INDEX

Greatness is not in where we stand, but in what direction we are moving. We must sail sometimes with the wind and sometimes against it - but sail we must and not drift, nor lie at anchor.

Oliver Wendell Holmes

(1809-1894) (American physician, professor, lecturer and author)

RESUMO

O tratamento atual para a Doença de Parkinson (DP), como a levodopa (L-DOPA) e os agonistas dopaminérgicos, pode efetivamente reverter os sintomas motores da doença. No entanto, além dos efeitos adversos associados aos fármacos, como a discinesia associada à L-DOPA, até a terapêutica farmacológica disponível não é capaz de prevenir o seu aparecimento ou de travar a evolução da doença. Assim, tem sido colocado muito enfâse na descoberta dos fatores etiológicos da DP Juvenil, particularmente na análise dos genes associados, de modo a propor um caminho provável para a sua modulação farmacológica. Estudos recentes têm proposto terapêuticas distintas que atuam na modulação intracelular do DJ-1. Por exemplo, o antioxidante Trolox, UCP0054278, fenilbutirato e pontes dissulfeto artificiais do DJ-1 aumentam a expressão dos níveis de DJ-1 *wild-type* e recuperam as funções do DJ-1 com consequente recuperação neuronal. Serão estas as ansiosamente aguardadas respostas inovadoras que podem conduzir à proteção dos neurónios dopaminérgicos? Estaremos na presença de fármaco úteis no tratamento da DP Juvenil?

ABSTRACT

Current treatment of Parkinson's disease (PD), such as levodopa and dopamine agonists, can effectively revert the disease's motor symptoms. However, on the top of drugs' side effects, such as L-DOPA-induced dyskinesia, the available pharmacological armamentarium is unable either to prevent its onset or to stop the evolution of the disease. Therefore, much emphasis has been put on unveiling etiologic factors of early onset PD, particularly the analysis of the associated genes, in order to propose a probable path for

pharmacological modulation. Recent reports have proposed distinct therapeutics acting on the intracellular modulation of DJ-1. For instance, the antioxidant Trolox, UCP0054278, phenylbutyrate and DJ-1's engineered disulfide bonds were considered to both increase DJ-1 wild-type expression as well as to promote the regain in DJ-1 functions with consequent neuronal recovery. Can these be the innovative and anxiously awaited therapeutics leading to dopaminergic (DAergic) neuroprotection and therefore to EOPD treatment?

KEYWORDS

Early onset Parkinson's disease, EOPD genetics, mitochondrial dysfunction, DJ-1 structure/functions, DJ-1 pharmacological modulation, UCP0054278, DJ-1's disulfide bonds, Trolox, phenylbutyrate

ABBREVIATIONS

3D	Three-dimensional
6-OHDA	6-hydroxy-dopamine
αSyn	α-synuclein
AD	Autosomal dominant
AMP	Adenosine monophosphate
AMPK	AMP activated protein kinase
AR	Autosomal recessive
ASK1	Apoptosis signal-regulating kinase 1
cAMP	Cyclic adenosine monophosphate
CNS	Central Nervous System
DA	Dopamine
DAergic	Dopaminergic
EOPD	Early onset Parkinson's disease
HSPs	Heat shock proteins
IR	Immunoreactivity
JNK	c-Jun N-terminal kinase
L-AP4	L-2-amino-4-phosphonobutyrate
LBs	Lewy bodies
L-DOPA	Levodopa
LRRK2	Leucine-Rich Repeat Kinase-2
MAO	Monoamine oxidase
mGlu	Metabotropic glutamate
MPP^+	1-methyl-4-phenylpyridinium

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

mRNA Mitochondrial RNA

mtDNA Mitochondrial DNA

mTOR Mammalian target of rapamycin

NO Nitric oxide

PD Parkinson's disease

PET Positron Emission Tomography

ROS Reactive Oxygen Species

SNc *Substantia nigra* pars compacta

TLR Toll-like receptors

UCP0054278 2-[3-(benzyloxy)-4-methoxyphenyl]-N-[2-(7-methoxy-1,3-benzodioxol-5-yl)ethyl]acetamide

wtDJ-1 Wild-type DJ-1

INTRODUCTION: CLINICO-PATHOLOGICAL CHARACTERIZATION OF EARLY ONSET PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative disorder, which is characterized primarily by the degeneration of dopamine (DA) neurons in a region in the midbrain, the *substantia nigra* pars compacta (SNc) (Dawson *et al.*, 2010). The SNc neurons project to forebrain areas, especially to the striatum, where the release of DA helps to regulate the firing of the thalamocortical motor circuits in the basal ganglia, in order to ensure proper planning and execution of movement (Duty, 2010). Thus, the pathological and anatomical hallmarks of PD include a relative selective loss of dopaminergic (DAergic) neurons in the SNc, which results in a decrease in DA availability, and the presence of Lewy bodies (LBs), enriched with aggregated forms of the presynaptic protein α-synuclein (αSyn). LBs are commonly found in surviving neurons of PD patients in several regions of the brain, such as SNc, locus coeruleus, dorsal nucleus of the vagus, parahippocampal gyrus and other brainstem and cortical regions.

PD's incidence is similar worldwide, being the second most common neurodegenerative disorder (after Alzheimer's disease) and the first if we include movement disorder (Bekris *et al.,* 2010). PD is believed to be a sporadic disease and age-related, with its higher rate in the sixth and seventh decades of life (de Andrade, 1996). In fact, its onset increases in elderly groups of the population, with over 1% of prevalence at the age of 65 years and over 4% by the age of 85 years. Also, approximately 20% of patients with PD report a family history of the disease (Bekris *et al.,* 2010).

A study led in five American and one French center, with a total of 1092 patients, revealed that the average age in which the symptoms begin is 57,1 years. PD cases starting at a very young age began to be described since late in the 19th century. For instance, in 1899,

Siehr described the first case in literature of two brothers with PD's manifestations starting at an early age. Since then, hundreds of similar cases, with two or more family members affected, have been reported. It is now a fact that these cases correspond to a subtype of PD – early or young onset PD, which is a juvenile form of parkinsonism defined as having an onset at or before the age of 45 years (Bogaerts *et al.,* 2008). This subtype, which is further discussed throughout this review, corresponds to about 5 to 10% of all cases (Kim *et al.,* 2006; Martin *et al.,* 2011). PD is diagnosed on clinical criteria and there is no definitive diagnostic test for PD; in fact the only way to confirm a diagnosis of PD is by brain biopsy (Borek *et al.,* 2006).

Clinically, PD is characterized by the following "cardinal signs": resting tremor, rigidity, bradykinesia and postural instability (Dekker *et al.,* 2003). The major motor manifestations are caused by the death of DAergic neurons in the SNc (Rappold *et al.,* 2010). Moreover, it is now known that symptoms of PD emerge as the concentration of striatal DA reduces below a threshold level of 70–80%. Alongside with the classical motor impairments, related symptoms, such as depression, bladder dysfunction, gastrointestinal disturbances and pain, can further weaken the quality of life of PD patients (Dekker *et al.,* 2003). The presence and specific presentation of the later features are used to differentiate PD from related parkinsonism disorders (e.g. medications, toxins and stroke-induced).

The differential diagnosis in a patient exhibiting parkinsonism includes neurodegenerative parkinsonian syndromes besides PD, such as progressive supranuclear palsy, multiple system atrophy and corticobasal ganglionic degeneration (Chahine and Stern, 2011). At present, the importance of distinguishing between PD and its differential diagnosis lies mainly in decisions regarding symptomatic management and prognosis. Hopefully in a near future, as neuroprotective and disease-modifying therapies emerge, distinction between them will be of even greater significance.

At present, levodopa (L-DOPA) and DA agonists are extremely successful at reversing the motor symptoms of PD. But they are quite ineffective in combating the degeneration of DAergic neurons in the SNc and their long-term use is associated with the appearance of disabling and hard to attenuate adverse effects (Duty, 2010). Currently, L-DOPA is still the mainly used drug (Bogaerts *et al.,* 2008). Although it has a first phase of dramatic benefit, some limitations become visible, specifically "DA resistant" motor signs (e.g. postural abnormalities and freezing episodes), "DA resistant" non-motor symptoms (e.g. autonomic dysfunctions, mood and cognitive impairment) and, sooner or later, psychosis, motor fluctuations and dyskinesias (Bogaerts *et al.,* 2008; Thanvi *et al.,* 2004). Additionally, L-DOPA does not cure the disease, nor does it prevent the development of clinical manifestations through the evolution of the disease. Current symptomatic therapies, besides oral drugs, include subcutaneous or intravenous DA agonists, continuous delivery therapy, transdermal patches, intraduodenal L-DOPA, and surgical interventions such as deep brain stimulation of the subthalamic nucleous, globus pallidum *pars interna*.

Since limitations of the current treatment (particularly L-DOPA) became evident, investigations have been developed to find alternative non-DAergic drugs.

The DJ-1 is a newly discovered gene, associated with EOPD. Its analysis brings new motivation into the investigation of PD's pathogenesis since it has enormous impact on DAergic cells. Thus, herein, we present a potential innovative pharmacological strategy for EOPD patients.

ETIOLOGIC FACTORS OF EARLY ONSET PARKINSON'S DISEASE

While EOPD has a monogenic basis, the etiology of sporadic PD (95% of the cases of PD) is not fully understood. Molecular genetic studies in PD cases identified mitochondrial dysfunction as a key feature of both sporadic and genetic causes of PD (Bogaerts *et al.,* 2008; Kim *et al.,* 2006). This mitochondrial hypothesis first arose after the discovery by Dr. Langston team in 1983 that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine), injected along with narcotics, reproduced PD-like symptoms. It became known that the metabolite MPP^+ (1-methyl-4-phenylpyridinium) is actively transported into DAergic neuron, enters mitochondria and selectively inhibits mitochondrial respiration at complex I of the electron transport chain. Therefore, the MPTP mouse model is the most widely employed in PD investigations.

Furthermore, studies in rats showed that chronic infusion of some environmental chemicals which are selective complex I inhibitors, such as herbicide paraquat, 6-hydroxydopamine (6-OHDA), and the pesticide rotenone, reproduced behavioral and neuropathological features of PD, i.e. induced DAergic neurodegeneration. These defects in complex I activity are constantly seen in SNc of patients with PD. Cybrid experiments, in which mitochondrial DNA (mtDNA) from PD patients is introduced into mtDNA-depleted cells, have shown that PD tissues show reduced mitochondrial respiratory chain complex I activity in the SNc, cerebral cortex, skeletal muscles and blood platelets (Larsen *et al.,* 2011). In fact, the defect in oxidative phosphorylation in PD is not merely confined to the brain, as studies found that this diminished complex I activity was also seen in PD patients' platelets as well as in their cybrid cells (cell lines engineered in order to enclose mitochondria derived from PD patients' platelets – this is useful for *in vitro* studies) (Dauer and Przedborski, 2003; Larsen *et al.,* 2011).

Besides the mitochondrial pathway, the involvement of reactive oxygen species (ROS) and oxidative stress in PD pathogenesis have also been suggested (Irrcher *et al.,* 2010). Loss of neuromelanin containing DAergic cells is characteristic of PD – this attracted attention to the auto-oxidation of DA. The normal metabolism of DA originates superoxide hydrogen peroxide, which can then form the hydroxyl radical in the presence of transition metals (Graham, 1978). In addition, DA is oxidized to a DA quinone, which happens spontaneously, non-enzymatically (Irrcher *et al.,* 2010). In the end, quinone modifies cellular macromolecules, which may contribute to DA-induced neurotoxicity.

