Is mitochondrial dysfunction a major cause for Parkinson's disease?

Research essay performed at the Cell Biochemistry group of the Groningen Biomolecular Science and Biotechnology Institute (GBB), University of Groningen, The Netherlands.

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by degeneration of dopamine producing neurons and cytoplasmic inclusions called Lewy bodies. Oxidative stress is said to have a great impact on dopaminergic neuronal death. However, mitochondrial dysfunction has been discussed widely in relation to PD. Mitochondria are vitally crucial organelles involved in various functions. Their most important role is energy metabolism where 90% of cellular energy in the form of ATP is produced through oxidative metabolism. Mitochondria are involved in other processes including regulation of calcium homeostasis, protein quality control and detoxification of reactive species. The direct link between mitochondrial dysfunction and Parkinson's disease (PD) was established by the Complex I impairment with patients exposed to neurotoxins like MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine). Identification of genes which are involved in the rare familial forms of PD by inhibiting complex I has further augmented the understanding and elevated the role of mitochondrial dysfunction in disease pathogenesis. Complex I inhibition and subsequent mitochondrial dysfunction could occur either as a cause or consequence of PD which is not well studied. This essay discusses about the mitochondrial dysfunction being the initial trigger and an important cause for the degeneration of dopaminergic neurons and accumulation of modified proteins.

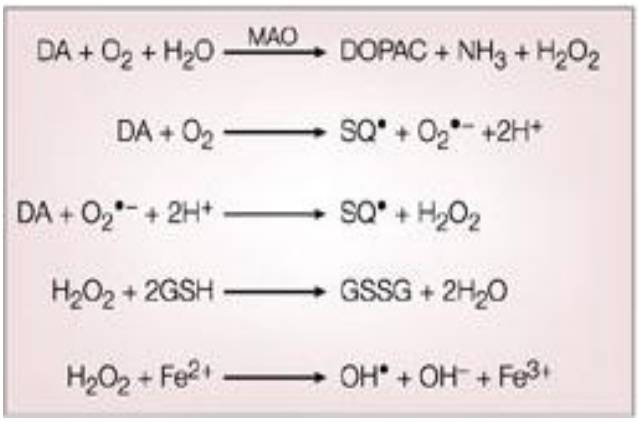
Contents

Parkinson's disease: Oxidative stress and mitochondrial involvement

Parkinson's disease (PD) is the second most common and progressive neurodegenerative disorder next to Alzhiemer **(Dauer et al., 2003)**. The prevalence of disease increases with age ranging from 1-2 % at 50 years to 5 % by the age of 85.Clinical symptoms of PD include rigidity, resting tremor, slowness of movement(bradykinesia) and postural instability**(Hardy et al., 2006).**The pathological hallmarks are characterized by progressive degeneration of dopamine producing neurons in the substantia nigra pars compacta (SNpc) region in midbrain and presence of cytoplasmic inclusions called Lewy bodies **(Sami et al., 2004,Lees et al., 2009 and Dickson et al., 2009)**. Though the dopaminergic neurons are less in number compared to other neurons, they play an important role in regulating various aspects of the brain's function with major contribution in control of movement. The production

and release of neurotransmitter, dopamine from these neurons allows messages to pass through the striatum, a part of forebrain that coordinates movement. The degeneration of dopaminergic cells results in the reduction of dopamine levels which blocks the signal transmission between pre-synaptic neuron (axons of dopamine producing nerve cells) and post-synaptic neuron (dendrites of striatum cells). So the regions of the brain that control movement no longer function properly. Parkinson's symptoms arise upon degeneration of majority of dopaminergic neurons (60-70%).

Oxidative stress induced by Reactive Oxygen Species (ROS), is considered to be an important cause for the degeneration of the dopamine neurons. The vulnerability of neurons to oxidative stress could be due to their presence in a harsh region of the brain the SNc which is dopamine rich and contains neuromelanin (dark pigment structurally related to melanin) and high iron content**.** While neuromelanin has been shown to bind neurotoxic and toxic metals that could promote neurodegeneration **(Good et al., 1992)**, iron present in the brain has the tendency to participate in a Fenton reaction which can result in the production of highly reactive hydroxyl radical (Fig 1). These hydroxyl radicals are observed to be heavily dependent on the presence of iron, which has been found to be elevated in the SN of PD patients **(Dexter et al., 1998**). In addition to the presence of high iron content, proper balance between oxygen metabolism and availability of antioxidant such as reduced glutathione (GSH) poses higher risk of oxidative damage to dopaminergic neurons **(Sies et al 1983).** The neurotransmitter dopamine itself can be a source of oxidative stress. Dopamine synthesized in the cytoplasm has to be sequestered immediately into monoaminergic vesicles. If unstored, cytosolic dopamine is thought to be capable of generating toxic reactive oxygen species (ROS) **(Dauer 2003)** via its catabolism in enzymatic and non-enzymatic process (Fig 1). The catabolism of dopamine occurs by auto-oxidation into toxic dopamine- Quinone species and H_2O_2.



Reactive oxygen species that are formed from oxidation of dopamine (DA) include **Superoxides (O_2 -), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH-**).

Hydrogen peroxide (H_2O_2) can be formed from DA both in enzymatic process catalysed by MAO and non-enzymatic process.

H_2O_2 thus formed can either oxidize reduced glutathione (GSH) to GSSG or participate in a Fenton reaction where it reaction with Fe2+ to form hydroxyl radical (OH-) and reduced iron Fe 3+

Fig 1 Oxidative metabolism of dopamine (DA)

