Mitochondria and Parkinson’s disease: genetic contributions to understanding the pathogenic process
Mitocôndria e doença de Parkinson: contribuições da genética no conhecimento do processo patogênico
Clécio de Oliveira Godeiro Júnior¹, Patrícia Maria de Carvalho Aguiar², André Carvalho Felício³, Henrique Ballalai Ferraz⁴

ABSTRACT
Parkinson’s disease is the second most common neurodegenerative disorder. Mitochondrial dysfunction plays a major role in the pathogenesis of this disorder. Studies on monogenic forms of parkinsonism have significantly contributed to understanding this process. We reviewed our understanding of the role of these genes in mitochondrial dysfunction in Parkinson’s disease.

Keywords: Parkinson disease/genetics; Parkinson disease/etiologia; Genes, mitochondrial

RESUMO
A doença de Parkinson é a segunda doença neurodegenerativa mais comum. A disfunção mitocondrial exerce um papel importante na patogênese desta enfermidade. Os estudos das formas monogênicas de parkinsonismo contribuíram de forma significativa para uma melhor compreensão deste processo. Revisamos como estes genes se adequam no entendimento do papel da disfunção mitocondrial na doença de Parkinson.

Descritores: Doença de Parkinson/genética; Doença de Parkinson/etiologia; Genes mitocondriais

INTRODUCTION
Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer’s disease, affecting approximately 1% of population aged over 60 years1-2. Diagnosis is made through medical evaluation and identification of bradykinesia in association with one of the following symptoms: resting tremor, muscular rigidity and/or postural instability1-2. It is a degenerative process that involves multiple areas of the central nervous system, presents morphological alterations as it progresses, particularly Lewy bodies, and affects the dorsal motor nuclei, olfactory nuclei and bulbs, followed by the locus coeruleus and substantia nigra pars compacta3.

Although the cause of PD is not known, it has been recognized for a long time that environmental and genetic factors are important. Experimental models of parkinsonism with toxins that inhibit mitochondrial function were the first indication that PD presented mitochondrial dysfunction. The mutant genes associated with parkinsonism promote structural or functional changes in the proteins that are directly or indirectly related to mitochondrial function or oxidative stress.
which reduces the amount of oxygen available to produce water. The oxidase-reductase reactions are related to the transfer of protons (H\(^+\)) through IMM, and this proton efflux creates a proton electrochemical gradient, known as the proton motive force (PMF). PMF is composed mainly of an electrical component called the mitochondrial membrane potential (MMP) and a pH transmembrane gradient. MMP is essential for mitochondria function since it generates the power to drive the flow of H\(^+\) and Ca\(^{2+}\) into the mitochondria. H\(^+\) enters the mitochondria through V complex. The reentrance of H\(^+\) depolarizes MMP and induces ADP phosphorylation to generate ATP\(^{1,2}\).

This oxidative phosphorylation process promotes the formation of numerous free radicals (FR) which are extremely reactive and cause oxidation of neighboring molecules through the extraction of one electron. Mitochondria are the main cellular source of FRs, particularly superoxide radicals, which are generated in the ETC complex I. The FRs can attack DNA and mitochondrial protein\(^{3,4}\).

Inhibition of ETC complex I activity leads to diminished ATP, jeopardizing all the cellular processes that are dependent on it, resulting in the formation of free radicals that cause oxidative stress. In the case of oxidative stress, mitochondrial permeability occurs as a result of transient pore opening, leading to membrane potential loss. This causes the outer mitochondrial membrane (OMM) to rupture and release proapoptotic proteins into the cytoplasm leading to caspase activation and subsequent cell death\(^{4,5}\).

Mitochondrial dysfunction and Parkinson’s disease

In Parkinson’s disease (PD), various studies have identified oxidative stress markers in dopaminergic cells\(^{6}\). Experimental models of parkinsonism induced by toxins, particularly, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, which are ETC complex I inhibitors, were the first objective connection between mitochondrial dysfunction and the development of PD\(^{6}\).

The development of parkinsonian symptoms in patients that had taken MPTP, a synthetic derivative similar to heroin, emphasized the possibility that oxidative stress secondary to mitochondrial dysfunction could be an etiological factor of PD\(^7\). MPTP is metabolized by the enzyme monoamine oxidase (MAO) in MPTP\(^{-}\) and MPP\(^{+}\) (1-methyl-4-phenylpyridinium). MPP\(^{+}\) has a high affinity through the dopamine (DA) transporter in the synaptic cleft and is recaptured by the dopaminergic neurons in the substantia nigra. In vitro experiments demonstrate that MPP\(^{+}\) is a ETC complex I inhibitor that progressively reduces mitochondrial ATP production and increases superoxide anion and free radical production\(^7\).