On the other hand, emerging data points to defects in mitochondrial morphology and dynamics as a source of ROS production thus contributing to increased sensitivity of neurons to oxidative stress (Irrcher *et al.,* 2010). Finally, apoptosis mitochondrial-driven was also implied in the degeneration of DAergic cells. Actually, an increased immunoreactivity for the effectors of the mitochondrial apoptotic pathway (e.g. caspase-3) has been detected in PD patients in *post-mortem* studies (Bogaerts *et al.,* 2008). Nevertheless, there is no consensus relating apoptosis to loss of DAergic neurons in PD. Therefore this issue warrants further investigation.

On the other hand, the goal of a wide range of recent studies has been the identification of genetic loci at which pathogenic mutations become associated with PD (Dekker *et al.,* 2003). In fact, over eleven genes and loci have been identified and reported as responsible for rare Mendelian forms of PD and provide important opportunities to understand the heterogeneity of this disease, plus the variety of pathogenic routes and their outcome (Table 1).

The majority of the identified genes has distinct pathogenic pathways, including ubiquitin-proteasome system dysfunction, oxidative stress and mitochondrial dysfunction. For instance, mutations in the autosomal recessive genes PINK1, parkin and DJ-1 may directly

cause mitochondrial dysfunction (selectively in the turnover of damaged mitochondria) in addition to other pathogenic individual roles (Cookson *et al.,* 2010). Additionally, these genes have been found to reside or translocate to the mitochondrial compartments, to participate in mitochondrial remodeling and to actively regulate mitochondrial quality control (Irrcher *et al*., 2010).

On another spectrum, dominant mutations in POLG, the catalytic subunit of mitochondrial DNA polymerase, cause parkinsonism in some families (Cookson *et al.,* 2010). Additionally, dominant mutations in LRRK2 (i.e. Leucine-Rich Repeat Kinase-2, a large multidomain containing protein and located to membranous structures) and αSyn are more commonly related to late onset PD.

Also, there are recent studies showing the relative frequencies of mutation categories dependent on ethnicity and familial history (Nuytemans *et al.,* 2010). For example, LRRK2 is the most frequently mutated PD gene so far, mainly in Caucasians, Asians and Latin Americans, among others. Due to the high prevalence of its mutations, this gene is extremely useful to study genotype-phenotype correlations (Alcalay *et al.,* 2010). In 2008 a study revealed that the most prominent phenotype of the upmost frequently reported LRRK2 mutation (G2019S) is an asymmetric levodopa-responsive parkinsonism with the common presence of tremor. The remaining six pathogenic LRRK2 mutations are far less frequent worldwide (Crosiers *et al.,* 2011). In a group of patients with known genes associated with EOPD, 6,7% had mutations in the parkin gene, the most frequently mutated gene associated with the disease, and about 0,2% with mutations in the DJ-1 gene. Although worldwide reports claim that DJ-1 mutations account for a small fraction of EOPD, they are the second most frequent cause for autosomal recessive (AR) EOPD (Vila and Przedborski, 2004). Moreover there is a growing interest on the study of this particular mutation.

The study of the monogenic PD families is of undeniable importance to achieve the most complete insight possible into the disease, its pathways and its clinical features (Crosiers *et al.,* 2011). On the other hand, these families will be crucial for the development of neuroprotective and disease-modifying therapies. Additionally, as they harbor presymptomatic mutation carriers, these families have a higher risk of developing PD and, as a result, are attractive subjects in future therapeutic trials. Additionally, the recognition of EOPD-linked genes has changed one's perspective of the pathophysiology of EOPD.

Table 1 – PD's genes. Display of some of the genes proven to be associated with inherited PD, either early or late onset, and their features (adapted from Martin *et al.*, 2011, Nuytemans *et al.,* 2010, Dekker *et al.*, 2003, Crosiers *et al.,* 2011)

PARK locus / *Gene*	**Map position**	**Inheritance**	**Phenotype**	**Clinical features**	**Pathology**
PARK1/4 / *SNCA*	4q21	AD	EOPD over the age of 21 years	Parkinsonism with common dementia; cognitive impairment and rapid disease progression	Lewy bodies
PARK2 / *parkin*	6q25-q27	AR	EOPD over the age of 21 years	Slow disease progression; good levodopa response; early motor fluctuations	Lewy bodies
PARK6 / *PINK1*	1p35-p36	AR	EOPD over the age of 21 years	Slow disease progression; good levodopa response; early motor fluctuations	Rarely shows Lewy bodies
PARK7 / *DJ-1*	1p36	AR	EOPD over the age of 21 years	Slow disease progression; good levodopa response; early motor fluctuations; focal dystonia and psychiatric symptoms	Unknown
PARK8 / *LRRK2*	12q12	AD	Late onset PD	Classical symptoms and signs	Lewy bodies
PARK9 / ATP13A2	1p36	AR	EOPD previous to 21 years	Parkinsonism with Kufor-Rakeb syndrome; pyramidal signs and dementia	Unknown
PARK15 / FBX07	22q12-q13	AR	EOPD previous to 21 years	Atypical parkinsonism with pyramidal signs and cognitive impairment	Unknown
Gaucher's locus / *GBA*	1q21	??	Late onset PD	??	Lewy bodies

THE ROLE OF DJ-1 IN EARLY ONSET PARKINSON'S DISEASE

As indicated in Table 1, mutations in the DJ-1 gene are linked with AR EOPD. DJ-1-related EOPD, as do sporadic forms of PD, also include resting tremor, postural tremor, bradykinesia and loss of postural reflexes, but with an asymmetric onset of symptoms before the age of 40 years in all cases identified so far (Dekker *et al.,* 2003). The disease incorporates variable degrees of severity and slow progression of symptoms, with continuous response to the L-DOPA treatment. Although rare, psychiatric co-morbidities were also reported, including psychotic episodes and anxiety in patients with the L166P and M26I mutations, respectively (Kahle *et al.,* 2009).

Linkage of this neurodegenerative disease to the PARK 7 locus was first identified in 2001, in a genetically isolated population in Southwest of The Netherlands that suffered from inherited EOPD (Dekker *et al.,* 2003). In fact, DJ-1 mutations has showed to be responsible for some cases of EOPD in the population from this region: a study including 220 randomly selected individuals revealed that four of them were heterozygous for the DJ-1 deletion, bearing an estimated mutant allele frequency of 1-2%. Parallel to this study, a survey conducted in the same population concluded that four out of six individuals with EOPD were homozygous for the DJ-1 deletion. Moreover, as this gene is known to be responsible for AR PD, its mutants can explain around 67% of EOPD in this region.

Being widely expressed in various tissues, subcellularly, DJ-1 is localized to the cytosol, mitochondrial matrix and inter-membrane space (Dawson *et al.,* 2010). This distribution is dynamic, with some DJ-1 fractions showing mitochondrial and nuclear translocations (Kahle *et al.,* 2009). Cytoplasmic DJ-1 locations have also been proven. In addition, it was also reported that DJ-1 protein was detected in extracellular spaces, such as

plasma and cerebrospinal fluids, suggesting that DJ-1 protein may function in both intracellular and extracellular spaces, and may be ultimately useful as a biomarker of PD (Inden *et al.,* 2011).

One of the many tissues generating DJ-1 protein is the brain, where the gene is thought to: (1) protect against oxidative stress responses, (2) function as a chaperone by refolding damaged proteins or by targeting and delivering damaged proteins to degradation (e.g. DJ-1 blocks αSyn aggregation) and (3) protect against mitochondrial dysfunction. Therefore, DJ-1 prevents neuronal damages as it has been shown both in *in vitro* and *in vivo* studies (Kumaran *et al.,* 2009; Logan *et al.,* 2010).

The expression of DJ-1 is up-regulated in both brains and cerebrospinal fluids in many neurological disorders, such as PD, Alzheimer's disease and stroke (Waragai *et al.,* 2007). Thus, detection of DJ-1 might be a diagnosis biomarker for these diseases and also determine theirs severity and evolution. Besides DJ-1 involvement in neurodegenerative diseases, other functions were assigned to DJ-1. For example, DJ-1 was first identified as an oncogene and its secretion has been recently observed in breast cancers (Tao *et al.*, 2003). It is also involved in the fertilization process in rat and mouse – in fact, a significant reduction in the amount of this protein on the surface of sperm makes it unable to fertilize eggs. In addition, it is related with androgen receptor signaling regulation and with mRNA (mitochondrial RNA) stabilization in response to cAMP (Kumaran *et al.,* 2009).

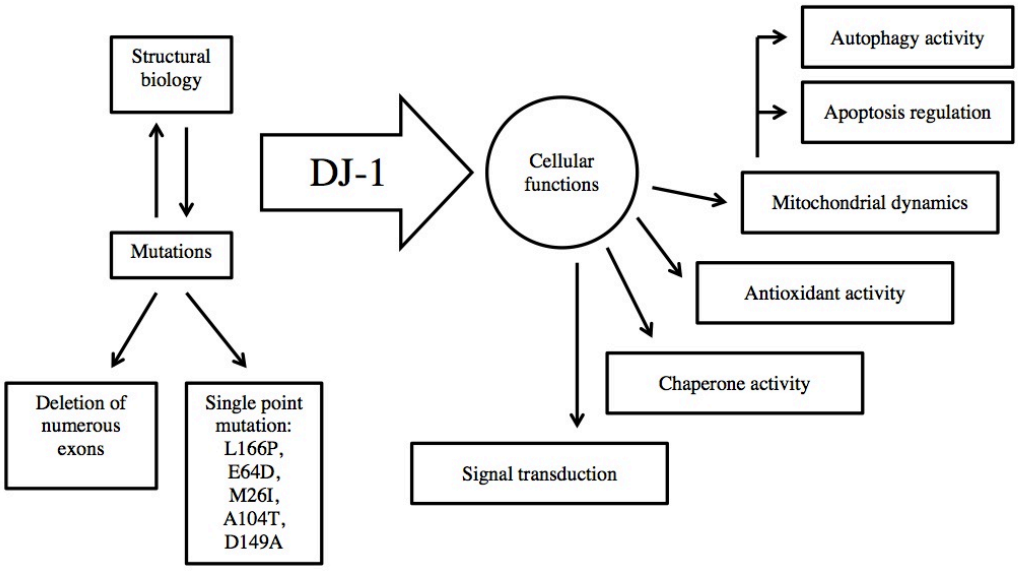

Figure 1. DJ-1 actions. Schematic representation of DJ-1's potential modification sites and cellular actions, further exploited in the following paragraphs.

Structural biology of PARK7/DJ-1 and its mutations

The opportune suggestion of the DJ-1 crystal structure provided a helpful support for the molecular understanding of its mechanisms of action (Kahle *et al.,* 2009). DJ-1 gene contains 8 exons (Moore *et al.,* 2003). Its protein exists as a homodimer, containing a structure with a six-strand parallel β-sheet sandwiched by α-helical arrangements – flavodoxin fold (Fig. 2). It contains 189 amino acid residues and belongs to the DJ-1/ThiJ/PfpI superfamily of proteins, which is present in various tissues (Thomas and Beal, 2007).