In addition to dopamine induced oxidative stress, mitochondrial dysfunction has been widely discussed in relation to Parkinson's disease. Why would neurons be critically dependent on mitochondria and why do mitochondrial dysfunction has a great impact on Parkinson's disease? Neurons are polarized cells consisting of a relatively small cell body, dendrites with multiple branches and a thin axon that can extend up to a meter long as in motor neurons. Transmission of movement

signals from pre-synaptic to post-synaptic neuron occurs by the release of neurotransmitters like dopamine at synapses. This process is called Synaptic transmission. Synaptic transmission requires ATP generation to fuel energy consuming process like exocytosis and endocytosis of neurotransmitters. Both glycolysis and mitochondria produce ATP at the synapse. While ATP production from Glycolysis can contribute to about 10% of total cellular ATP, mitochondria on the other hand are thought to produce more than 90% of cellular ATP through oxidative phosphorylation. Neurons would more likely and critically be dependent on mitochondria for ATP supply indicating that proper mitochondrial function is required for survival of neurons **(Nicholls and Budd, 2000).** In addition to ATP production, mitochondria are dynamic organelles facilitating their transport to the locations with greater energy demands. This suggests why mitochondria are abundant at axons and synaptic terminals which require more energy **(Rowland et al. 2000, Shepherd and Harris 1998)**. The energy consuming process at synapses or presynaptic neurons involve generation of action potential, synapse assembly and synaptic transmission (**Lee and Peng, 2008 and Verstreken et al., 2005**). Mitochondria present at synapses in addition to ATP production, buffer Ca2+ homeostasis. Loss of mitochondria at axonal terminals result in aberrant synaptic transmission in Drosophila mutant Milton due to insufficient ATP supply or altered Ca^{2+} transients **(Ma et al., 2009**) .Thus ATP depletion and impaired $Ca2^{+}$ homeostasis due to mitochondrial dysfunction could have a major effect on survival of dopamine producing neurons.

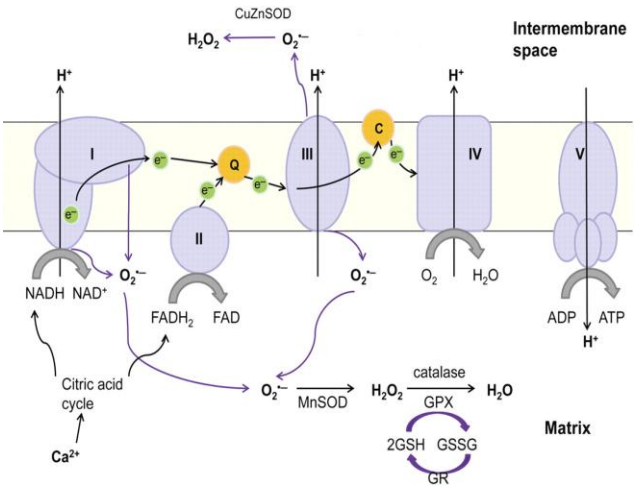
Several hypotheses for the progressive and selective neurodegeneration in PD linked with mitochondrial dysfunction have been proposed. One such hypothesis is, the selectively vulnerability of dopaminergic neurons to oxidative stress induced by complex I inhibition **(Vila and Przedborski, 2003)**. Complex-I is one of the subunits of the Electron transport chain present in the inner mitochondrial membrane. Mitochondria use this complex to produce ATP in a process known as oxidative phosphorylation. The study by Vila and Przedborski observed Parkinson's disease like symptoms among drug users exposed to a compound MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine), a synthetic by-product of heroin production **(Langston et al., 1983)**.This lipophilic compound has the tendency to diffuse blood brain barrier and get converted into active form MPP+. The MPP+ thus formed is known to selectively inhibit complex I of electron transport chain resulting in respiratory chain inhibition and thereby ATP depletion. This indicates the direct evidence between mitochondrial dysfunction and Parkinson's disease. However the major question in the field of PD is whether mitochondrial dysfunction could be cause or consequence of PD. This essay focuses on functional impairment of mitochondria such as bioenergetics dysfunction, oxidative stress, calcium dysregulation, impaired protein quality control in relation to Parkinson's disease pathogenesis and thereby concluding mitochondrial dysfunction as a major cause of PD.

Mitochondrial electron transport chain dysfunction in PD: Is there a link between dopamine induced oxidative stress and bioenergetic dysfunction?

As discussed before, oxidative stress induced by dopamine metabolism and mitochondrial dysfunction are said to be important factors related to PD. It is essential to know whether these two factors are interdependent on each other or bring out degeneration in an individual and different manner. This could probably be explained by assessing mitochondrial function and dysfunction. The predominant physiological function of mitochondria is the generation of ATP by oxidative phosphorylation but additional functions include the generation and detoxification of reactive oxygen species, apoptosis, and calcium homeostasis, degradation of misfolded proteins by ubiquitin

proteasome system and the transport of the organelles themselves to correct locations within the cell **(Jiang et al., 2004, Szabadkai et al., 2008, Chan 2006, Cadenas et al., 2000 and Lass et al., 1997)**. Abnormalities in any of these processes could be termed as mitochondrial dysfunction. Of all these functions, mitochondrial ATP generation is crucial in particular at neuronal synapses which have been previously discussed.

Mitochondrial ATP is produced by combination of electron transport chain complexes and ATP synthase. The electron transport chain complexes (I, II, III and IV) and ATP synthase (Complex V) are located in the inner mitochondrial membrane (Fig 2). The electron transport chain becomes the site for oxidative phosphorylation, the function of which is to produce ATP from products of Citric acid cycle, fatty acid and amino acid oxidation **(Chan 2006).** This is accomplished by the transport of electrons between complexes causing movement of protons (H+ ions) from the matrix to the intermembrane space resulting in the generation of proton concentration gradient. This concentration gradient can then be used by ATP synthase to produce ATP. During the process of oxidative phosphorylation, electrons tend to leak from the electron chain complexes (I and III) which could react with molecular oxygen to form superoxide (O_2-), a free radical with unpaired electrons **(Taksheige et al 1979 and Beyer 1992)**. Superoxide formation occurs at relatively low levels which can be detoxified by an enzyme Superoxide dismutase (MnSOD) present in the mitochondrial matrix (Fig 2). It acts as an antioxidant defense system by catalysing the conversion of superoxide (O_2-) to hydrogen peroxide (H_20_2) which is then converted to water by reduced glutathione (GSH) **(Lass et al 1997 and Andersen et al 2002).** Though electron leakage occurs at relatively low levels, it could still be a major source of oxidative stress both to mitochondria and other organelles **(Cadenas et al 2000)**. Thus dysfunction in the ATP production by means of inhibition of any of the electron transport chain complexes could result in the higher leakage of electrons thereby producing more reactive oxygen species.



