits mitochondrial fusion through ubiquitin-mediated degradation of the fusion protein Mitofusin (Mfn). Mitochondrial transport is regulated by the Pink1/Parkin pathway. Here, Parkin-mediated degradation of the adaptor protein Miro is thought to detach a dysfunctional mitochondrion from kinesin motor proteins, halting its transport. Finally, when a dysfunctional mitochondrion cannot be repaired, it is cleared through mitophagy. Under basal conditions, the protein kinase Pink1 is imported into the mitochondrial intermembrane space, where it is cleaved by 2 proteases and subsequently degraded. However, upon mitigation of the MMP, Pink1 is no longer cleaved. The protein then phosphorylates and activates the E3 ubiquitin ligase Parkin. Parkin, along with E2 ligases such as Rad6, initiates mitophagy by ubiquitinating target proteins.

Figure 2.

The multiple hit model of Parkinson's disease (PD). Top panel: In familial parkinsonism, for example due to loss of function of Parkin, several key aspects of mitochondrial homeostasis are mildly affected, including energy production, protein folding (UPRmt), or dynamics (fission and fusion). These defects interact and amplify one another, but a second hit, such as elevated endogenous reactive oxygen species (ROS) or cellular activity, is likely required to trigger neuronal dysfunction and loss in certain vulnerable cell types, such as in DA neurons. Bottom panel: In idiopathic PD, multiple hits, including both common and rare genomic variations at diverse susceptibility loci, may in combination cause similar, subtle, and distributed defects in mitochondrial function. α -Synuclein pathology, ROS, potential environmental factors, and the widespread cellular effects of aging further degrade

mitochondrial activity. In a potential feedback mechanism, mitochondrial dysfunction may also promote α -synuclein aggregation, which in turn may further amplify mitochondrial defects, ultimately leading to neuronal dysfunction and cell death.

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Gene	Mitochondrial phenotypes				References
	Function	Fly	Rodent	Human	
		<p><i>Lof^{d1}</i>; (↓) mtDNA level; (↓) respiration; (↓) ATP level</p> <p>Mito morphology</p> <p><i>Lof^{d1}</i>; abnormal</p>	<p><i>Lof^e</i>; (↓) CI activity; (↓) CI assembly; (↓) ATP; (↓) respiration; (↓) ψ_m; (↓) UCPs expression</p> <p>Mito morphology/dynamics</p> <p><i>Lof^e</i>; (↑) fragmentation; (↓) fusion <i>Mutant O/E^c</i>; (↑) Fragmentation</p>	<p><i>Lof^e</i>; (↓) ψ_m</p> <p>Mito morphology/dynamics</p> <p><i>Lof^e</i>; (↑) fragmentation <i>Mutant O/E^c</i>; (↑) fragmentation <i>WT O/E^c</i>; (↑) elongated mitochondria</p>	<p>al. 2010, Hao et al. 2010, Heo et al. 2010, Irrcher et al. 2010, Krebichl et al. 2010, Kwon et al. 2011, Nagakubo et al. 1997, Thomas et al. 2011, Wang et al. 2012a</p>
<i>LRRK2</i> Dominant	Kinase/GTPase activity	<p>Mito morphology</p> <p><i>Mutant O/E^b</i>; abnormal</p>	<p>Mito functions</p> <p><i>Mutant O/E^c</i>; (↓) ψ_m</p> <p>Mito morphology/dynamics</p> <p><i>WT and mutant O/E^c</i>; (↑) fission</p>	<p>Mito function</p> <p><i>Lof^e</i>; (↓) ψ_m; (↓) ATP; (↓) CI, CII, CIV; (↑) UCPs expression <i>Mutant O/E^c</i>; (↓) ψ_m; (↑) UCPs expression</p> <p>Mito morphology/dynamics</p> <p><i>Mutant O/E^c</i>; (↑) fragmentation</p>	<p>Cherra et al. 2013, Moriboy et al. 2010, Niu et al. 2012, Paisán-Ruiz et al. 2004, Papkovskaia et al. 2012, Wang et al. 2012b</p>
<i>ATP13A2</i> Recessive	Lysosomal ATPase	NA	<p>Mito morphology</p> <p><i>Lof^e</i>; (↑) fragmentation</p>	<p>Mito function</p> <p><i>Lof^e</i>; (↓) ATP; (↑) mtDNA level; (↑) oxygen consumption rate</p> <p>Mito morphology</p> <p><i>Lof^e</i>; (↑) fragmentation</p>	<p>Grünewald et al. 2012, Ramirez et al. 2006, Ramonet et al. 2012, Schultheis et al. 2004</p>
<i>FBXO7</i> Recessive	E3 ubiquitin protein ligase subunit	NA	NA	<p>Mito function</p> <p><i>Lof^e</i>; (↓) Parkin recruitment to mitochondria</p>	<p>Burchell et al. 2013, Ilyin et al. 2000, Shojaei et al. 2008</p>
<i>Vps35</i> Dominant	Subunit of the retromer complex	NA	NA	<p>Mito function</p> <p><i>Lof^e</i>; (↓) delivery of MAPL from mitochondria to peroxisomes</p>	<p>Braschi et al. 2010, Edgar & Polak 2000, Vilarinho-Güell et al. 2011</p>

^a Abbreviations: CI, complex I; CIV, complex IV; CV, complex V; ETC, electron transport chain; *Gof*, gain-of-function; *Lof*, loss-of-function; MAPL, mitochondrial-anchored protein ligase; mtDNA, mitochondrial DNA; NA, not available; O/E, overexpression; UCPs, mitochondrial uncoupling proteins; WT, wild type; ψ_m , mitochondrial membrane potential.

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- ^b Overexpression in vivo animal model.
- ^c Overexpression in vitro cell culture.
- ^d Loss of function in vivo animal model.
- ^e Loss of function in vitro cell culture.