Just a year after the identification of the PARK7 locus being related to PD, mutations in the DJ-1 gene are identified. Indeed, DJ-1 mutations were first identified in consanguineous relatives with autosomal recessive PD in two different families (Dauer and Przedborski, 2003). One retained a deletion thought to inhibit the protein function, whereas the second carried a missense mutation (insertion of a proline into an α-helical region, which leads to

mutant DJ-1 accumulation in the mitochondria).

Additionally, studies of an Italian family with autosomal recessive EOPD revealed two mutations in the homozygous state, a missense mutation in exon 7 resulting in the E163K substitution and the other located in the promoter region of this gene. Since then, several studies took place, resulting in the identification of two types of disruptions of DJ-1 in PD patients: (1) the first corresponds to a deletion of numerous exons, causing a stop in the production of the DJ-1 protein; (2) the second disruption includes a single point mutation. Hague and his collaborators (2003), following the sequence of coding exons 1 through 7 of DJ-1 in 107 cases of EOPD, found a subject with 24 years, which had a frameshift mutation in the first exon and a splice mutation in the seventh. Both these mutations are thought to result in the protein's functional loss. This individual's EOPD included asymmetric onset and an robust response to treatment with L-DOPA.

DJ-1's structural perturbations associate with five different PD linked homozygous loss-of-function mutations in the gene encoding DJ-1: L166P, E64D, M26I, A104T, and D149A, being L166P the most frequent mutant protein (Kahle *et al.,* 2009). The mutations have different sites of expression in the crystal structure of DJ-1: M26I and A104T are highly buried within the interior of the monomer, whereas L166P is located between the two helices that outline the dimer interface, and both E64D and D149A are relatively surface exposed (Fig. 2) (Malgieri and Eliezer, 2008). Crystal from E64D and M26I display slight local perturbations, whereas the classical L166P mutation in DJ-1 prevents its dimerization by disrupting the α-helix G of the C-terminal, leading to a dramatic destabilization and functional loss due to degradation by the proteasome (Kahle *et al.,* 2009). The familial DJ-1 mutation M26I and E64D, together with H_2O_2 induced cysteine 106 oxidation and cleavage, have proven to destabilize DJ-1 (Thomas and Beal, 2007).

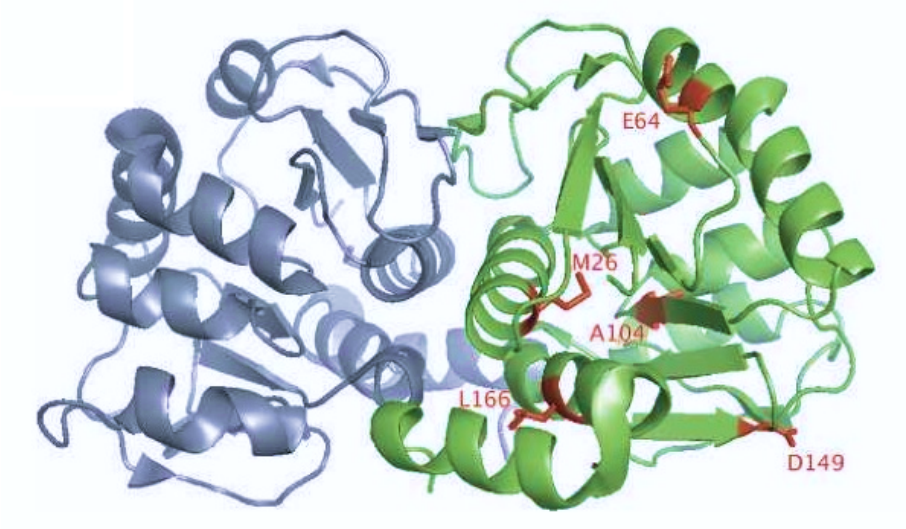

Figure 2. wt DJ-1 conformation. Representation of the crystal structure of the wild-type DJ-1 (wtDJ-1) dimer, with one monomer in grey and the other in green. The sites of PD-linked mutations are labeled in red (Malgieri and Eliezer, 2008).

For example, in studies from consanguineous Dutch and Italian relatives affected with EOPD, L166P mutation underlied disease in homozygous individuals, while heterozygous carriers were unaffected, suggesting that the total loss of function of DJ-1 is pathogenic (Lockhart *et al.,* 2004). This result confirms that DJ-1 causes AR EOPD, as it only manifests the disease in homozygosity. In fact, unlike in parkin and PINK1 mutation cases, the heterozygosity of DJ-1 appears compatible with a normal striatal presynaptic DAergic neurotransmitter system (Guo *et al.,* 2011).

Data from several studies show that L166P has a marked protein-folding defect, disrupting the dimer formation, which has a dissimilar cytoplasmic distribution (Tao *et al.,* 2003). Therefore it is associated with rapid protein turnover and elevated tendency to produce large protein complexes (Malgieri and Eliezer, 2008; Olzmann *et al.,* 2004). In cell culture, the L166P mutant is expressed at a lower steady state level when compared to wtDJ-1. In addition, it shows an increased turnover rate. In part, proteasome inhibitors revoke these

effects. L166P protein is believed to be polyubiquitnated, implying a role for the ubiquitin/proteasome system in the turnover of this mutant, among other possible degradation pathways (Olzmann *et al.,* 2004). Moreover, it is ineffective in eliminating H_2O_2 inside the cell and preventing their induced death. It is clearly considered functionally inactive. From what is currently known, substitutions at different sites of the DJ-1 protein destabilize its structure to a lesser extent (Madian *et al.,* 2011). In fact, data obtained from mutations compared with data from the wtDJ-1 shows that, unlike L166P mutation, E64D mutation's structure is almost unperturbed and closely resembles the wtDJ-1. E64D mutation probably modulates DJ-1's interactions with binding partners and further compromises its function without perturbing the structure. Finally, the remaining three mutants have distinct structural rearrangements accompanied by a decrease in the protein's thermodynamic stability. This wide range of disparities is likely to underlie the functional differences between the mutants and the wtDJ-1 and are, in turn, likely responsible for the consequent pathogenicity (Malgieri and Eliezer, 2007). Appropriate localization in cells is also thought to be required for DJ-1 to operate properly; while wtDJ-1 is essentially localized in the cytoplasm and nucleus, L166P is mainly found in the mitochondria (Taira *et al.,* 2004). Further genetic investigation on the DJ-1 promoter and untranslated mRNA sections can deliver additional insight into its molecular genetics.

Neuropathology of DJ-1

The pathology in EOPD triggered by DJ-1 mutations is still not completely understood, but *pre/ante-mortem* studies indicated neuropathological changes in the brains of DJ-1 patients: Positron Emission Tomography (PET) neuroimaging showed severe DA depletion in homozygous DJ-1 mutation carriers; transcranial ultrasound revealed the PD-

characteristic *substantia nigra* hyperechogenicity in a E64D-mutated DJ-1 patient, which was comparable to other genetically related PD patients, although less outstandingly than in sporadic PD patients (Kahle *et al.,* 2009). Furthermore, PET scanning allows *in vivo* analysis of the nigrostriatal deficit in both hereditary and sporadic PD patients. Using a DA transporter ligand in affected patients and asymptomatic carriers of mutations in the parkin, PINK1 and DJ-1 gene, it was demonstrated that the mapping results are identical, as in all cases abnormal nigrostriatal dysfunction was evident. This suggests the existence of a common pathogenesis among these mutations. Many recent investigations disclosed that, in the normal human brain, DJ-1 is moderately expressed in neurons and in astrocytes throughout the Central Nervous System (CNS). Examination of sporadic neurodegeneration in PD patients revealed that DJ-1 is strongly expressed in their reactive astrocytes.

Several studies took place in order to map DJ-1's distribution in PD patients' brain using monoclonal antibodies, which recognized a single band corresponding to DJ-1 from human frontal cortex. In 2004, Bandopadhyay and his coworkers applied Western blotting and immunoreactivity (IR) in order to localize this protein in the frontal cortex and *substantia nigra*, regions invariably involved in PD (Bandopadhyay *et al.,* 2004). This study led to the result that DJ-1 is an abundant protein in human frontal cortex both in control cases and in idiopathic PD. Moreover these authors showed that DJ-1 IR was predominantly noticeable in the astrocytes in control cases and also in PD's patients' frontal cortex, whereas neurons displayed slight or no DJ-1 IR. That is to say that the major cell type expressing DJ-1 is glial instead of neuronal. Nevertheless, only a few LBs showed faint DJ-1 IR, localized to its outer halo, which indicates that this protein is not a vital component of LBs and is therefore improbable to take part in their formation.

In addition, Kumaran and his coworkers used quantitative real-time PCR (Polymerase Chain Reaction) to compare DJ-1 mRNA levels in both *post-mortem* PD and control brain

regions, in order to determine if DJ-1 is involved in the pathogenesis of sporadic forms of PD (Kumaran *et al.,* 2009). DJ-1 mRNA levels revealed region-specific decreases in putamen, frontal cortex, parietal cortex and cerebellum in PD (30–60%) compared to controls. However, an up-regulation was seen in the amygdala (90%) and entorhinal cortex (39%).

Cellular functions of DJ-1

Biochemically, DJ-1 oxidizes, shifting to more acidic forms. These oxidative modifications have been found in brain samples from PD and Alzheimer disease patients (Kahle *et al.,* 2009) (further see *Antioxidant activity*).

DJ-1 is a cytoprotective protein acting to promote mitochondria-stabilizing anti-oxidant and anti-apoptotic mechanisms (Larsen *et al.,* 2011). Though the pathogenic mechanisms underlying PD are not well known, growing evidence has linked DJ-1 deficiency to the impairment of mitochondrial connectivity, fusion rates, membrane potential, respiratory capacity and more recently autophagy, further explained ahead (Irrcher *et al.,* 2010).

Thus, when DJ-1 goes out of function the cells go awry, as DJ-1 is a cytoprotective protein acting to promote mitochondria-stabilization and exhibiting anti-oxidant and chaperone activities. Therefore, mitochondrial instability, oxidative stress and protein mishandling are intimately related with DJ-1 mutations and underlie the pathogenic cascade seen in PD (Fig. 3) (Vila and Przedborski, 2004).