Complex I (**NADH coenzyme Q reductase**; labeled I) accepts electrons from the citric acid cycle electron carrier nicotinamide adenine dinucleotide (**NADH**), and passes them to coenzyme Q (**ubiquinone**; labeled Q), which also receives electrons from complex II (**succinate dehydrogenase**; labeled II). Q passes electrons to complex III (**cytochrome bc1 complex**; labeled III), which passes them to cytochrome c (labeled as c). c passes electrons to Complex IV (**cytochrome c oxidase**; labeled IV), which uses the electrons to reduce molecular oxygen to water. Complex V(**ATP synthase**;labelled V) allows the transport of protons back to matrix where the stored energy is used to phoshphorylate ADP to ATP Electrons(e^-) leak from complex I and III to form Superoxide(O_2) and H_20_2(cataysed by MnSOD)

Fig 2 Mitochondrial Electron Transport chain and oxidative phosphorylation

In relation to the link between mitochondrial dysfunction and Parkinson's disease attributed by the observation of complex I deficiency in the post-mortem of substantia region pars compacta brain

region of PD patients suggests complex I deficiency as one of the fundamental causes of Parkinson's disease **(Schapira et al., 1990, Mizuno at al., 1989 and Langston et al., 1983)**. This complex I impairment could be due to oxidative damage to complex I, thereby misassembly of its subunits, all of which are characteristics of isolated PD mitochondria **(Parker at al., 2008)**. One of the possible reasons for the susceptibility of complex I to the oxidative damage could be the presence of thiol groups and iron sulphur centers within this enzyme complex which are prone to oxidation. The biochemical defect that was observed with complex I deficiency was similar to PD like symptoms observed with young drug abusers exposed to MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine), a synthetic by-product of heroin production (**Langston et al., 1983)**. MPTP has the tendency to cross the blood brain barrier where it gets converted to active metabolite MPP+. MPP+ is produced from MPTP in a reaction catalysed by monoamine oxidase (MAO). Selective neurodegeneration to dopaminergic neurons occur by MPP+ which is taken up by dopamine transporter **(Hekkila et al., 1984)**. Once they are within the dopamine neurons, they selectively inhibit mitochondrial complex I and the inhibition of which affects respiratory chain function resulting in the ATP depletion, increased oxidative stress, cytoplasmic calcium overload and excitotoxicity eventually causing neuronal damage and death **(Nicklas et al., 1985)**. Complex I inhibition could be followed by impaired electron flow to other complexes. Thus with the electrons that are present in the electron transport chain complexes for a longer time than normal, oxygen could react to produce superoxide which then would be released into matrix for further conversion to H_20_2.

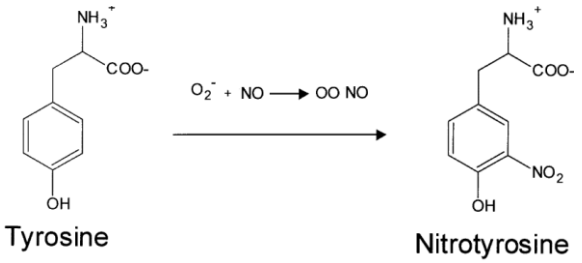
The increase in oxidative stress from complex I inhibition is now evident and could provide us with an explanation for the selective vulnerability of dopaminergic neurons to complex I inhibition. In case of complex I inhibition followed by insufficient ATP production, the loading of dopamine into vesicles and release into synaptic cleft would be impaired. This complex I inhibition could be the cause for higher amount of unstored dopamine available for oxidation and oxidative stress imposed on dopaminergic neurons by its oxidative products. In search for the link between DA induced oxidative stress and mitochondrial dysfunction, it is now clear complex I inhibition results in bioenergetics dysfunction and ROS formation which brings about the oxidative damage to Mitochondria and dopaminergic neurons. This further suggests that mitochondria as target of ROS in addition to source of ROS formation and mitochondrial dysfunction the major trigger for dopamine induced oxidative stress of neurons.

Impact of mitochondrial complex I dysfunction in PD

The mitochondrial energy failure due to complex I inhibition causes a build-up of electrons prior to blockage at the sites and results in the formation of Reactive oxygen species (ROS). Reactive oxygen species are chemical molecules with an unpaired or odd number of electrons and as a result, are very unstable and highly reactive with the tendency to cause damage to our cells**(Halliwell 1992).** Oxidative stress induced by ROS are deleterious to neurons and are known to accelerate aging. Every living cell possess sophisticated and elaborated antioxidant defense systems which include several enzymes and non –enzymatic mechanisms to protect against the rising threats of reactive species to endure the cell with the minimal damage.The increased stress results from the imbalance betwen oxidation and antioxidant systems indicated by progressive increase in ROS production when the cell exceeds its antioxidant capacities. The ROS family includes wide range of chemical species such as superoxide anions ($\bullet O^-$), hydroxyl radicals ($\bullet OH^-$) and hydrogen peroxide(H_2O_2)**(Armstrong et al.,1978).**Electron leakage from complexes I and III can contribute to the generation of superoxide radicals**(Turrens et al.,**

1980). The reactivity and proximity of superoxide along with the availability of biological targets including SO_2, NO and susceptible iron -sulphur centers could determine its fate within mitochondria. Superoxide dismustase (SOD) present in the mitochondrial matrix acts as the first line of antioxidant defense against superoxide by catalysing the conversion of superoxide into molecular oxygen followed by hydrogen peroxide formation **(Zemlan et al., 1989)**. Unlike superoxide which is not easily diffusible, hydrogen peroxide thus formed in the mitochondrial matrix readily diffuses to cytosol and other biological membranes where it could gain access to other cellular components **(Valko et al., 2007)**. This suggests that superoxide generated within mitochondria as a result of Electron Transport or respiratory chain inhibition would contribute to mitochondrial oxidative stress, while H_2O_2 that can readily access other biological mebranes may exert oxidative damage both inside and outside of mitochondria **(Jenner 2003)**.

