Mitochondria in Parkinson Disease

Back in Fashion With a Little Help From Genetics

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Parkinson disease is a devastating neurodegenerative disorder with no known cure. Impairment in mitochondrial dysfunction is thought to play a major role in the pathogenesis. Recent genetic advances suggest that mitochondrial dysfunction may be the primary defect. Drugs that target the mitochondria may therefore represent the best hope for disease-modifying therapies in Parkinson disease.

Arch Neurol. 2006;63:649-654
THE PREGENETIC ERA

The discovery of the effects of MPTP in humans had 3 major forms of impact. First, MPTP was able to recapitulate many of the features of parkinsonism in animals, including mice and primates, and provided researchers with robust and reproducible animal models of PD that could be used to study mechanisms of neuronal loss as well as test potential therapeutic compounds.7 Second, MPTP was demonstrated to be a selective inhibitor of complex I of the electron transport chain, and complex I inhibition and hence mitochondrial dysfunction have been suggested to play a causal role in PD.8 Third, the discovery of the effects of MPTP rekindled interest in the environmental toxin hypothesis of PD and motivated an extensive search into MPTP-like substances among pesticide compounds that had previously been linked to PD development via epidemiologic studies. A biochemical link between the effects of MPTP and idiopathic PD was established when several groups demonstrated that complex I was selectively reduced in the substantia nigra of the human brain, the major site of neuronal loss.3–5 Since then complex I defects have been demonstrated in a variety of other tissues, including muscle and platelets of patients with PD.9 The most compelling evidence to suggest a primary role of complex I dysfunction in PD pathogenesis has been the demonstration that rats developed parkinsonism after exposure to the herbicide rotenone, another complex I inhibitor. In addition to levodopa-responsive symptoms, the rats exhibited both selective dopaminergic neuronal loss and remarkably Lewy body–like inclusions.10

GENETICS

The entire structure and function of cells are encoded by thousands of genes present mainly within our chromosomes in the nucleus (nuclear DNA). Mitochondrial DNA is the only source of extranuclear DNA within cells and consists of a circular genome. Human mitochondrial DNA encodes just 13 proteins that are all components of the electron transport chain and OXPHOS system. However, the remaining 850 proteins required for mitochondrial structure and function are encoded by the nuclear DNA.

Studying the genetic basis of PD informs us in 2 ways. First, it enables us to identify mutations in genes that cause mendelian-inherited PD. Although familial PD makes up a small proportion of PD overall, the discovery of these genes has implicated novel molecular pathways that may be involved in all forms of PD. Second, adopting a population genetics approach informs us about the relationship between common variation in certain genes and its role in increasing susceptibility to PD.

MITOCHONDRIAL GENETICS AND PD

Studies11,12 that use cell cybrid systems in which platelets from patients with PD with complex I defects are fused with mitochondrial-deficient cell lines (p0 cells) show transmission of the complex I defect to the cybrid cells and strongly suggest that complex I defects originate from the mitochondrial genome. These cybrid lines are associated with increased oxidative stress and were recently found to be sufficient to cause the formation of aggregates, suggesting a link to Lewy bodies.13 Furthermore, Swerdlow et al14 reported a kindred in which both the apoC gene and the tRNAGlu gene were transmitted as a Mendelian trait. In addition, a cluster of haplogroups, namely, J, T, U, and K, were all less common in PD cases compared with controls, but it was significant only when all the haplogroups were pooled. A meta-analysis of association studies (published between 1966 and 1999) reported that the only mitochondrial variant positively associated with PD was the 4366A>G polymorphism in the ND3 gene.22 The mechanism by which these mitochondrial variants alter mitochondrial function remains unclear, and there is no direct evidence that these variants reduce reactive oxygen species generation. Moreover, using a tagging single nucleotide polymorphism approach, our laboratory did not find such an association between mitochondrial variants and PD in 800 white patients with PD (P. Sleiman, unpublished data, 2005).

NUCLEAR GENES AND PD

The use of classic positional cloning strategies has heralded an exciting wave of PD research, leading to the discovery of 6 nuclear genes im-

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plicated in mendelian forms of PD: α-synuclein, parkin, DJ-1, UCH-L1, PINK1, and LRRK2. Most recently, mutations in another gene, Omi/HtrA2, have been identified in sporadic PD cases. The ubiquitin proteasome system (UPS) is an essential pathway that is involved in the removal of misfolded and damaged proteins from neurons. The discovery of α-synuclein and the reports that mutations of α-synuclein could interfere with the UPS suggested that defective proteolysis was an important pathway in PD pathogenesis. Moreover, the discovery of mutations in UCH-L1 and particularly the E3 ubiquitin ligase, parkin, provided the strongest evidence of the role of UPS dysfunction in PD pathogenesis. Extrapolating from these studies, McNaught et al demonstrated that inhibition of the proteasome with natural inhibitors could also reproduce parkinsonism in animals. How does UPS dysfunction fit into our understanding of PD from a mitochondrial perspective? Since the UPS requires adenosine triphosphate, UPS dysfunction would be predicted to occur as a downstream consequence of impairment of oxidative phosphorylation. Furthermore, the UPS is critical for the removal of damaged proteins, including oxidized proteins that might accumulate from oxidative stress secondary to mitochondrial dysfunction. Hence, there appears to be close interplay between these 2 processes, with dysfunction in one likely to lead to dysfunction in the other by feed-forward and feedback mechanisms.