Mitochondrial genetics and Parkinson’s disease

Only 13 proteins have been encoded in human mitochondrial DNA (mtDNA) and they are ETC and oxidative phosphorylation system components\(^5\). Therefore, one hypothesis to consider is the fact that complex I dysfunction derived from mtDNA is responsible for oxidative stress in PD. Experiments using hybrid PD cells that demonstrated increased production of reactive oxygen species as well as of enzymes that degrade them, support this hypothesis\(^8\).

Various specific mutations of mitochondrial genes were identified in patients with matrilineal inheritance parkinsonism, including cytochrome b and 12sRNA genes. A study using hybrid mtDNA cells from 15 family members with matrilineal inheritance parkinsonism demonstrated an increased production of free radicals and diminished complex I activity in these cells when compared to hybrids produced from patrilineal inheritance mtDNA\(^9\).

Some studies were conducted to investigate polymorphisms of the mitochondrial genome and their relation to idiopathic PD. One study demonstrated that polymorphisms affecting the haplogroups J and K of the mitochondrial genome, reduced the production of reactive oxygen species by complex I in these cells, suggesting that this polymorphism could reduce the risk of PD. The lower occurrence of the K haplogroup in the ND3 gene, containing the single-nucleotide polymorphism (10398G>A), is found more often in PD patients than normal controls. Another interesting observation is the fact that the haplogroup cluster (U, K, J and T) is less common in PD patients than controls. In fact, the only mtDNA polymorphism that has a true association with PD is 4366A>G, in the tRNA\(^{Glu}\) gene. The mechanism through which these polymorphisms alter mitochondrial function is still not clear\(^{10-11}\).

Even though these observations are ambiguous, they indicate a greater possibility that decisive genetic factors of PD exist. Mitochondrial dysfunction is evident and even though mtDNA encodes proteins involved in ETC and oxidative phosphorylation, the remaining proteins required for perfect functioning of this system are synthesized from nuclear DNA.

Nuclear DNA and Parkinson’s disease

PD is usually sporadic but can also be inherited. However, patients with a family history account for less than 10% of all cases. There are at least six genes described as the cause of familial parkinsonism: SNCA – PARK1, UCHL-1 – PARK5 and LRRK2 – PARK8, attributed to autosomal dominant hereditary PD; and parkin – PARK2, PINK1 – PARK6 and DJ1-PARK7, attributed to autosomal recessive hereditary PD\(^{12}\).
PARK1 – SNCA – Alpha-synuclein

The first encouraging evidence in relation to the association of a genetic component with the PD etiology, was the discovery of the A53T mutation in the alpha-synuclein gene in patients with inherited autosomal dominant parkinsonism, which is the main component of the Lewy bodies. Since these structures are identified in both the familial and sporadic forms of PD, it is possible that alpha-synuclein abnormalities are part of the pathogenesis of this disorder.(13)

The exact function of alpha-synuclein has not yet been clearly defined, but it is believed that it could play a part in protecting neurons from injury since it is associated with a nerve ending chaperone, preventing neurodegeneration.(14) In vitro experiments demonstrate that wild-type or mutant alpha-synuclein are able to form (oligomeric) protofibrils or assume fibrillar configurations, but were not able to establish which formation type would be neurotoxic(15). Some evidence indicates that protofibril formations are the main cause of neurotoxicity. Both alpha-synuclein mutations, A53T and A30P, promote the formation of protofibrils, but only A53T causes fibril formation while A30P inhibits fibril formation.(15). Protofibril formation is increased and stabilized by dopamine quinones (products of dopamine oxidation) and could be the cause of greater alpha-synuclein toxicity in the substantia nigra. The protofibrils bind to the synaptic vesicles, creating pores and membrane permeability, leading to an excessive release of dopamine in the cytosol. The wild-type alpha-synuclein appears to be neurotoxic, since its phosphorylation promotes the formation of protofibrils and filaments in vitro and in vivo.(16).