Oxidative stress induced by formation of highly reactive species has the capacity to damage macromolecules like Proteins, Lipids and DNA **(Eşrefoğlu 2009**). The most common mechanisms of protein damages are post translational modifications such as Protein carbonylation and nitration. In carbonylation, the amino acid side chains of proteins are oxidized by ROS to form carbonyl groups (aldehydes and ketones) **(Floor et al.,1998)** while peroxynitrite participates in protein nitration that occurs by covalent attachment of nitro group ($-NO_2$) adjacent to hydroxyl group on the aromatic ring of tyrosine residues (Reiter et al.,2000)(Fig 3) Peroxynitrites (OONO) are Reactive Nitrogen Species (RNS) generally formed by the reaction of superoxide (o2-) with nitric oxide (NO) (Fig 3)



Reaction of superoxide(O_2-) with nitric oxide(NO) results in peroxynitrite formation(OONO). Nitration of tyrosine residues in protein occurs by the attachment of nitro group (NO_2) from peroxynitrites to form Nitrotyrosine.

Fig 3 Nitration of Tyrosine by Peroxynitrite

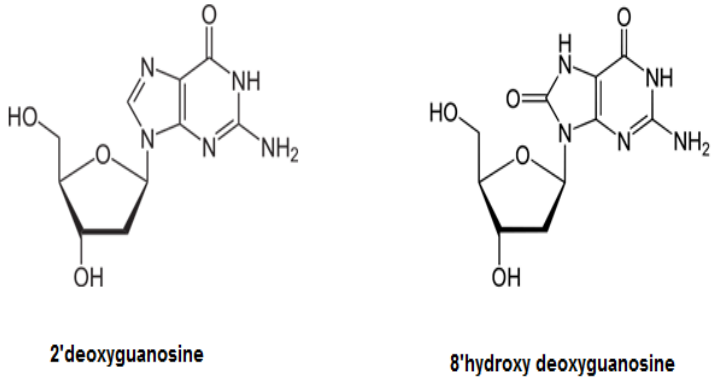
The nitration of the tyrosine residues in the protein by peroxynitrite results in the formation of 3-Nitrotyrosine,which is one of the common oxidative stress biomarkers that are said to cause cell death and are observed to be present in human brain of PD patients and rodents**(Good et al., 1998 and Reiter et al., 2000)** . This could be supported by the loss of enzyme activity of mitochondrial superoxide dismutase (MnSOD) which was the first protein to be nitrated in vivo**(Thompson et al., 2000;2001).** Tyrosine 34 nitration in Mn-SOD results in complete enzyme inactivation contributing to an increase in oxidative stress as it can no longer act as antioxidant defense againt superoxides or other highly reactive species**(Thompson et al., 1999).** So higher the superoxides present, higher would be the rate of peroxynitrite formation which could damage the macromolecules at higher degree.In addition to post translational modifcation of MnSOD, α-Synuclein a familial gene was found to be nitrated by peroxynitrite, which accelerates its oligomerization and aggregation**(Giason et al.,200and Chen et al.,2001)**. Aggregated and misfolded α-Synuclein form Lewy bodies, the pathological hallmark of

Parkinson's disease.Mn-SOD is mitochondrial protein while α-Synuclein associates with mitochondria under pathological conditions.It is widely accepted that thiol groups and iron sulphur clusters present in Complex I subunit of Electron Transport Chain are prone to oxidation. This observation was further confirmed by presence of Peroxynitrite mediated oxidation of cysteine residues in complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), and complex III (cytochrome *c* reductase) as well as complex V (ATP synthase, all of which participate in bioenergetic function by ATP production**(Castro et al.,2002 and Hodara et al., 2002)**In addition to the oxidative damage imposed on either mitochondrial or mitochondrial associated proteins, there are evidences that show tyrosine-hydroxylase ,a rate limiting enzyme involved in dopamine synthesis undergoes tyrosine nitration and cysteine oxidation mediated by Peroxynitrite thus contributing to Parkinson's disease**(Souza et al.,2001 and Saddi et al.,2002).** Other than impairing the antioxidant defense system present in mitochondrial matrix, Peroxynitrite affects the antioxidant defense system in brain regulated by reduced glutathione(GSH). GSH is particulary known to protect the brain from reactive nitrogen species. The oxidation of GSH by peroxynitrite results in the oxidized product (GSSH) thereby reducing GSH levels**(Dexter et al.,1992).** The decrease in GSH levels comes with an in increase in peroxynitrite levels which contributes to higher cellular oxidative stress. Overall, the protein modifications by peroxynitirite damages the antioxidant system and inhibits the respiratory chain complexes both of which contributes to increase in oxidative stress and neuronal damage.

Next to process of protein damage ,oxidative damage to lipid molecules occurs through Lipid peroxidation. It is the process where the hydrogen atom in lipid molecules react with reactive oxygen species to form lipid radicals and lipid peroxides. The end products of lipid peroxidation are reactive aldehydes such as malondialdehyde(MDA) and 4 hydroxynonenal(HNE). HNE serves as the major oxidative stress biomarker of lipid peroxidation**(Dexter et al.,1989 and Sayre et al.,1997)**.Phospholipids are particulary vulnerable to lipid peroxidation as they contain polyunsaturated fatty acids.Considering any single membrane or double membrane organelle as phospholipid bilayers, the modification of phospholipids could alter the permeability and fluidity of the membrane. In mitochondria where both these factors are of great importance, lipid peroxidation can have major effect on mitochondrial homeostasis. Affecting the membrane permeability would change the mitochondrial membrane potential thereby affecting the ATP production, calcium homoeostasis and passage of ions or molecules. These factors are more than sufficient to damage mitochondria resulting in the increase in reactive oxygen species which could then cause insurmountable effects to other organelles finally leading to neurodegeneration.Cardiolipin, one of the major phosholipids that is required for activity of Complex I, III and IV of electron transport chain was observed to damaged**(Dexter et al.,1986)** . In addition to lipids affecting the membrane properties, they have the tendency to react with proteins by protein-lipid cross linkage thereby contributing to oxidative damage of mitochondrial proteins, inactivation and accumulation of modified proteins. One plausible mechanism for this crosslinkage could be the modification of cysteine thiol group or amino group of lysine by hydroxynonenal. Since thiol group are prone to oxidative damage and HNE is said to have properties like reactive oxygen species, it increases the chance of thiol oxidation.