A primary role of oxidative stress and mitochondrial dysfunction in PD has finally been confirmed by mutations in nuclear-encoded mitochondrial genes, including PINK1, DJ-1, and most recently Omi/HtrA2. PINK1 is a mitochondrial protein kinase that may play an important role in protecting mitochondria from oxidative stress and apoptosis. This protection is lost by a G309D missense mutation of the gene, and it was recently demonstrated that this mutation results in reduced kinase activity, suggesting that phosphorylation of as yet unknown substrates may be critical in conferring protection for neurons after oxidative stress. Another recent study has also confirmed that PINK1 protects against apoptotic stress and showed that overexpressed wild-type PINK1 reduced cytochrome c release, caspase cleavage, and poly(adenosine-diphosphate-ribose) polymerase cleavage. Notably, these effects were abrogated by a “kinase dead” mutant, thus supporting the notion that the protective effect of PINK1 is mediated by phosphorylation.

Although the function of DJ-1 remains unknown, studies of the endogenous protein confirm that DJ-1 is localized to the matrix and the intermembranous space of mitochondria under normal conditions. Moreover, recent data from DJ-1 knockout mice revealed increased sensitivity of the mice to MPTP, suggesting that DJ-1 may play a role in mitochondria to reduce oxidative stress. This is supported by in vitro data showing that DJ-1 can reduce the formation of reactive oxygen species in cell lines and that it undergoes transformation to an alternative species with a more acidic isoelectric point in the presence of oxidative stress. Interestingly, the protease Omi/HtrA2 is also localized to the intermembranous space, where it is released into the cytosol during apoptosis and removes the inhibition of caspases. It remains unknown whether Omi/HtrA2 interacts with DJ-1 or PINK1.

Fundamentally important discoveries in Drosophila melanogaster and mice models of parkin mutations have revealed a critical role of parkin in the maintenance of mitochondrial function and protection against oxidative stress. In Drosophila parkin mutants, Whitworth et al observed mitochondrial abnormalities and increased oxidative damage associated with loss of a specific cluster of dopaminergic neurons. Moreover, overexpression of glutathione S-transferase rescued flies from neurodegeneration. In parkin knockout mice, nigrostriatal dysfunction was associated with decreased expression of proteins involved in mitochondrial function, including complex I subunits. In vitro studies have also shown that parkin can protect against ceramide-induced mitochondria-dependent apoptosis with reduction in cytochrome c release. The same study also reported parkin localization in the outer mitochondrial membrane. Thus, parkin may play a role in degrading oxidized mitochondrial proteins and be a critical point of convergence for the UPS and mitochondria.

Although α-synuclein is localized predominantly in synaptic terminals, significant evidence links α-synuclein mutations to oxidative stress. Overexpression of mutant α-synuclein sensitizes neurons to mitochondrial toxins, such as MPTP and 6-hydroxydopamine, and interestingly one study reported that α-synuclein knockout mice are more resistant to MPTP.

Although all these studies support mitochondrial dysfunction and highlight a close link to the UPS, further work is necessary to dissect these pathways and determine how the genes interact. Data are accumulating on an interaction between parkin and α-synuclein, and recently parkin was also shown to associate with and enhance the stability of mutant DJ-1, although this does not appear to be via parkin-dependent ubiquitylation. It remains unknown how the 2 most recently discovered genes, PINK1 and LRRK2, interact with the known genes.

RELEVANCE TO CLINICAL PRACTICE

Robust biological data exist to support the development of mitochondrial agents in the treatment of PD (Figure). However, drug development in humans requires agents to be safe and well tolerated as well as being able to penetrate the blood-brain barrier to achieve efficacious concentrations, and these constraints have possibly accounted for why relatively few compounds to date have been evaluated in human trials. Furthermore, sensitive biomarkers to monitor treatment effects are lacking. Nevertheless, some notable successes have occurred. The compounds can be divided into those that (1) modulate the mitochondrial electron transport chain, (2) modulate mitochondrial apoptosis via effects on mitochondrial outer membrane permeabilization, and (3) generally reduce oxidative stress in mitochondrial...
Encouraging results have been reported for coenzyme Q₁₀ (CoQ₁₀), an antioxidant and electron transporter for mitochondrial complexes II and III. Previously, CoQ₁₀ was found to be low in platelet mitochondria from patients with PD and correlated with reductions in complex I activity. Treatment with CoQ₁₀ has also been shown to protect mice against a variety of neurotoxins, including MPTP. Data from a phase 2 study demonstrated dose-dependent improvements in Unified Parkinson’s Disease Rating Scale scores in patients with PD taking oral CoQ₁₀, which were highest for patients taking 1200 mg/d. Coenzyme Q₁₀ is a carrier molecule for the electron transport chain. In other neurodegenerative diseases characterized by mitochondrial dysfunction, such as Huntington disease and Friedreich ataxia, CoQ₁₀ has not been shown to significantly improve neurologic outcome or symptoms, although in a small open study of patients with Friedreich ataxia, CoQ₁₀ in combination with vitamin E appeared to improve cardiac bioenergetic function. Furthermore, the same group recently reported their 4-year results for patients with Friedreich ataxia receiving CoQ₁₀ (400 mg/d) and vitamin E and showed that the bioenergetic improvement in cardiac and skeletal muscle of these patients was sustained during the 4-year period; however, no benefit in clinical neurologic parameters occurred. Therefore, the CoQ₁₀ findings, although promising in PD, need to be validated by larger randomized controlled trials.