The presence of alpha-synuclein, ubiquitin and proteasome subunits in the Lewy bodies, suggests the involvement of alpha-synuclein in the etiology of the ubiquitin-proteasome system (UPS) dysfunction in PD.(16). UPS is essential for degrading damaged proteins, including those that are oxidized and/or phosphorylated, that can be formed from cellular oxidative stress, which can be caused by mitochondrial dysfunction.(17).

Alpha-synuclein dysfunction has an indirect and significant action on mitochondrial neuron function. The increased alpha-synuclein expression makes the neurons more susceptible to oxidative stress and to damage from toxins, such as MPP+ and 6-hydroxydopamine.(18). There is currently strong evidence that genetics are involved in the association between parkinsonism and mitochondrial dysfunction. Although there is no evidence for mitochondrial localization, alpha-synuclein interferes in mitochondrial function.

UCHL-1

A missense mutation (I93M) in the gene that encodes ubiquitin carboxyl-terminal hydrolase L1 (UCHL-1) was identified in two members of a German family with autosomal dominant hereditary parkinsonism.(19). UCHL-1 belongs to the family of deubiquitinating enzymes, that are responsible for the hydrolysis of polyubiquitin chains into monomeric ubiquitin. The polyubiquitin chains are essential to target proteins for degradation by UPS, which is jeopardized in PD(20). Mutations of the UCHL-1 gene diminish UPS catalytic activity by 50%.(17). Hence, there does not appear to be a direct connection between the mutation of this gene and mitochondrial dysfunction.

LRRK-2

The acronym LRRK-2 stands for leucine-rich repeat kinase-2. Mutations of this gene are responsible for 5% to 6% of cases of familial PD and roughly 1.6% of sporadic cases. The encoded protein for this gene is called dardarin and it has multiple complex domains: N-terminal leucine-rich repeat domain, GTPase ROC/COR domain, mitogen-activated protein kinase kinase kinase (MAPKKK) and C-terminal WD40 repeat domain,(21). These characteristics suggest that this protein plays the role of an intracellular marker.

Dardarin is able to promote autophosphorylation, could be related to OMM and binds to the parkin protein,(22). In vitro experiments demonstrated three mutations associated with PD, two in the kinase domain (G2019S and I2020T) and one in the GTPase ROC/COR domain (R1441C), that increase the autophosphorylation of dardarin, suggesting a gain-of-function mechanism.(23).

Even though mitochondrial localization for dardarin is possible, it is more likely that this protein promotes alpha-synuclein phosphorylation, contributing to the formation of Lewy bodies. As such, in vivo experiments are required for a better understanding of this protein function.(24).

Parkin

Mutations of the parkin gene are common and correspond to roughly 50% of cases of autosomal recessive parkinsonism, especially for those with early onset before 21 years of age. A large variety of mutations was reported for this gene. The protein encoded by the parkin gene is usually found in the Lewy bodies of familial and sporadic PD patients.(25). The mutations that abolish parkin activity appear to be associated with the lack of Lewy body formation. Nevertheless, mutations with reduced, but not abolished, activity have Lewy bodies. Molecular biology studies have demonstrated that parkin protein dysfunction interferes in both UPS function and mitochondrial energy metabolism, which, when modified,
could promote the development of parkinsonism\textsuperscript{(26)}.

The molecular structure of the parkin protein is characterized by two RING (really interesting new gene) domains separated by an IBR (inbetween RING) domain. The RING domain functions as an E3 ubiquitin ligase. E3 ligases catalyze the addition of ubiquitin molecules to lysine residues of damaged proteins and the polyubiquitin chains are markers for the removal and degradation of the first cells by the proteasome system\textsuperscript{(27)}. Therefore, parkin dysfunction causes UPS dysfunction, leading to an accumulation of damaged proteins which could be responsible for neurotoxicity and the onset of cellular apoptosis\textsuperscript{(26)}. The parkin protein protects against neurotoxicity induced by alpha-synuclein in vitro and in vivo. The interaction between these two proteins could be direct, since parkin degrades altered oxidized forms of alpha-synuclein, or indirect because parkin retrieves neurons from alpha-synuclein induced proteasome dysfunction\textsuperscript{(28)}.