Oxidative damage to DNA occurs by modification of nucleic acids which are building blocks of DNA. Unlike the nuclear DNA that are protected by molecular chaperones such as histones, mitochondrial DNA does not possess any chaperone mediated defense mechanisms to protect against

oxidative damage. Other major reason why mitochondrial DNA(mtDNA) could be sensitive to oxidative damage is the presence of mtDNA at the site of ROS formation i.e mitochondrial matrix confirmed by higher level of mtDNA deletions in substantia nigra brain region of PD patients**(Bender et al., 2006)**.Reactive oxygen species that are formed as a result of respiratory chain inhibition or from toxic insult has the tendency to attack mtDNA in particular deoxyribose sugars, purine and pyrimidine bases by direct or indirect mechanisms. Highly reactive species like superoxides, nitric oxide and hydrogen peroxides do not tend to react with nucleotides but hydroxyl radicals have greater tendency to create single strand or double strand breaks in DNA**(Kratysberg et al.,2006).**In addition to those physical damages,they tend to produce 8- hydroxydeoxyguanosine, one of the most common and important biomarkers of oxidative stress**(Kikuchi et al., 2011, 2009)**. 8- hydroxydeoxyguanosine are formed by the oxidation of deoxyguanosine, a guanosine purine base lacking an hydroxy group at position-2 (Fig 4)



Oxidation of deoxyribose sugar (**2'deoxyguanosine**) in DNA by hydroxy radical (OH) results in the formation of (**8'hydroxy deoxyguanosine**), a marker for DNA oxidative damage.

Fig 4 Oxidation of deoxyguanosine by hydroxy radical

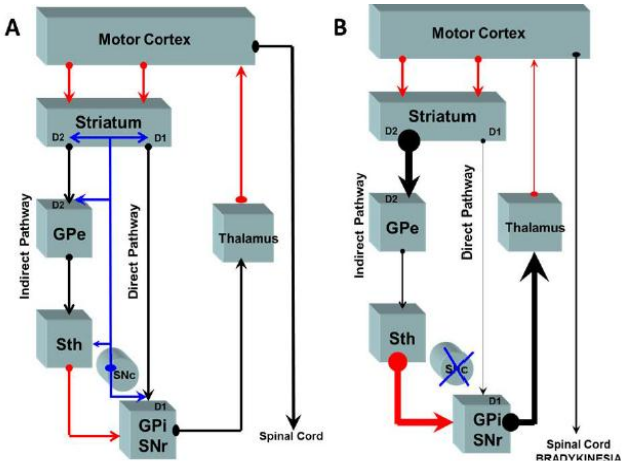
Hydroxy radicals that are present tend to react with the aromatic rings of 2' deoxyribose sugars to generate various saccharide DNA radicals formed as a result of modifications in all positions indicating overall sensitivty**(Kharapko et al.,2009)**. In particular, nitrogen bases of the aromatic rings with C5-C6 double bonds of pyrimidines and C4, C5, and C8 of purines are shown to poses higher risk of oxidative damage**(Fukui et al., 2009).**These evidences suggest that mitochondria DNA are more vulnerable to modifications with a greater effect induced upon transcription of damaged mtDNA and translation to proteins. The translation could neither occur if all it occurs, the proteins thus synthesized would not be functional resulting in the impairment of mitochondrial function thus contributing to human aging and neurodegenrative disorders as in Parkinson's disease**(Malkus et al.,2009).**

Mitochondrial dysfunction in PD : Beyond bioenergetic defect and oxidative stress

Impairment of respiratory chain resulting in ATP depletion and ROS formation has been shown to bring out more consequences ultimately leading to neurodegeneration.In addition to bioenergetic dysfunction and oxidative stress, ca2+ induced excotoxicity is being widely discussed in relation to Parkinson's disease**(Sheehan et al.,1997)**. Neurons rely on calcium signalling to mediate synaptic transmission and calcium overload is a major source of neurotoxicity.Calcium induced neuronal death is thought to occur by increase in intracellular calcium concentration resulting from the activation of ionotropic glutamate receptors : NMDA and AMPA **(Choi 1988)**. These two receptors are differentially distributed in basal ganglia cells and there are evidences which show presence of these receptors in dopamine producing neurons. AMPA receptors are permeable to sodium ions but blocks calcium ions

from entering, while NMDA receptors are calcium permeable but are blocked by magnesium in a voltage dependent manner. The pre-synaptic neurons release Glutamate which activates AMPA receptors at the post synaptic membrane. The activation of AMPA receptors allows influx of sodium ions which brings out neuronal depolarization at the synapse.This further removes the magnesium block enabling extracellular glutamate to activate the NMDA receptor causing calcium influx**(Chan et al.,2009).**Presynaptic neuron could be any of the glutaminergic neurons which produces glutamate neurotransmitter and post-synaptic neuron or receiving neuron could be dopaminergic neuron in substantia nigra pars compacta. This suggests that process of dopaminergic cell death may not be entirely cell autonomous resulting from oxidative stress and bioenergetic dysfunction but also increased glutamatergic transmission may contribute to excitotoxicity**(Khodorov 2004)**.

Inorder to combine all these factors in a nutshell,it would be appropriate to describe Parkisnon's disease as the result of an imbalance in the motor networks that would stimulate and/or inhibit the initiation of movements by dopamine release**(Albin 1982 and Penny 1989)**. There are two main pathways that have been studied in basal ganglia that regulates the movements : Direct and indirect pathway (Fig 4).Both the pathways are modulated by the substantia nigra pars compacta (SNc), which produces dopamine**(Obeso 2000)**. In direct pathway, binding of dopamine to dopamine receptors (D1) in basal ganglia favors the stimulation of movement by stimulating GABAergic neurons while in indirect pathway, dopamine binding to its receptors (D2) in basal ganglia inhibit movement by inhibition of GABAergic neurons. In competence to the dopamine regulation, GABAergic neurons in indirect pathway are stimulated by excitatory neurotransmitters such as acetylcholine and glutamate. The loss of dopamine would result in the imbalance between direct and indirect pathway thereby increasing the inhibitory effect of movement as characterized by bradykinesia (Fig 5) **(Bezard et al., 2009).** As indirect pathway is stimulated by acetylcholine and glutamate, their levels would be much higher in the absence of dopamine. This could probably explain why dopaminergic neurons in PD brains are susceptible to glutamate induced excitotoxicity which is attributed by higher influx of calcium (**Greene et al., 1996).**As discussed before, dopamine loss could occur due to auto oxidation of cytoplasmic dopamine formed as a result of mitochondrial dysfunction. This suggests for an indirect role of mitochondria in excitotoxicity.