In the second group of agents, creatine, a safe and commonly available nutritional supplement used by athletes, was shown to be neuroprotective against MPTP models. Creatine is converted into phosphocreatine and prevents opening of the mitochondrial permeability transition pore (mPTP) that spans the IMM and the outer mitochondrial membranes (OMM). This is associated with release of proapoptotic proteins, cytochrome c (Cyt c), and the apoptosis-inducing factor (AIF). These factors induce caspase activation and apoptosis and can be modified by proteins of the Bcl-2 family. In addition, ROS can inhibit the ubiquitin proteasome system (UPS) that leads to the accumulation of damaged and misfolded proteins that eventually form aggregates called Lewy bodies. The effect of drugs and recently discovered mendelian genes is illustrated. Bax indicates Bel-2–associated X protein; MPP⁺, N-methyl-4-phenylpyridinium; arrows, activation; side whiskers, inhibition; and terms in rectangular boxes, drugs.
tions; however, several studies have found that it may exacerbate dopamine loss in MPTP models. A caveat is that most of these therapeutic studies have been undertaken in MPTP models that do not recapitulate Lewy body formation, so the data need to be interpreted cautiously. Furthermore, the timing and dosing regimen of MPTP often varies among studies, and such factors need to be considered when comparing results from separate studies. Recently, Fornai et al demonstrated that long-term infusion of MPTP in mice caused the formation of ubiquitin-positive and α-synuclein-positive inclusions with severe dopaminergic cell loss and behavioral abnormalities. Moreover, these features were reversed by dopamine agonists, and it may be that therapeutic studies in such models are more likely to lead to the discovery of compounds that will also be beneficial in humans. Several compounds that act against the Bcl-2 family (including Bax) have been developed, such as humanin peptides; however, they remain in the preclinical phase and have not been evaluated in PD models. One of the challenges of these compounds will be to enhance their specificity to prevent a generalized inhibition of apoptotic pathways that might lead to cancer.

In the final group, several antioxidants hold promise for PD, including dopamine agonists (eg, ropinirole hydrochloride or pramipexole dihydrochloride) and the plant extract Ginkgo biloba. Pramipexole has been shown to protect against MPTP neurotoxicity in primates and can inhibit mitochondrial membrane depolarization and cytochrome c release in cells. In humans, promising but as yet preliminary evidence of disease-modifying effects for pramipexole has been reported in the comparison of the Agonist Pramipexole vs Levodopa on Motor Complications in Parkinson’s Disease (CALM-PD) study that used fluorodopa F 18 positron emission tomography to monitor disease progression. A recent study demonstrated that genetic or pharmacologic chelation of iron prevented MPTP-induced oxidative stress. Iron catalyzes the formation of reactive oxygen species and can increase mitochondrial oxidative stress, and this agent may be beneficial in PD. In fact, the metal chelator clioquinol has shown some benefit in phase 2 clinical trials of Alzheimer disease.

CONCLUSIONS

Despite much research, many questions remain about the role of mitochondrial dysfunction in PD. In particular, how do the newly discovered genes DJ-1 and PINK1 fit into the MPTP/complex 1 paradigm of pathogenesis? Given that PINK1 is a kinase, what are its targets, and how does phosphorylation mediate its neuroprotective role against apoptotic stress? These questions are likely to be answered once postmortem brain material is available from patients with mutations and knockout animal models have been developed. Greater understanding is likely to lead to a more extensive array of drug targets for PD in the future.

Accepted for Publication: January 9, 2006.

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Author Contributions: Study concept and design: Muqit, Gandhi, and Wood. Acquisition of data: Muqit, Gandhi, and Wood. Analysis and interpretation of data: Muqit and Wood. Drafting of the manuscript: Muqit and Wood. Critical revision of the manuscript for important intellectual content: Muqit, Gandhi, and Wood. Obtained funding: Muqit, Gandhi, and Wood. Study supervision: Wood.

Funding/Support: This study was supported by Medical Research Council (MRC) program grant G0400000 and grants from the Parkinson’s Disease Society (Dr Wood). Dr Muqit was an MRC Clinical Research Training Fellow. Dr Gandhi is a Wellcome Trust Clinical Research Training Fellow.

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