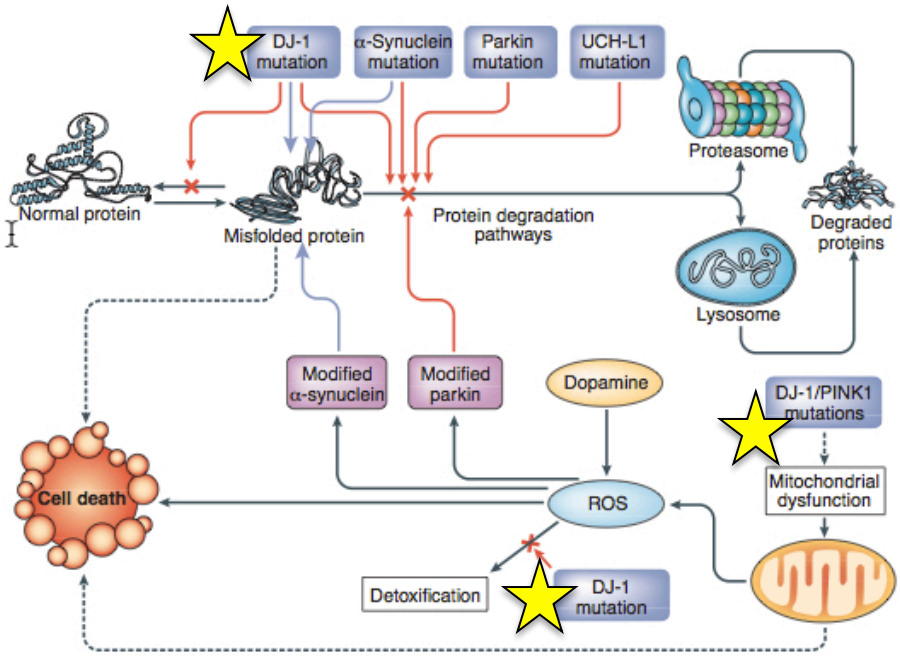

Figure 3. Representation of PD's genetic mutations and their pathogenic cascade (Vila and Przedborski, 2004). DJ-1 actuation sites were emphasized with yellow stars. The DJ-1 protein, as well as αSyn, may be misfolded (blue arrows) and overload the degradation pathways of ubiquitin and lysosome. DJ-1 chaperone activity may also be altered by its mutations, which interrupt the refolding of the damaged proteins or their delivery to degradation (red arrows). PD's oxidative stress may be originated from the reduced capacity of mutated DJ-1 to detoxify ROS. In part, mitochondrial dysfunction might be associated with altered activity and mislocation of both DJ-1 and PINK1.

- ***Mitochondrial dynamics***

Pharmacological inhibitors of mitochondrial complex I, such as rotenone, indubitably cause parkinsonism in experimental conditions (Larsen *et al.,* 2011; Mullet and Hinkle, 2011). DJ-1 deficit, seen in cultured neurons from mouse brain and lymphoblast cells derived from DJ-1 patients, display aberrant mitochondrial morphology (Irrcher *et al.,* 2010). In fact, it was demonstrated that mitochondria is considerably more fragmented in cells with DJ-1 loss,

which contributed to oxidative stress-induced sensitivity to cell apoptosis. Thus, the protein is over-expressed in PD astrocytes and its loss unables the cells to protect neurons against pesticides like rotenone. Additionally, it is known that, only in the presence of pesticides that inhibit mitochondrial complex I activity (like rotenone, pyridaben, fenazaquin, and fenpyroximate), do impairments induced by DJ-1 deficit occur (Mullet and Hinkle, 2011). Moreover, epidemiological studies imply that exposure to pesticides, herbicides, and other environmental toxins that inhibit mitochondrial complex I can lead to excess production of ROS and increased incidence of sporadic PD (Choi *et al.,* 2006). Furthermore, knockout flies of DJ-1α and DJ-1β, the two DJ-1 homologues in *Drosophila*, revealed a selective sensitivity to environmental toxins (e.g. paraquat and rotenone) (Bogaerts *et al.,* 2008). Both these homologues are associated to enlarged and swollen mitochondria. Also, astrocyte mitochondrial behavior differs between sub-cellular regions and it can be affected by the physical presence of neurons in the culture (Larsen *et al.,* 2011). Current studies recognized that the aberrant mitochondrial phenotype could be rescued, in DJ-1's absence, by the expression of Pink1 and parkin, the two PD-linked genes also involved in regulating mitochondrial dynamics and quality control (Irrcher *et al.,* 2010).

It was first revealed that complex I activity was reduced in the SNc of PD patients in 1990 and, since then, a growing number of investigations took place, to evidence that DAergic neuronal cells without DJ-1 exhibit abnormal mitochondrial respiratory chain complex I activity, associated with a defect in its formation (Heo *et al.*, 2012). Therefore, the majority of the neurons that persist in SNc, the most affected region of the brain in PD, exhibit a defect in complex I of the mitochondrial electron transport chain and show signs of oxidative damage (Madian *et al.,* 2011). Pathological damage of the mitochondrial respiratory chain disturbs electron transfer, generating oxidative stress that results in mitochondrial dysfunction (Heo *et al.,* 2012). It ultimately leads to cell death via apoptosis, thus ensuring

mitochondrial quality and adequate energy supply, which is crucial for correct neuronal performance.

So far, it seems that one of the most important functions of DJ-1 is to maintain mitochondrial membrane potential, as the DJ-1 null cells possess mitochondria with reduced rate of oxygen consumption and altered membrane potential (Heo *et al.,* 2012). Furthermore, DJ-1 appears to be involved in removing damaged mitochondria by activating mitophagy. Particularly, PD patients with altered DJ-1 displayed smaller mitochondria and, specifically in these cases, mitochondrial fragmentation is thought to be due to a defect in complex I, although the exact mechanism is still unknown.

Curiously, DJ-1 deficient mice show neither anatomical nor neuronal gross abnormalities, and have normal numbers of DAergic neurons. Still, their nigrostriatal pathway is dysfunctional, conducting to higher DA concentrations and more cellular oxidative stress. In conclusion, DJ-1 impact on complex I of mitochondria might be one of the main pathological mechanisms for the deterioration of DAergic neurons in PD, particularly in AR EOPD (Heo *et al.,* 2012).

A recent study by Wang and his collaborators (2012), using confocal and electron microscopic analysis, revealed that M17 human neuroblastoma cells with wtDJ-1 over-expression showed elongated mitochondria. On the other hand, these same cells with over-expressed DJ-1 mutations associated with PD (e.g. L166P) displayed great increase of fragmented mitochondria as well as structural damage of the cell. This ultimately confirms the stated above – DJ-1 mutation over-expression in a cell result in its mitochondrial dysfunction, particularly impairment of mitochondrial bioenergetics. This increases neuronal vulnerability to oxidative stress or neurotoxins, such as rotenone.

The cell survival pathway, when under starvation conditions, is autophagy. Thus, when cells are put under stress factors, such as treatment with rotenone or simply subjected to hypoxia, autophagic cell death is induced. A recent study provides examples of the existence of both apoptosis and autophagy in the same cell (Chen and Gibson, 2008). It is established that mitochondria play an important role in the regulation of both types of cell death.

- ***Autophagic activity***

There is mounting evidence linking autophagic activity to AR EOPD (Thomas *et al.,* 2011). Some PD-linked genes, as Pink1, parkin and DJ-1, seem to regulate mitophagy, i.e. mitochondrial autophagy, in response to mitochondrial damage. In 2010, Thomas and his collaborators developed an investigation using M17 human DAergic neuroblastoma cell lines firmly expressing shRNA (i.e. short hairpin RNA) against DJ-1, in addition to nonsense shRNA controls selected and cloned in parallel. In this sequence, they were able to prove that loss of DJ-1 leads to deficit in mitochondrial polarization, fragmentation of mitochondria and accumulation of markers of autophagy (LC3 punctae and lipidation) around mitochondria in DAergic cells.

DJ-1 function may either contribute to the regulation of autophagy or mitigate the downstream effects of ROS, as studies have shown that ROS can up-regulate autophagy (Irrcher *et al.,* 2010; Thomas *et al.,* 2011). Accordingly, lack of DJ-1 increases autophagic activity and consequently increased cellular turnover.

Considering that DJ-1 may participate in the Pink1/parkin pathway led Irrcher and his partners to suggest that DJ-1 could modulate Pink1/parkin activity and thereby regulate autophagic activity (Irrcher *et al.,* 2010). There is evidence suggesting that ROS up-regulate autophagy (Chen and Gibson, 2008). In fact, these authors provided strong evidence that mitochondrially-generated ROS production is involved in the induction of autophagy. These

results were established through treatment with mitochondrial toxins inhibiting the electron transport chain, including rotenone, in transformed and cancer cells. On the contrary, these toxins failed to induce autophagy in normal astrocytes. ROS involvement was ascertained by the formation of autophagosomes and autolysosomes. Therefore, DJ-1 function may either directly regulate autophagy or mitigate the effects of ROS in autophagy up-regulation. These hypotheses warrant further studies.

Moreover, autophagy can be promoted by AMPK (AMP activated protein kinase - a main energy sensor and also controls the cellular metabolism to preserve energy homeostasis) but it can be inhibited by mTOR (the mammalian target of rapamycin - an essential regulator of cell-growth integrating the growth factor and nutrient signals) (Kim *et al.,* 2011). In fact, under glucose deprivation, AMPK directly activates the autophagy-initiating kinase Ulk1, while mTOR prevents Ulk1 activation, in part, trough the disruption of Ulk1 and AMPK interaction. In this line of thought, DJ-1 may directly regulate additional upstream modulators of autophagy, including mTOR and AMPK (Vasseur *et al.,* 2009). Actually, in cancer, DJ-1 behaves as an oncogene handling Akt-mediated cell survival, although its mechanism of action is not completely understood. The tumor's adaptation to hypoxic conditions (in order to survive and progress) is mostly mediated by transcription factor HIF1, which has its stabilization partially dependent on the PI3K/Akt/mTOR pathway. Over-expression of DJ-1 positively regulates Akt. Therefore, DJ-1 might be implied in sustaining HIF1 transcriptional activity under hypoxia. This statement was verified through a study of human cell lines and transformed mouse fibroblasts, which showed that DJ-1 expression is critical for the activity of Akt and mTOR. Furthermore, DJ-1 increases AMPK activity that acts as a metabolic sensor, particularly during hypoxia. A rapid induction of both AMPK activity and HIF1 stabilization suggests that the AMPK and HIF1 systems work in parallel during hypoxic states, and are ultimately regulated by DJ-1.

- ***Apoptosis regulation***

The main proteins that interact with DJ-1 in DAergic neuronal cells are p54nrb and pyrimidine tract-binding protein-associated splicing factor (PSF), two nuclear proteins acting as multifunctional regulators of RNA transcription and metabolism, being PSF required for *in vitro* splicing of pre-mRNA (Xu *et al.,* 2005). In order to clarify the specific molecular functions of DJ-1 in these neurons, an impartial tactic was employed by affinity purification and mass spectrometry so as to identify the major interacting proteins, followed by protein interaction assays and co-localization analysis. Furthermore, Xu and his coworkers (2005) showed that DJ-1 developed a nuclear complex with p54nrb and PSF. Both these proteins have homologous RNA recognition motifs and establish heterodimeric complexes capable of binding RNA, in addition to regulate gene transcription and binding to DNA. The wtDJ-1 inhibits the transcriptional silencing activity of the PSF, thus indirectly preventing the PSF-induced neuronal apoptosis. It also induces PSF over-expression, thus directly preventing apoptosis. Both DJ-1 and p54nrb work to prevent oxidative stress and cell death induced by mutant αSyn. As DJ-1 mutants exhibit decreased nuclear distribution and increased mitochondrial localization, there is diminished interaction with p54nrb and repressor PSF (Xu *et al.,* 2005). Therefore, these mutations, by decreasing DJ-1 stability and nuclear distribution, leave DAergic neurons vulnerable to apoptosis and may therefore contribute to the pathogenesis of EOPD.