D1, D2- dopamine receptors for excitation and inhibtion respectively**, Gpe :** Globus palidium external**, Sth:** Subthalamus **"Gpi-SNr":** Globus palidium interna and substantia nigra recticula

A) <u>Direct Pathway</u> : Cortex (stimulates) → **Striatum** (inhibits) → **"SNr-GPi" complex** (less inhibition of thalamus) → **Thalamus** (stimulates) → **Cortex** (stimulates) → **Muscles, etc.** → (<u>hyperkinetic state</u>)

<u>Indirect pathway</u> : Cortex (stimulates) → **Striatum** (inhibits) → **GPe** (less inhibition of STN) → **STN** (stimulates) → **"SNr-GPi" complex** (inhibits) → **Thalamus** (is stimulating less) → **Cortex** (is stimulating less) → **Muscles, etc.** → (<u>hypokinetic state</u>)

B)<u>Indirect pathway</u> : Cortex (stimulates)→ **Striatum** (high inhibition) → **GPe** (less inhibition of STN) → **STN** (higher stimulation) → **"SNr-GPi" complex** (higher inhibition) → **Thalamus** (is stimulating less) → **Cortex** (lesser stimulation) → (<u>hypokinetic) state</u>)

Fig 5 The basal ganglia motor circuits in A) Normal brain B) Parkinsonian brain

So how would mitochondria contribute directly to ca2+ induced neuronal death? It is being widely accepted that other than ATP production, mitochondria regulates ca2+ homoeostasis and this function is tightly regulated and tends to be more important in synaptic transmission. In neurons, mitochondria play an important role in buffering and maintaining stable calcium levels as calcium over load can cause cell death **(Castellani et al.,2002)**. When cytosolic calcium levels are highher, mitochondria take up ca2+ through a uniporter or a transient rapid mode in a voltage dependent manner and release them slowly when calcium levels subside**(Gunter et al., 2000)**. This suggests that mitochondrial dysfunction could result in reduced ATP production thereby altering mitochondrial membrane potential and facilitating the release of calcium into cytosol**(Sherer et al.,2002)**. Higher the cytoplasmic calcium levels, higher would be the Nitric Oxide levels. Nitric oxides thus formed by calcium regulated nitric oxide synthase (NOS) can react with superoxides to form Peroxynitrites, both of which tend to damage the macromolecules.Along with the alteration of membrane potential , reduced ATP levels may affect the activity of Na+/K+ ATPase resulting in the neuron depolarisation. The depoloarised neuron can undergo cell death by a ca2+ induced excitotoxic process due to lower ATP levels or ROS generated by inhibition of respiratory chain. Both of these factors release calcium by opening of mitchondrial permeability transition pore(MPT) present in the mitochondrial pore. MPT in addition to calcium release, facilitates the release of cytochrome c which interacts with apoptotic proteins like caspases to induce cellular apoptosis. Thus possible hypothesis for calcium induced cell death could be that defect in complex I could alter membrane potential thereby resulting in calcium overload and cell death.This was confirmed from the study where Complex I inhibition in SH-SY5Y cells developed cellular damage thus suggesting that mitochondrially driven or mitochondrial excitotoxcity could be a major factor in Parkinson's disease**(Sherer et al.,2001)**.

Genetic causes of PD and underlying mitochondrial dysfunction

Familial PD cases that attribute to mutations in specific genes contribute to lesser extent while the idiopathic PD causes caused by the environmental and intrinsic factors account for 90%.Both forms of PD are characterizied by selective degeneration of dopaminergic neurons in the Substantia Nigra pars compacta. Mutations in PARK1 (α synuclein) and LRRK2 are autosomal dominant while autosomal recessive mutations include PARK2 (Parkin), PARK7 (DJ-1) and PARK8 (PINK1)**(Klein et al.,2006 and 2012)**.As discussed so far, it is more convincing that mitochondrial dysfunction could have a greater contribution in pathogenesis of PD which can further be supported with an increasing evidence of familial genes that directly or indirectly affect mitochondrial function.

α-Synuclein. The *α-Synuclein* gene encodes for a soluble presynaptic protein with 140 amino acids. This protein has the tendency to misfold and aggregate due to its central hydrophobic non-*β*-amyloid domain**(Kim et al.,2008)**.Misfolded and aggregated *α-Synuclein* inclusions are observed in Lewy bodies, one of the pathologies of sporadic PD**(Spillantini et al.,1998)**. Three missense mutations A53T,A30P and E46K in *α-Synuclein* localize to amphipathic N-terminal region and are said to be the cause for autosomal dominant inherited form of PD**(Conway et al.,2000).**The synaptic plasticity of *α-Synuclein* allows it to adapt various conformations from monomers to protofibrils which can further polymerize to form fibrils and Lewly bodies.From the study of transgenic mouse bearing A53T synuclein,the polymerisation of *α-Synuclein* into amyloid fibrils were accelerated with development of

dysmorphic brain and spinal cord mitochondria**(Martin et al., 2006)**.In the substantia nigra pars compacta region, *α-Synuclein* is considered to have a role in loading and reloading of synaptic vesicles, Vesicle MonoAmine Transporter 2(VMAT2) with dopamine.In addition to this, it has been observed that *α-Synuclein* translocates and asscoiates with inner membrane of mitochondria under pathological conditions such as ROS induced acidic cytosolic environement**(Devi et al.,2008 and Cole et al.,2008)**. The association of synuclein protein with mitochondria resulted in complex I impairment ,increased ROS production , protein tyrosine nitration and a decreased mitochondrial transmembrane potential**(Parihar et al.,2009)**. Post translationally modified *α*-synuclein assciated with mitochondria was significant in PD-vulnerable brain regions: SNpc and striatum**(Hsu et al.,2000)**.These modifications include c-terminal truncation, ubiquitination, phosphorylation and nitrated *α*-synuclein Thus, biochemical abnormalities interact with genetic modifications to the *α*-synuclein gene which results in the impairment of mitochondrial function and subsequent neuronal degeneration**(Parihar et al.,2008)**.