New evidence indicates a connection between parkin and mitochondrial energy metabolism. The complex I activity is selectively reduced in peripheral leucocytes in patients with parkinsonism related to parkin mutations. An experimental model with \textit{Drosophila} demonstrated that parkin has a role in maintaining mitochondrial function and preventing oxidative stress. Fruit flies without parkin developed a mitochondrial pathology characterized by reduced life expectancy, apoptosis, muscular degeneration in the wings and male sterility\textsuperscript{(29)}. Another study using the same model, demonstrated progressive degeneration in a select group of dopaminergic neurons and increased oxidative damage\textsuperscript{(29)}. Mammal models reinforce parkin’s role in maintaining mitochondrial function. Deletion of exon 3 in mice resulted in nigrostriatal dysfunction and reduction of proteins involved in mitochondrial function and oxidative stress, including subunits of I and IV complexes\textsuperscript{(26)}.

Parkin could be directly involved in maintaining mitochondrial integrity since it has already been identified in OMM, where it performs an essential role in the prevention of edema and mitochondrial rupture secondary to ceramide toxicity. Indirectly, parkin can maintain mitochondrial function through its ubiquitin ligase action. Since oxidative damage from free radicals is a consequence of ETC, normal parkin can help remove damaged proteins, directing them to UPS, while mutated parkin allows them to accumulate\textsuperscript{(26,28)}.

**DJ-1**

Mutations of the DJ-1 protein are very rare and correspond to just 1% to 2% of cases of early onset parkinsonism\textsuperscript{(30)}. Its subcellular distribution is primarily cytoplasmic with a small amount associated with the mitochondria\textsuperscript{(31)}. DJ-1 is a member of the superfamily ThiJ/PpI/DJ1, homologous to various procariotic proteins, such as chaparones and ThiJ/PpI proteases\textsuperscript{(32)}. This protein participates in oxidative stress response both as a redox sensor or an antioxidant. Ablation of DJ-1, or deletion of the small interfering RNA (siRNA) or the gene, makes the live cells susceptible to oxidative stress. These cells can be saved by the increased expression of the DJ-1 wild-type protein, but not by the mutant protein (L166P)\textsuperscript{(33)}.

Despite reported evidence, it is unlikely that DJ-1 participates its cellular protection function only through antioxidative mechanisms, since its ability to apprehend reactive oxygen species is limited\textsuperscript{(31-32)}.

DJ-1 nuclear function is well established but the mitochondrial function is not. Therefore we do not know if the mutations with greater localization in the mitochondria are a result of mitochondrial function gain or difficult access to the nuclear proteins\textsuperscript{(24)}.

**PINK1**

The PINK1 gene encodes an acid protein with 581 amino acids which presents a N-terminal mitochondrial targeting motif, a highly conserved serine/threonine domain and an autoregulatory C-terminal domain. At a cellular level this protein acts as a mitochondrial kinase. Mutations in PINK1 cause a type of familial autosomal recessive parkinsonism\textsuperscript{(34)}, and is the second most common type after parkin.

The PINK1 protein can perform a role in cellular protection in situations of stress that affect the mitochondrial membrane potential. Since most mutations in this gene affect the kinase domain, it is possible that abnormal phosphorylation of the PINK1 target proteins represents the pathogenic mechanism, causing an abnormal response to oxidative stress and neurodegeneration\textsuperscript{(34)}. The mutations located outside the kinase domain can affect protein function through interference in the mitochondrial localization or processing.

Loss of PINK1 function interferes in mitochondrial function and cellular viability under stress. An experiment using a lineage of neuroblastomas with the G309D mutation, exposed to cellular stress with the proteasome inhibitor, MG-132, demonstrated a reduction in the mitochondrial membrane potential and increased cell death in relation to cells with wild-type PINK1. In baseline conditions, no differences were found\textsuperscript{(34)}. This suggests that the integrity of PINK1 is essential for cellular protection from oxidative stress. In agreement with this impression, the increase of the wild-type PINK1 expression demonstrated a reduction in the release of
cytochrome c from the mitochondria in baseline and stress situations. The lowered release of cytochrome c results in a diminished production of proapoptotic enzymes: caspase 3, caspase 7, caspase 9 and poly-ADP-ribose polymerase (35).

CONCLUSION

Although all these data demonstrate that mitochondrial dysfunction and its direct association with UPS are part of the PD pathogenesis, further information on these metabolic paths is required. The recent identification of causal PD genetic mutations and the proteins involved is an excellent starting point for this difficult task. PINK1 is highlighted, since, to date, this is the only protein that has been identified in the mitochondria and when altered, it causes mitochondrial dysfunction.

The ever increasing pathogenic path information is making the dream of more efficient therapeutic instruments and neuroprotection mechanisms to combat parkinsonism a reality.

REFERENCES