- ***Antioxidant activity***

Every organism reacts to toxic environmental stresses, such as ROS, by over-expressing a set of heat shock proteins (HSPs), which may act as molecular chaperones to assist the folding of other proteins (Shendelman *et al.,* 2004). DJ-1, like HSP31 (an E. coli

ThiJ domain protein), functions as a protein chaperone to protect cells from ROS. This activity was quantified though the suppression of heat-induced aggregation of citrate synthase and glutathione S-transferase, two well-known protein chaperone assays. They undergo heat-induced aggregation during incubation, which was effectively suppressed by the addition of DJ-1. In fact, DJ- 1 chaperone activity is comparable to that of a well-described small cytoplasmic chaperone, human HSP27 (further see *Chaperone activity*).

DA is a highly toxic compound, which leads to generation of ROS due to its catabolism, via monoamine oxidase (MAO) or via auto-oxidation (Lev *et al.,* 2009). DA is synthesized in the cytosol and rapidly sequestered into synaptic vesicles, where the low vesicular pH and the absence of MAO limits its break-down, opposite to what happens in cytosol. Therefore, DA-producing neurons (specially SNc Daergic neurons) are particularly susceptible to oxidative stress. That is because neurotransmitters produced by other neurons are not as toxic as DA due to its action as a mitochondrial toxin (by inhibition of the electron transport chain) (Lev *et al.,* 2009). This way, DA-exposure leads to abnormal mitochondrial morphology and to up-regulation of DJ-1, which plays a neuroprotective role by increasing cell resistance to DA toxicity and diminishing intracellular ROS. It was suggested by Lev and his collaborators (2009) that the MAP kinases pathway, through activation of ERK1/2, triggers DA-induced DJ-1 up-regulation.

While mutations in DJ-1 gene set off loss-of-function, the wtDJ-1 has an important job in neuroprotection. The wtDJ-1 both in humans and rats includes three cysteine residues at amino acid numbers 46, 53 and 106 (C46, C53 and C106, respectively) (Inden *et al.,* 2011). The C106 is the easiest oxidized DJ-1 residue and is believed to be the key residue involved in the acidic shift in the DJ-1 isoelectric point. Oxidation of the sulfhydryl group to a sulfinic acid on cysteine residue C106 generates the '2O' form, which is the active form of a DJ-1 variant with improved neuroprotective function (Madian *et al.,* 2011). Thus, stimulating the

conversion of DJ-1 to the 2O form may be therapeutically beneficial for PD patients.

Also, the peripheral cysteine residues C53 and C46 in the dimer's interface might represent a second redox center within DJ-1, which might be able to control the oxidation and therefore the activation of DJ-1. Regardless of the biological implications of C106 oxidation, the overall structure of oxidized DJ-1 does not differ from the reduced form (Kahle *et al.,* 2009). In fact, some familial mutations that depend on a single amino acid substitution are believed to disrupt DJ-1 activity by interfering with conversion of the protein to its 2O form (Madian *et al.,* 2011). For instance, M26I mutant interferes with the ability of DJ-1 protein to go through oxidation into the 2O form, by altering the geometry of the active site surrounding C106, even though M26I simply concerns slight conformational changes instead of a global unfolding of the protein. Interestingly, in this rare mutation of DJ-1, M26I, a methionine residue in its structure is mutated into isoleucine and can be detected as oxidized (Choi *et al.,* 2006). This specific and reversible methionine oxidation, in addition to others in different mutant proteins, might regulate DJ-1 activity by intracellular redox status.

In fact, upon ROS exposure, by insults including paraquat or H_2O_2, and in addition to over-expression, DJ-1 undergoes a isoelectric point shift from 6.2 to a more acidic 5.8, being these resulting isoforms more frequent in brains and cerebrospinal fluid of PD patients, either sporadic or inherited, than in controls (Lev *et al.,* 2009). This finding suggests that DJ-1 may work as a sensor in case of increased cytoplasmic ROS, and its fast up-regulation can be a first line defense mechanism of DAergic neurons to counteract DA toxicity.

Thus, DJ-1 is thought to influence the expression of specific antioxidative enzymes inside the cell (Kahle *et al.,* 2009). As previously referred, DJ-1 is an important regulator of antioxidative gene induction, thus functioning as an important antioxidant protein mediator (Thomas and Beal, 2007). In response to oxidative stress, DJ-1 can up-regulate glutathione synthesis, due to the ability of DJ-1 to interact with mRNA transcripts related to glutathione

metabolism (Thomas *et al.,* 2011). It specifically mediates the generation of glutamate cysteine ligase, the rate-limiting enzyme of glutathione biosynthesis. Overall, the lack of DJ-1 increases sensitivity to ROS.

Also, and since DJ-1 has the intrinsic ability to undergo self-oxidation in order to eliminate H_2O_2, it functions as a scavenger of ROS (Thomas and Beal, 2007). Furthermore, it was also found that mutations of DJ-1 abolished this activity, leading to hypersusceptibility to H_2O_2-induced cell death (Taira *et al.,* 2004).

As stated before, reduced mitochondrial complex I activity coexisting with increased oxidative stress has been linked to PD (Dauer and Przedborski, 2003). In effect, *in vitro* studies indicate that complex I abnormalities may subject cells to oxidative stress and energy failure.

- ***Chaperone activity***

A study by Shendelman and her collaborators (2004) disclosed the purpose of DJ-1 as a redox-regulated molecular chaperone, activated in an oxidative cytoplasmic environment. Every organism reacts to toxic environmental stresses by overexpressing a set of HSPs (further see *Antioxidant activity*) and, in this cases, DJ-1 acts as a protein chaperone. Its activity was proved and quantified though the suppression of heat-induced aggregation of citrate synthase and glutathione S-transferase, which undergo heat-induced aggregation and can be effectively suppressed by the addition of DJ-1. Molecular chaperones characteristically present great stability to thermal stress and the ultraviolet-circular dichroism spectrum (mainly used to investigate the secondary structure of proteins) of wtDJ-1 is coherent with a well-folded protein. In contrast, the spectrum of the L166P mutant protein is representative of a partly unfolded polypeptide, suggesting it presents a significant loss of DJ-1 helical structure. The mutant protein does not exhibit a thermal unfolding transition in the range

studied (0–90 ºC), whereas thermal denaturation of wtDJ-1 presents a thermal unfolding transition at about 75 ºC.

Also, its chaperone activity extends to αSyn, a protein implicated in PD pathogenesis, where its aggregation leads to the development of prominent intracytoplasmic neuronal inclusions, the LBs (Shendelman *et al.,* 2004). DJ-1 alterations are more frequent than αSyn mutations although both genes are associated with EOPD (Batelli *et al.,* 2008). A variety of experiments were undertaken to determine if DJ-1 acted as a chaperone for αSyn. Due to wtDJ-1 structural analogy to HSP31, this protein prevents the aggregation of αSyn, which is believed to be a crucial step in PD's etiology (Zhou *et al.,* 2006). It was demonstrated that native DJ-1 (i.e. unoxidized) did not reduce αSyn fibrillation – there were not stable interactions between αSyn and native DJ-1. Nevertheless, DJ-1 is easily oxidized by the addition of two oxygen atoms, forming the sulfinic acid of Cys106, which is simulated by the addition of H_2O_2. Although oxidation of Cys106 to the sulfinic acid had minor effect on DJ-1 structure, the resulting protein was very successful in preventing αSyn fibrillation.

In vitro, monomeric αSyn is disordered in a dilute solution (Shendelman *et al.,* 2004). The incubation of purified wild-type human αSyn for about 2h at 55 ºC results in the generation of high molecular weight soluble multimers (protofibrils). These αSyn protofibrils are an intermediate in the development of mature amyloid fibrils, which are not formed in this treatment, as determined by Congo red staining. It is a fact that wtDJ-1 successfully restrains the αSyn protofibrils generation. In spite of this, both L166P mutation and HSP27 failed to restrain the formation of the protofibrils. Consistently, wtDJ-1 inhibited Congo red-positive αSyn fibrils generation, whereas L166P did not. That is to say, wtDJ-1 seems to restrict the establishment of αSyn fibrils by preventing formation of αSyn protofibrils. On the other hand, further *in vitro* studies were performed in order to confirm DJ-1 chaperone function. Neuroblastoma cells were used (as αSyn aggregates here to form both protofibrils and mature

amyloid fibrils when exposed to oxidative stress) and it was conclude that DJ-1 over-expression also inhibits the aggregation of αSyn by stimulation of HSP70 (Batelli *et al.* 2008). Also, immunohistochemical analyses were performed in neuroblastoma cells transfected with αSyn along with DJ-1 or control vector. Over-expression of αSyn in these cells stimulates the growth of cytoplasmic aggregates. As expected, added over-expression of wtDJ-1 drastically decreases the number of cells containing αSyn aggregates. Interestingly, DJ-1 appears not to co-localize with αSyn aggregates, which suggests that it acts at an early step in the formation of mature aggregates.

The interface between αSyn and DJ-1 has been further explored in cellular models, particularly using human neuroblastoma cells, suggesting that DJ-1 is involved in preventing αSyn toxicity (Batelli *et al.,* 2008). HSP70 also prevents αSyn toxicity both *in vivo* and i*n vitro* and it was consequently suggested that αSyn neuroprotective activity depends upon HSP70 induction. In fact, the study by Batelli and her coworkers (2008) showed that DJ-1 and αSyn are involved in the antioxidant response against H_2O_2 and 6-OHDA, with HSP70 being a common downstream mediator. Both DJ-1 and αSyn promote HSP70 up-regulation but at different levels. This is demonstrated by the fact that DJ-1 increases HSP70 at mRNA and at protein expression level, whereas αSyn was unable to modify HSP70 mRNA. This might be because αSyn increases HSP70 protein level by a chaperone-like stabilization mechanism or by an action that reduces HSP70 degradation over time. In addition, DJ-1 expression was proven to be relevant not only for its action on HSP70 but also in controlling αSyn performance, which can be shifted from a soluble, neuroprotective condition to an aggregated, neurotoxic context if DJ-1 is down-regulated.

In conclusion, DJ-1 harbors chaperone activity toward a range of substrates and, along with HSP70, DJ-1 may become a target to treat PD for their response to oxidative stress and their involvement in αSyn solubility-related cell function.

- ***Signal transduction***

Upon DA intoxication, DJ-1 is induced via extracellular signal-regulated kinases, thus activating a neuroprotective pathway. Remarkably, over-oxidized damaged DJ-1 protein is not reduced and can accumulate in PD patients' brains (Kahle *et al.,* 2009). And even though the easily oxidizable wtDJ-1 has biochemical reactivity and its 3D structure has the alleged flavodoxin fold as core secondary structure arrangement (further see *Structural biology of PARK7/DJ-1 and its mutations*), it does not function as a direct antioxidative enzyme *in vivo.* That being said, it is more likely that DJ-1 impels antioxidative gene regulation so to perform its powerful antioxidative effects. In fact, high-levels of DJ-1 expression can facilitate the prosurvival phosphatidylinositol 3-kinase–Akt pathway by promoting Akt phosphorylation in immortalized knockout mouse embryo fibroblasts exposed to H_2O_2. However, unstable mutants of DJ-1 failed to do so, leading to DAergic neurodegeneration.