Parkin, PINK1 and DJ-1. Parkin, PTEN- induced putative kinase I(PINK1) and DJ-1 are genes that code for proteins that are crucially involved in mitochondrial function and resistance to oxidative stress and have been linked with PD. The parkin gene encodes a cytosolic 465 amino-acid protein with a ubiquitin- like (UBL) domain at the N-terminus and an RBR (RING between RING) domain close to the C-terminus. Like other RING finger proteins, parkin acts as an E3 ubiquitin ligase which mediates the attachment of ubiquitin moieties to lysine residues of substrate proteins and mark those proteins for degradation by proteasome**(Moore 2006).** Thus, mutations to parkin may cause loss of this ligase activity resulting in accumulation of toxic substrates and neurodegeneration**(West 2004).**Relating it to the sporadic PD where Lewy bodies consits of aggreagated *α-Synuclein*, oxidatively modified and misfolded parkin was observed to present in the brain of PD patients**(Pawlyk et al., 2003)**., suggesting the failure of Parkin's E3 ligase activity and impairment in Ubiquitin Proteasome System (UPS).There are increasing evidences that suggest that presence of cysteine-rich RBR domain renders parkin vulnerable to post translational modifications and inactivation by severe oxidative stress **(Winkholker et al.,2003 and Schlehe et al,2008)**.It has also been suggested that Parkin may be involved in mitochondrial function and protection from mitochondrially generated ROS. A reduction in subunits of complex I and IV along with increased oxidative stress was observed in the brain tissue of parkin null mice marked by the presence of increase lipid peroxidation and protein modifications**(Palacino et al.,2004)**.This is further supported by reduced mitochondrial Complex I activity observed in Parkinsonian's patients with Parkin mutations and the increased age- dependent or rotenone (complex I inhibitory toxin) induced DA neurodegeneration and mitochondrial abnormalities in Parkin mutant drosophila**(Elibol et al.,2004 and Wang et al., 2007)**.

PINK1 is a conserved serine/threonine kinase associated with mitochondria via a short N-terminal mitochondrial targeting sequence (**Silvestri et al., 2005**).It is known to phosphorylate serine/ threonine residues in mitochondrial TNF-receptor-associated protein 1(TRAP1) the activation of which prevents oxidative stress-induced cytochrome c release and apoptosis**(Pridgeon et al., 2007**).Phosphorylation of serine 142 in HtrA2, one of the substrates of Parkin, is significantly increased in sporadic PD brains of humans, but virtually abolished in PD patients with PINK1 mutations **(Plun-Favreau et al., 2007)**.Knockdown of PINK1 has contributed to development of mitochondrial dysfunction by reduction in membrane potential, increase in ROS formation, dysregulation of calcium

homoestasis and heightened senstitivity to apoptosis **(Wood-Kaczmar et al., 2008)** while G309D and W437X PINK1 mutations in PD patients displayed lower respiratory activity, increased glycolysis and elevated levels of lipid peroxidation. Recent studies suggest the interaction between Parkin and PINK1 in removing damaged mitochondria.PINK1 tends to accumulate on the outer mitochondrial membrane and recruites Parkin under conditions of mitochondrial depolarization to promote mitophagy, the selective degradation of damaged mitochondria by lysosomes**(Xiong et al.,2009)**.Thus,loss of PINK1 blocks parkin recruitment to dysfunctional mitochondria which results in accumulation of impaired mitochondria and a subsequent buildup of ROS and apoptotic proteins.

DJ-1 is a 189 aa cytosolic protein, the mutation of which leads to autosomal recessive PD**(Bonifati et al.,2003)**. Oxidation of cysteine 106 to cysteine-sulfinic acid promotes the mitochondrial translocation of DJ-1 and is critical for DJ-1 function in this organelle(**Canet-Aviles et al.,2004 and Meulener et al.,2006)**.There are reports that suggest the role for DJ-1 as redox molecular chaperone by inhibiting α-synuclein aggregate formation and preventing neuronal death**(Kahle et al.,2009)**.A direct mitochondrial-DJ-1 association was observed when cells were exposed to neurotoxins. The observation that DJ-1 rapidly translocates to mitochondria under conditions of oxidative stress suggest the presence of mitochondrial targets for DJ-1**(Junn et al.,2009 and Blackinton et al.,2009)**. Possible targets of DJ-1 could be subunits of mitochondrial respiratory complexes, in particular complex I, because loss of DJ-1 is associated with increased sensitivity to complex I inhibitors. The importance of DJ-1 in mitochondrial activity was confirmed by its association to NADH dehydrogenase and nuclear and mitochondrial DNA encoding subunits of complex I . This was further supported by hypersentivity of DJ-1-deficient Drosophila to oxidative stress and environmental toxins implicated in PD**(Meulener et al.,2005).**The role of DJ-1 in sporadic PD forms is consistent with age related increase in levels of DJ-1 modifications, leading to significant increase in oxidative stress and inactivation of the protein's function**(Menzies et al.,2005 and Yang et al., 2005).**

Overall, genes involved in familial PD are either mitochondrial proteins or associated to mitochondria upon certain conditions and acts as crucial players in maintanenance of mitochondrial homoestasis by cellular apoptosis, removal of misfolded or aggregated proteins and dysfunctional mitochondria. Mutation in any of these genes brings about the initial effect to mitochondria which then acts as a causal factor in PD. In comparison to sporadic PD, where mitochondrial complex I was greatly inhibited resulting in the ATP depletion and oxidative stress by ROS formation, mutation of these genes also contribute to respiratory chain inhibtion in particular complex I impairment and oxidative damage to macromolecules like proteins, lipids and DNA by ROS products.The evidences which were putforth above suggests that mitochondrial complex I inhbition is common in both cases of PD and oxidative stress incurred upon respiratory chain defect could lead to mitochondrial dysfunction which then causes neurodegeneration.This is in line with the fact that both environmental factors and genetic factors could be the cause for sporadic PD from the perspective that familial genes are posttranslationally modified by environmental factors.