These conclusions were provided by several studies reported by Kahle and his associates, where DJ-1 was proven to suppress the stress-activated p38MAPK and c-Jun N-terminal kinase (JNK) pathways (Kahle *et al.,* 2009). Both these pathways are associated with ROS-dependent signaling of upstream MAPK kinase kinases. DJ-1 protein suppressed the stimulation of MAPK/extracellular signal-regulated kinase 1 by ultraviolet-light and, by this mean, the JNK signaling cascade leads to cell death. Also, DJ-1 mutations eliminate the wtDJ-1 suppressing activity of apoptosis signal-regulating kinase 1 (ASK1), potentially contributing to neurodegeneration due to enhanced ASK1 activity. This signaling pathway, which is ROS-dependent, is activated by tumor necrosis factor and Toll-like receptors (TLR). It is known that DJ-1 regulates TLR4 signaling in astrocytes. It was recently seen that primary astrocyte cultures of DJ-1 knockout mouse provide disproportionately high amounts of nitric oxide (NO). This cytotoxic effect is a reply to lipopolysaccharide, due to selective induction

of type II NO synthase via a ROS- and p38MAPK-dependent pathway. Likewise, DJ-1-silenced astrocytes offer minor neuroprotection for primary neuronal culture exposed to rotenone.

Hence, the DJ-1 gene associated with EOPD, not only plays a part as an antioxidative and antiapoptotic regulator in neurons, but also has an important transcellular role in the management of astrocytic neuroinflammatory impairment (Kahle *et al.,* 2009). To highlight this assumption, we must notice the fact that DJ-1 is moderately expressed in neurons when compared with its great up-regulation in acute and chronic human neurodegenerative conditions, such as stroke or PD.

PHARMACOLOGICAL MODULATION IN PRE-CLINICAL STUDIES

As lack of DJ-1 has revealed to be one of EOPD causes, it is reasonable to assume that its over-expression might interrupt its development. In fact, DJ-1 improves the defense mechanisms against oxidative stress, as displayed in the later chapter. Therefore, recent studies are being published worldwide concerning DJ-1's pharmacological modulation.

In 2010 a study took place in order to investigate the restoration of DJ-1 mutants' function through engineered disulfide bonds (Logan *et al.,* 2010). The main hypothesis was based on the idea that if the 'weakest structural link' in a mutant structure could be recognized, it might be fortified with a covalent bond in order to prevent the succeeding downstream unfolding actions. This strategy has been well succeeded before. This study was accomplished through high-technologic methods for protein expression and purification, simulating molecular dynamic, differential scanning fluorimetry and electron microscopy analysis among other complex techniques. As mentioned before, it was suggested that DJ-1's mutations undermine its dimeric structure. In an attempt to stabilize the resulting protein, the weakest regions of the mutated protein were researched and, consequently, a residue was mutated [Val 51 (V51) to cysteine (C51) in the L166P, A104T or M26I familial mutant background] in order to produce a symmetric disulfide bridge with the pre-existing Cys 53 on the opposite subunit. Consequently, it was found that the introduction of this disulfide linkage had several benefits. It stabilized two of the mutants (A104T and M26I) against both thermal and chemical denaturation, it displayed an increased ability to search for ROS and, also, it reinstated the chaperone-like function, preventing αSyn aggregation and resulting formation of LBs. Unfortunately, the L166P mutant is too unstable and presumably highly deleterious to DJ-1's structure, causing dramatically low cellular levels of protein and making it apparently

unfeasible to be saved through the introduction of V51C. Through this work, it was ultimately demonstrated that the structural defects in DJ-1 mutants can be reinstated into nearly the wtDJ-1, recovering its stability and antioxidant function by covalently strengthen the most vulnerable parts of its structure with correctly placed disulfide bridges. Future probably involves the development of similar molecules.

Also, using the X-ray crystal structure of DJ-1 oxidized at C106 and the three-dimensional coordinate data of over 30,000 chemical compounds, modulators of DJ-1 binding were explored by *in silico* virtual screening. Among the results, 2-[3-(benzyloxy)-4-methoxyphenyl]-N-[2-(7-methoxy-1,3-benzodioxol-5-yl)ethyl]acetamide (UCP0054278) was found to be the most promising and to have the highest binding constant (docking score) toward the oxidized C106 region, being this binding reasonably specific of wtDJ-1 protein (C106 oxidation is essential for DJ-1 to exert an antioxidant response). Also, *in vitro* experiment corroborated that 6-OHDA- or rotenone-induced cell death was significantly inhibited by treatment with UCP0054278 in a concentration-dependent manner (Inden *et al.,* 2011; Yanagida *et al.,* 2009).

The current investigation was based on the effect of the peripheral administration of UCP0054278 on either 6-OHDA– or rotenone-induced DAergic cell death in the models previously proposed (Inden *et al.*, 2011). UCP0054278 passes through the blood–brain barrier and binds to endogenous DJ-1 protein. The resulting complex performs neuroprotective effects against ROS-mediated DAergic degeneration. It is thought that the UCP0054278 / DJ-1 complex may eradicate ROS activity through the auto-oxidative mechanism, by stimulating mitochondrial complex I by maintaining its activity and preventing its loss after the action of neurotoxins. In addition, this complex may increase DJ-1–induced anti-oxidative and anti-apoptotic initiation, acting synergistically to prevent both ROS production and cell death. In

fact, this study reflected that either pre-treatment or post-treatment with UCP0054278 tended to improve 6-OHDA–induced behavioral dysfunctions and DAergic deficits, although the therapeutic concentration remains uncertain. Still, pre- and post-treatment with UCP0054278 have also shown synergistic neuroprotective effects.

The intranigral co-injection of UCP0054278 along with 6-OHDA prevented DAergic neural cell death and repaired the motion defect in the rat model of PD (Inden *et al.*, 2011). Additionally, the intra-striatal pre-injection with UCP0054278 inhibited neurodegeneration induced by occlusion of the middle cerebral artery, and lead to reperfusion in rats. In spite of this, the effect of the peripheral administration of UCP0054278 on *in vivo* PD model is still unclear. Even though 6-OHDA models have the benefit of providing a quantifiable motor deficit, the blood-brain barrier becomes damaged by the intranigral injection of the compound. Also, in these models, there is absence of LBs. Likewise, rotenone-induced behavioral dysfunctions and DAergic deficits were significantly reduced by treatment with UCP0054278. In this case, the inhibition was proven to be concentration-dependent and this model resembles EOPD symptoms although long-term treatment *per os* with rotenone is required.

In a previous study by Yanagida and his collaborators (2009), the neuroprotective influence of UCP0054278 in focal ischemia-induced degeneration in rats' brains was determined (Yanagida *et al.,* 2009). This DJ-1 modulator inhibits hydrogen peroxide–induced cell death as well as the production of ROS in normal brain cells, but not in DJ-1–knockdown cells. This suggests that UCP0054278 interacts with endogenous DJ-1, increasing its antioxidant and neuroprotective responses. It was the first *in vivo* description of UCP0054278 as a neuroprotective DJ-1 modulator in a brain ischemia, suggesting that UCP0054278 may bind to the SO_2H-oxidized C106 region in endogenous DJ-1 protein and thus maintain DJ-1 functions as anti-oxidative and anti-apoptotic agent. This study was the one that widely

opened the door to PD's pharmacological modulation, implying that DJ-1 stimulatory modulators, such as UCP0054278, were valuable in neuroprotective treatment against a wide range of oxidative stress-mediated disorders.

Further investigation took place in 2010, based on the premises that DAergic neurons with defects in PINK1 or DJ-1 developed mitochondrial structural damages, serious complex I deficits and expanded production of ROS (Shim *et al.,* 2010). Up to the present time, mitochondrial defects have primarily been described in DAergic tissue samples from PD patients aside from transgenic mouse and fruit fly models of the disease. Deficiency of PINK1 or DJ-1 genes ultimately triggers more severe mitochondrial impairment in DAergic neurons than in non-DAergic cells, which might explain the selective loss of DAergic neurons in familial EOPD patients. The investigation by Shim and his colleagues (2010) employed homogeneous DAergic cells from mouse embryos with a deficit in PINK-1 or DJ-1, in order to assess the biochemical and structural origin of mitochondrial malfunction in DAergic neurons from familial PD cases. These authors demonstrated that DAergic neuronal cells in DJ-1 knockout mice revealed exponential increase in the sensitivity to oxidative stress and MPTP, both known to aim for mitochondria, more exactly triggering complex I abnormalities, which are rather specific of PD and can be dangerously present in DAergic neurons in familial PD. Moreover, these cells with a deficiency in either PINK1 or DJ-1 displayed multiple mitochondrial anomalies, such as structural variances, decreased respiratory complex I activity, or considerably abnormal complex IV activity (exclusive of PINK1-deficient cells). Several data gathered throughout the study concluded that the complex I deficits can be drastically improved by an antioxidant called Trolox (water-soluble vitamin E analogue with a broad antioxidant spectrum) (Davies *et al.,* 1988). In wild-type neuronal cells, Trolox did not seem to influence complex I activity or other cell enzymes. However, Trolox radically

augmented complex I enzyme activity by 100% in the DJ-1-deficient cells and by 70% in the cells with deficient PINK1 (in these cells, Trolox also seems to increase complex IV activity by a lesser degree). This ultimately allowed to suggest that an antioxidant therapy promotes recovery from oxidative damage in complex I subunits.

Focusing on a distinct point of view, through *in vitro* cell cultures it was recently found that sodium phenylbutyrate and sodium butyrate increase DJ-1 protein expression by 300% in the N27 DA cell line after two days of treatment, due to modulation and increased gene transcription by combing Sp1 to the DJ-1 promoter (Zhou *et al.,* 2011). Phenylbutyrate is a histone deacetylase inhibitor, increasing acetylation levels of histones H3 and H4, thereby promoting transcriptional activation. It has been shown to be neuroprotective in *in vivo* studies, more specifically in adult C57BL/6 mice models of Huntington disease, spinal muscular atrophy and amyotrophic lateral sclerosis. This compound also rescues cells from oxidative stress, objectively protecting DAergic neurons against MPTP toxicity (which leads to PD-like symptoms), as well as rotenone-induced cell death. Its long-term administration also prevents mutant αSyn aggregation, by reinforcing DJ-1 chaperone activity, thus stopping the formation of LBs and inhibiting age-related deterioration in motor and cognitive function. This discovery allows the conclusion that drugs that can increase DJ-1 gene expression probably decelerate, or may even prevent, the progression of PD by moderating oxidative stress generation and activity as well as protein aggregation.

Table 2 – Pharmacological modulation. A review of the studies described concerning DJ-1's pharmacological modulation.

Drug / strategy	Method	Result	Publishing
Engineered disulfide bonds	***In vitro*** Protein expression and purification, simulating molecular dynamic, differential scanning fluorimetry and electron microscopy analysis.	Mutants' stabilization against thermal and chemical denaturation. Increased ability to search for ROS.Reinstatement of chaperone-like function, preventing αSyn aggregation.	Logan *et al.* 2010
UCP0054278	***In vivo*** 6-OHDA–microinjected rats and rotenone-treated mice as acute and chronic animal models of PD respectively	UCP0054278 / DJ-1 complex has an auto-oxidative mechanism (eradicating ROS) by stimulating mitochondrial complex I and preventing DJ-1 loss. The complex increases DJ-1–induced anti-oxidative and anti-apoptotic functions.	Inden *et al.*, 2011
Antioxidant Trolox	***In vivo*** mouse embryos with a deficit in PINK-1 or DJ-1	Increased complex I enzyme activity by 100% in the DJ-1-deficient cells and by 70% in the cells with deficient PINK1 – consequent oxidative damage recovery in complex I subunits.	Shim *et al.,* 2010
Sodium phenylbutirate and sodium butyrate	***In vitro*** N27 DA cell line	Increase DJ-1 protein expression by 300% after two days of treatment	Zhou *et al.,* 2011
	In vivo adult C57BL/6 mice	Recue cells from oxidative stress and protects DAergic neurons against MPTP and rotenone. In long-term, prevent αSyn aggregation	

FUTURE DIRECTIONS

A lot has been analyzed concerning PD's therapeutic in the past few years. However, further *in vitro* and *in vivo* studies related to DJ-1's potential to pharmacological modulation are required. They will hopefully uncover real improvement into the modification of mitochondrial dysfunction and many other sources of cellular instability related to EOPD.

Future studies should aim to examine whether DJ-1 modulation in laboratory may bring real hope to PD patients. However, it is still too early to say whether any of the previously described strategies will be able to be effective in clinical trials, by offering any degree of long-term potential in the treatment of PD. Nevertheless, the advent of improved active drugs for DJ-1 modulations is taking giant steps in the current literature, with investigations searching for alternative and innovative forms of acting in DJ-1's cellular functions in animal models, which should enable due attention to be paid to these issues in a very near future.

Additional studies examining drugs targeting DJ-1 mutations should also clarify whether structural changes will have any bearing on the long-term utility of this approach.

It is vital to establish accurate disease models of PD in order to understand its pathophysiology and to develop therapeutic strategies previous to clinical trials (Inden *et al.,* 2011). The ideal PD model should have the following features: (1) easily detectable parkinsonian motor deficits, (2) selective and gradual loss of DA neurons developing with age and (3) production of LB–like cytoplasmic inclusions. Unfortunately, the life span of rodents is much shorter than that of humans, however age-dependency can be overcome by the use of neurotoxins, such as MPTP, rotenone and 6-OHDA.

To date, investigations in the DJ-1 mutations modification fields, either chemical or structural, have still not progressed into the final preclinical model of PD - the primate treated

with MPTP. This model is the most clinically relevant model in which to monitor the long-term effectiveness of current PD's treatment agents, both given alone or in combination with a low dose of L-DOPA (Duty, 2010). As such, studies in the MPTP-treated primate will provide invaluable insight into the expected clinical potential of targeting DJ-1 in EOPD using the mentioned strategies, as well as revealing unpredicted side effects that might arise from whichever therapeutic plan in study. This is a rather unexplored field with need for a deep scrutiny in future researches, as it might reveal any inconvenient effect leading to the rejection of a now supposed to be revolutionary treatment.

While the outcome of longer-duration clinical trials is still awaited, these up-to-date pre-clinical investigations of pharmacological modulation of DJ-1 mutants offer hope that drugs targeting DJ-1 gene might be revolutionary and, not only treat EOPD and PD's sporadic forms, but also prevent its symptoms entirely.

CONCLUDING REMARKS

PD, the second most frequent progressive neurodegenerative disorder, is characterized by the pathological selective loss of DAergic neurons from the SNc and the presence of intracytoplasmic proteinaceous inclusions, the LBs. The etiology of this disease remains unclear, as most cases occur in sporadic idiopathic form. Nevertheless, the recent investigation of genetic mutations in rare familial cases of EOPD has provided tremendous insight into its molecular pathogenesis and great ideas on the disease's management have further appeared in the latest investigations.

But how can advances in research turn into real changes in patients' life? In the current literature, only a few articles relate to this issue – the pharmacological modulation of DJ-1. Further *in vivo* studies in this area, particularly concerning engineered DJ-1's disulfide bonds that were only studied *in vitro*, would be required in order to help develop effective therapies for complex I deficits, mitochondrial dysfunction and other causes of cellular instability related to EOPD, in which mutations of DJ-1 take part.

Results from some reports cited in the present review advocate that DJ-1 stimulatory modulators are likely to be valuable for practical neuroprotection in cytoprotective treatment in a wide range of oxidative stress–mediated disorders, particularly EOPD (Inden *et al.,* 2011; Yanagida *et al.,* 2009). These studies were accomplished through *in vivo* models, particularly mice. Also, an *in vitro* investigation by Logan and his coworkers, along with others in the current literature, contribute to a wide search for drug-like molecules affecting protein stability (Logan *et al.,* 2011). Additionally, antioxidant treatment of DJ-1 in DAergic cells from mice embryos is supported by the Trolox-caused improvement of mitochondrial complex I deficits (Shim *et al.,* 2011). This ultimately leads to functional DAergic neuron recovery in EOPD.

Concerning Inden's 2011 report about UCP0054278 / DJ-1 complex practical benefits, I believe that UCP0054278 may be the most innovative and greatest breakthrough drug for treating DJ-1-caused PD in a hopefully near future. The results of improvement were seen both *in vitro* and *in vivo* and raise the possibility that this drug may be the type of DAergic neuroprotective innovative drug awaited for the treatment of EOPD and other oxidative stress–mediated disorders. Further studies are required, and several other potential DJ-1 stimulatory modulators are certainly to be discovered.

With the mentioned results in mind, the ultimate conclusion to be understood is that the reinstatement of DJ-1 function by gene therapy or by pharmacological means is exponentially developing inside the scientists' community. Thus, DJ-1 seems to be emerging as a remarkable target for the treatment of EOPD and even of other chronic neurodegenerative diseases.

This futuristic foresight brings hope of brighter times ahead, drawing the line to several new treatments, innovative ones, with expected greater results. Hopefully, in a not so far future, we might be able to treat people before they develop the first symptom.

REFERENCES

Alcalay RN, Caccappolo E, Mejia-Santana H, Tang MX, Rosado L *et al.* (2010) Frequency of known mutations in early onset PD; implication for genetic counseling: the CORE-PD study. *Archives of Neurology* 67:1116–1122

Batelli S, Albani D, Rametta R, Polito L, Prato F *et al.* (2008) DJ-1 Modulates α-Synuclein Aggregation State in a Cellular Model of Oxidative Stress: Relevance for Parkinson's Disease and Involvement of HSP70. *PLoS ONE* 3(4):e1884

Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM *et al.* (2004) The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* 127:420-430

Bekris LM, Mata IF, Zabetian CP (2010) The Genetics of Parkinson Disease. *Journal of Geriatric Psychiatry and Neurology* 23(4): 228–242

Bogaerts V, Theuns J, van Broeckhoven (2008) Genetic findings in Parkinson's disease and translation into treatment: a leading role for mitochondria? *Genes, Brain and Behavior* 7:129-151

Borek LL, Melissa MA, Friedman JH (2006) Non-Motor Aspects of Parkinson's Disease. *CNS Spectrum* 7:541-554

Chahine LM, Stern MB (2011) Diagnostic markers for Parkinson's disease. *Current Opinion in Neurology* 24:309 – 317

Chen Y, Gibson SB (2008) Is mitochondrial generation of reactive oxygen species a trigger for autophagy? *Autophagy* 4(2):246-248

Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST *et al.* (2006) Oxidative Damage of DJ-1 Is Linked to Sporadic Parkinson and Alzheimer Diseases. *The Journal of Biological Chemistry* 281(16):10816-10824

Cookson M R, Bandmann O (2010) Parkinson's disease: insights from pathways. *Human Molecular Genetics* 19: R21–R27

Crosiers D, Theuns J, Cras P, Van Broeckhoven C (2011) Parkinson's disease: Insights in clinical, genetic and pathological features of monogenic disease subtypes. *Journal of Chemical Neuroanatomy* 42:131–141

Dauer W, Przedborski S (2003) Parkinson's Disease: Mechanisms and Models. *Neuron* 39:889-909

Davies MJ, Forni LG, Willson RL (1988) Vitamine e analogue trolox c. e. s. r. and pulse-radiolysis studies of free-radical reactions. *Biochemical Journal* 255(2):513-522

Dawson TM, Ko HS, Dawson VL (2010) Genetic Animal Models of Parkinson's Disease. *Neuron* 66: 646–661

De Andrade LAF (1996) Considerações a Respeito da Doença de Parkinson de Início Precoce. *Arquivo de Neuropsiquiatria* 54(4):691-704

Dekker MCJ, Bonifati V, van Duijn CM (2003) Parkinson's disease: piecing together a genetic jigsaw. *Brain* 126:1722-1733

Duty S (2010) Therapeutic potential of targeting group III metabotropic glutamate receptors in the treatment of Parkinson's disease. *British Journal of Pharmacology* 161:271-287

Graham D. G. (1978) Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Molecular Pharmacology* 14:633-643.

Guo J, Wang L, He D, Yang QO, Duan Z *et al.* (2011) Clinical features and [11C]-CFT PET analysis of PARK2, PARK6, PARK7-linked autosomal recessive early onset Parkinsonism. *Neurology Science* 32:35–40

Hague S, Rogaeva E, Hernandez D, Gulick C, Singleton A *et al.* (2003) Early-Onset Parkinson's Disease Caused by a Compound Heterozygous DJ-1 Mutation. *Annals of Neurology* 54(2):271-274

Heo JH, Park JH, Kim SJ, Seo KS, Han JS *et al.* (2012) DJ-1 Null Dopaminergic Neuronal Cells Exhibit Defects in Mitochondrial Function and Structure: Involvement of Mitochondrial Complex I Assembly. *PLoS ONE* 7(3):e32629

Inden M, Kitamura Y, Takahashi K, Takata K, Ito N *et al.* (2011) Protection Against Dopaminergic Neurodegeneration in Parkinson's, Disease–Model Animals by a Modulator of the Oxidized Form of DJ-1, a Wild-type of Familial Parkinson's Disease–Linked PARK7. *Journal of Pharmacological Science* 117:189 – 203

Irrcher I, Aleyasin H, Seifert EL, Hewitt SJ, Chhabra S *et al.* (2010) Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. *Human Molecular Genetics* 19(19):3734-3746

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery & Psychiatry* 79:368-376

Kahle PJ, Waak J, Gasser T (2009) DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. *Free Radical Biology & Medicine* 47:1354–1361

Kim JM, Lee KH, Jeon YJ, Oh JH, Jeon SY *et al.* (2006) Identification of Genes Related to Parkinson's Disease Using Expressed Sequence Tags. *DNA Research* 13:275-286

Kim J, Kundu M, Viollet B, Guan KL (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature Cell Biology* 13(2):132-141

Kumaran R, Vandrovcova J, Luk C, Sharma S, Renton A *et al.* (2009) Differential DJ-1 gene expression in Parkinson's disease. *Neurobiology of Disease* 36:393–400

Larsen NJ, Ambrosi G, Mullett SJ, Berman SB, Hinkle DA (2011) Dj-1 Knock-Down Impairs Astrocyte Mitochondrial Function. *Neuroscience* 196:251-264

Lev N, Ickowicz D, Barhum Y, Lev S, Melamed E, Offen D (2009) DJ-1 protects against dopamine toxicity. *Journal of Neural Transmission* 116:151–160

Lockhart PJ, Lincoln S, Hulihan M, Kachergus J, Wilkes K et al. (2004) DJ-1 mutations are a rare cause of recessively inherited early onset parkinsonism mediated by loss of protein function. *Journal of Medical Genetics* 41:e22