Discussion : A hypothetical model for mitochondrial dysfunction in PD

One of the plausible hypothesis for mitochondrial dysfunction as the cause for PD could be that complex I inhibition attributing as the initial and major trigger which then brings out many consequences(Fig 6). There are accumulating evidences that suggest both genetic factors and

environmental factors such as exposure to herbicides, pesticides and drugs impair Complex I by oxidation of iron –sulphur clusters**(Sriram et al.,1998)**

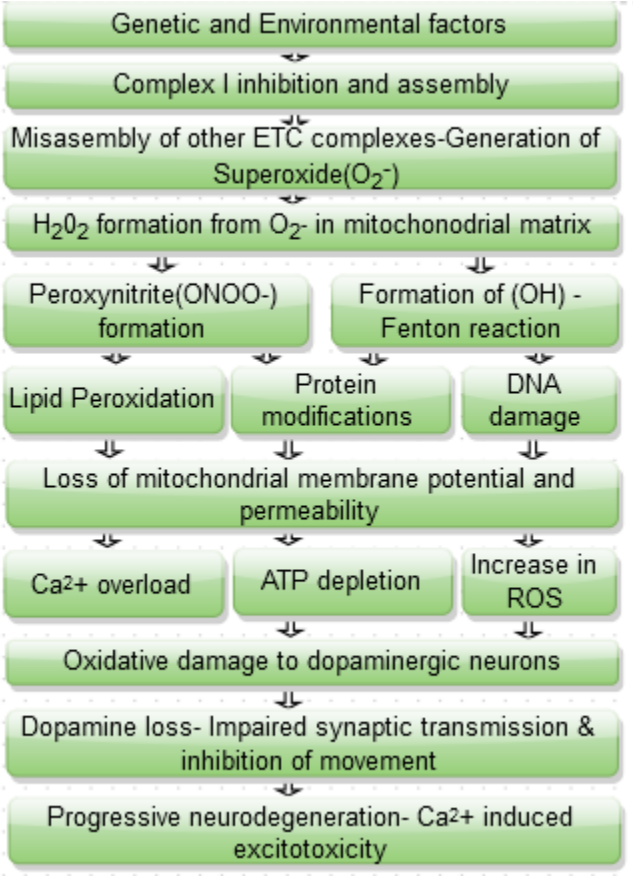


Fig 6 Hypothetical model for mitochondrial dysfunction in PD

Inhibition of Complex I impairs electron transfer to other electron transport chain complexes and affects its assembly. The electrons present in the missassembled complexes tend to leak from the inner mitochondrial membrane to matrix resulting in superoxides formation .The superoxide thus formed are further converted to hydrogen peroxide which diffuses to cytosol. Once H_20_2 is in the cytoplasm, it has the tendency to react with Fe2+ in a fenton reaction and produce hydorxy radical which attributes to the oxidative DNA damage**(Cadenas et al.,2000 and Zhang etal.,1999)**. The hydrogen peroxide that are still present in the mitochondrial matrix reacts with Nitric oxide to form Reactive Nitrogen species, peroxynitrite. Now the oxidative stress imposed on mitochondria increases by the formation of reactive oxygen and nitrogen species which could damage macromolecules**(Tsang et al.,2009)**

While lipid molecules undergo peroxidation induced by peroxynitrite, proteins are post translationally modified by peroxynitrite and hydroxyl radical.As a result of lipid peroxidation, protein modification and DNA damages, the permeability of the mitochondrial membrane is affected with a decrease in mitochondrial membrane potential**(Dexter et al.,1986,Floor et al.,1998 and Sayre1997)**. Thus, lower the mitochondrial membrane potential, higher would be the release of calcium to the cytosol by opening of mitochondrial permeability transition pore. Calcium homoestasis that are being bufferd by mitochondria is thus dysregulated**(Gunter et al.,2000)**.Now with the misassembled complexes, ATP production no longer takes place resulting in the depletion of ATP. The electrons that are not transfered between complexes and present within electron chain complex subunits for longer time tend to leak and produce more Rreactive oxygen Species(ROS)**(Armstrong et al., 1978)**. Increase in cytoplasmic calcium levels, ROS formation and ATP depletion creates a favourable environment for dopaminergic neurodegeneration to occur.Dopamine molecules which are generally stored in vesicles are released into cytoplasm as vesicle loading requires ATP. Now the cytosolic dopamine can oxidize to form quinones and dopamine adducts. These oxidative products thus formed increases the oxidative stress induced on neurons. Though the cytosolic dopamine levels that are present would be lower, the release of dopamine at the synapses cannot occur as exocytosis requires ATP.With a greater effect on synaptic transmission due to ATP depletion and Ca2+ dysregulation, the execution of movement would

be inhibited. This could increase the glutamate levels which stimulates inhibition and results in higher Ca2+influx**(Greene etal.,1996)**. Now along with bioenergetic dysfunction and oxidative stress, Ca2+ induced excitotoxicity adds up to the factors that cause progressive degeneration of dopamine neurons.

Concluding remarks and putative treatment strategies for PD

Overall, complex I inhibition affects other functions of mitochondria which leads to damaged and dysfunctional mitochondria. Thus dysfunctional mitochondria can no longer contribute to synaptic tranmission or neuronal survival thereby degeneration occurs as a consequence of mitochondrial dysfunction.When considering the cause for Parkinson's disease,it is important to take into account that bioenergetic dysfunction,oxidative stress, protein accumuation ,apoptosis and calcium dysregulation are not isolated events but surely are interrelated and occur as a result of mitochondrial impairment. For example,in case of complex I inhibition , the leakage of electrons favours reactive oxygen species(ROS) formation. ROS stress thus formed tends to attack other complexes and macromolecules lipids, proteins and DNA resulting in increased oxidative stress. The consequence of which protein degrardation pathways, ATP production, calcium regulation and antioxidant systems are compromised. There are existing therapies which target mitochondrial dysfunction in particular complex I deficiency such as Coenzyme 10(COQ10), creatine and SS peptides which are observed to beneficial than therapies that target dopamine loss such as administration of Levodopa and Carboxydopa. Thus neuroprotective methods targeting the mitochondrial dysfunction could be a novel therapy in treatment of Parkinson's disease.

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