Logan T, Clark L, Ray SS (2010) Engineered disulfide bonds restores chaperone like function of DJ-1 mutants linked to familial Parkinson's disease. *Biochemistry* 49(27):5624-5633

Madian AG, Hindupur J, Hulleman JD, Diaz-Maldonado N, Mishra VR *et al.* (2011) Effect of single amino acid substitution on oxidative modifications of the Parkinson's disease-related protein, DJ-1. *Molecular & Cellular Proteomics* 11:M111.010892

Malgieri G, Eliezer D (2008) Structural effects of Parkinson's disease linked DJ-1 mutation. *Protein Science* 17:855–868

Martin I, Dawson VL, Dawson TM (2011) Recent Advances in the Genetics of Parkinson's Disease. *Annual Review of Genomics and Human Genetics* 12:301–325

Moore DJ, Dawson VL, Dawson TM (2003) Genetics of Parkinson's Disease: What Do Mutations in DJ-1 Tell Us? *Annals of Neurology* 54(3):281-282

Mullet SJ, Hinkle DA (2011) DJ-1 deficiency in astrocytes selectively enhances mitochondrial Complex I inhibitor-induced neurotoxicity. *Journal of Neurochemistry* 117(3):375-387

Nuytemans K, Theuns J, Cruts M, van Broeckhoven C (2010) Genetic Etiology of Parkinson Disease Associated with Mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 Genes: A Mutation Update. *Human Mutation* 31(7):763-780

Obeso JA, Rodriguez-Oroz MC, Goetz CG, Concepcion M, Kordower JH et al. (2010) Missing pieces in the Parkinson's disease puzzle. *Nature Medicine* 16:653-661

Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q *et al.* (2004) Familial Parkinson's Disease-associated L166P Mutation Disrupts DJ-1 Protein Folding and Function. *The Journal of Biological Chemistry* 279(9):8506-8515

Rappold PM, Tieu K (2010) Astrocytes and Therapeutics for Parkinson's Disease. *Neurotherapeutics* 7:413–423

Shendelman S, Jonason A, Martinat C, Leete T, Abeliovich A (2004) DJ-1 Is a Redox-Dependent Molecular Chaperone That Inhibits a-Synuclein Aggregate Formation. *PLoS Biology* 2(11):1764-1773

Shim JH, Yoon SH, Kim KH, Han JY, Ha JY *et al*. (2011) The antioxidant Trolox helps recovery from the familial Parkinson's disease-specific mitochondrial deficits caused by PINK1- and DJ-1-deficiency in dopaminergic neuronal cells. *Mitochondrion* 11:707-715

Taira T, Saito Y, Niki T, Iguchi-Ariga SMM, Takahashi K, Ariga H (2004) DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Reports* 299:256-259

Tao X, Tong L (2003) Crystal Structure of Human DJ-1, a Protein Associated with Early Onset Parkinson's Disease. *The Journal Of Biological Chemistry* 278: 31372–31379

Thanvi BR, Lo TCN (2004) Long term motor complications of levodopa: clinical features, mechanisms, and management strategies. *Postgraduate Medical Journal* 80:452-458

Thomas B, Beal MF (2007) Parkinson's disease. *Human Molecular Genetics* 16(2):R183-R194

Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M *et al.* (2011) DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Human Molecular Genetics* 20(1):40–50

Vasseur S, Afzal S, Tardivel-Lacombe J, Park DS, Iovanna JL, Mak TW (2009) DJ-1/PARK7 is an important mediator of hypoxia-induced cellular responses. *Proceedings of the National Academy of Sciences* 106(4):1111-1116

Vila M, Przedborski S (2004) Genetic clues to the pathogenesis of Parkinson's disease. *Nature Medicine* 10:S58-S62

Waragai M, Nakai M, Wei J, Fujita M, Mizuno H et al. (2007) Plasma levels of DJ-1 as a possible marker for progression of sporadic Parkinson's disease. *Neuroscience Letters* 425:18–22

Whang X, Petrie TG, Liu Y, Liu J *et al.* (2012) Parkinson's disease-associated DJ-1 mutations impair mitochondrial dynamics and cause mitochondrial dysfunction. *Journal of Neurochemistry* 121:830-839

Xu J, Zhong N, Wang H, Elias JE, Kim CY et al. (2005) The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis. *Human Molecular Genetics* 14(9):1231-1241

Yanagida T, Kitamura Y, Yamane K, Takahashi K, Takata K et al. (2009) Protection Against Oxidative Stress-Induced Neurodegeneration by a Modulator for DJ-1, the Wild-Type of Familial Parkinson's Disease-Linked PARK7. *Journal of Pharmacological Sciences* 109:463-468

Zhou W, Zhu M, Wilson MA, Petsko GA, Fink AL (2006) The Oxidation State of DJ-1 Regulates its Chaperone Activity Toward α-Synuclein. *Journal of Molecular Biology* 356:1036-1048

Zhou W, Bercury K, Cummiskey J, Loung N, Lebin J, Freed CR (2011) Phenylbutyrate Up-regulates the DJ-1 Protein and Protects Neurons in Cell Culture and in Animal Models of Parkinson Disease. *The Journal of Biological Chemistry* 286(17): 14941–14951

APPENDIX I - INSTRUCTIONS FOR PREPARATION AND SUBMISSION OF BRITISH JOURNAL OF PHARMACOLOGY REVIEW ARTICLES

A note from our Senior Reviews Editor on reviews that consider possible therapeutic applications of new drugs, including pure substances derived from natural products:

"My advice is to start by focusing on establishing a concentration- (in vitro) and dose-response (in vivo) profile of the range of actions (on receptors, enzymes and channels etc., in vitro and on hemodynamics, tumour growth etc. in vivo), so that the molecular specificity and selectivity of the substance can be related to the benefits it obtains in disease models. In other words, interrogate not just what it might be good for based on extrapolation from individual molecular actions, but also explore the identity of the mechanism. You should then be in a position to say either what the mechanism is, or what it might be (and what more we need to know). At this point you will need to consider whether the mechanism is unique, and what the potential advantages vs. available drugs might be. Here you need to consider the strength of the evidence (all too often such articles generate a list of possibilities without any real insight into the bottom line - this should be avoided). Finally, accepting that proof is likely to be lacking, you may finish by exploring what more we would need to know before a significant investment and clinical development would be warranted".

Preparation

- Specific instruction on arranging review content, formatting references etc. are shown below
- Inclusion of published material (figures) should be avoided. If such material is used, authors must obtain and provide the BJP with full permissions.

- Length: 5000-8000 words.
- As a general point, review articles sometimes fail peer review because referees find the manuscript lacks balance, and feel that that material has been selected with bias to support the author's personal viewpoint. Personal viewpoints are valuable, but they should not take precedence over dispassionate reportage, and they should always be clearly presented within the relevant section as viewpoint rather than masquerade as firm deduction. Published material that goes against the author's viewpoint must be cited appropriately and not disregarded. Viewpoint can be used creatively at the end of a review to inform a section on future directions, and this is the preferable place for it.

1. **Title Page:** Title should be brief and snappy. Below it, please provide an abbreviated title for use as page header (50 characters maximum). Please include the names and addresses of authors. Author names should be written Initials followed by Family name. In order to ensure correct citation of your article on PubMed, should your article be accepted for publication, please include spaces in-between author's initials on the title page of your manuscript e.g. A E Smith. If author's initials do not appear on the title page of your manuscript according to these guidelines they may appear incorrectly on PubMed until your article has been typeset and published in an issue. The author to whom correspondence should be sent should be identified in a footnote, and an e-mail address must be given.
2. **Summary Page:** Summary to be arranged as a single paragraph.
    - **a.** must not exceed 250 words
    - **b.** should start with the context, summarise the content and end with a strong statement
    - **c.** to be followed by keywords (10 maximum)
    - **d.** then to be followed by all non standard abbreviations used in the paper

3. Text: Use headers (typed in italics) to break up the review into a sequence of small sections, approximately 400–1000 words long. It is desirable to begin with an introductory section (this need not necessarily be entitled Introduction) that contains concise statements about the context, issues and the aims of the review. Please don't number the sections.

4. Acknowledgements: Please list sources of financial support, and sources of any drugs that were *gifted* by manufacturers.

5. Statement of conflict of interest: Please list the nature of any financial links with manufacturers of any of the materials or devices described in the manuscript. This includes relevant past as well as relevant present consultancy with the pharmaceutical industry or regulatory agency. Industry based authors please acknowledge whether or not you are employed by a company that sells one or more of the drugs or devices mentioned in your article.

6. References: All references in the text to be included in the Reference List and vice versa. The bibliography should be in alphabetical order. Items 'in press' may be cited. Manuscripts not yet accepted for publication must not be cited. Referencing style:

a. **Paper in a journal**—Clements-Jewery H, Hearse DJ & Curtis MJ (2005). Phase 2 ventricular arrhythmias in acute myocardial infarction: a neglected target for therapeutic antiarrhythmic drug development and for safety pharmacology evaluation. Br J Pharmacol 145: 551–564.

b. **Book chapter**—Meesmann W (1982). Early arrhythmias and primary ventricular fibrillation after acute myocardial ischaemia in relation to pre-existing coronary collaterals. In Early arrhythmias resulting from myocardial ischaemia. ed Parratt, J.R. McMillan: London, pp 93–112.

c. **Meeting abstract**—Wenger TL, Lederman SN, & Strauss HC (1985). Effects of flecainide in dogs with coronary occlusion and reperfusion. Circulation, 72 (suppl. III):225.

d. **Other (e.g. government guideline)—**FDA (2002). ICH Draft Consensus Guideline, S7B Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals: US Department of Health and Human Services.

7. **Tables**: Each on a separate page at the end of the manuscript, paginated as part of the paper. The format should be:

   a. At the top,

   b. The content, with headers in italics. Numbers aligned left, and asterisks (*) to show significance.

   c. At the bottom, the number ('Table X') followed by a short title ('effect of X on haemodynamics') and any necessary explanations of the nature of values (e.g., % or mean ± s.e.m., *P < 0.05 compared with what, etc.) and the sources of any material not your own, or material published elsewhere.

8. **Figure legends**: Each on a separate page after any tables, paginated as part of the paper.

9. **Figures**: Please use colour in cartoons if it helps clarity, but please try to avoid lurid clashes (yellow on a purple background with red borders, etc). Regarding general points:

   a. keys to symbols and histograms should appear on the Figures themselves, and not in the respective legends.

   b. 'box style' figures are not in keeping with the Journal style; line drawings etc must have only left-hand and bottom axes.

c. minimum resolution for production-quality figures (required on acceptance) is to be 300 dpi for colour Figures or black and white halftones and 600 dpi for line illustrations. For online manuscript submission, lower resolution files are acceptable.

d. when submitting via the web, please name your Figure files according to the convention "figure1.jpg", "figure2.tif" etc (using the correct file extension), and label the figures themselves with the name of the corresponding author and the figure number (jones_fig1, etc).

Submission

- Manuscript peer review will be moderated by the commissioning editor, or (for unsolicited reviews) the 'reviews editor'.
- If you would like to submit your article to the reviews editor for a pre-submission content check, please send it as an email attachment (text in Word and figures in PowerPoint). The address is michael.curtis@kcl.ac.uk
- Submission proper must be done via the journal web page. Please follow the links at http://mc.manuscriptcentral.com/bjp