Review

Genetic findings in Parkinson’s disease and translation into treatment: a leading role for mitochondria?

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Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder and in most patients its aetiology remains unknown. Molecular genetic studies in familial forms of the disease identified key proteins involved in PD pathogenesis, and support a major role for mitochondrial dysfunction, which is also of significant importance to the common sporadic forms of PD. While current treatments temporarily alleviate symptoms, they do not halt disease progression. Drugs that target the underlying pathways to PD pathogenesis, including mitochondrial dysfunction, therefore hold great promise for neuroprotection in PD. Here we summarize how the proteins identified through genetic research (α-synuclein, parkin, PINK1, DJ-1, LRRK2 and HTRA2) fit into and add to our current understanding of the role of mitochondrial dysfunction in PD. We highlight how these genetic findings provided us with suitable animal models and critically review how the gained insights will contribute to better therapies for PD.

Keywords: Genetics, Mitochondria, Neuroprotection, Nuclear-encoded proteins, Oxidative stress, Parkinson’s disease, Treatment

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provide direct or indirect evidence for a major role of mitochondrial dysfunction in PD.

In this review we aim to highlight the recent genetic data on mtDNA polymorphisms and the nuclear-encoded SNCA, parkin, PINK1, DJ-1, LRRK2 and HTRA2, and focus on the relevance of these genes for mitochondrial function. Pathogenetic theories focus on a combination of genetic and environmental risk factors, and thus we will also briefly consider environmental exposures relevant to mitochondrial dysfunction in PD. Our current understanding of the mitochondrial pathway to PD provided us with tools to create better animal models, as well as interesting entry points for therapy. These entry points will be discussed along with therapeutic drugs acting on them. Some of the drugs are already in clinical use; others are still in the primary stages of evaluation, but show great promise for modifying PD progression.

Mitochondria

**Function and structure**

The primary function of mitochondria is the generation of cellular energy in the form of adenosine 5'-triphosphate (ATP) by oxidative phosphorylation. In addition to energy production mitochondria play a role in the metabolism of e.g. amino acids and lipids, as well as intermediate metabolic pathways, calcium homeostasis and free radical scavenging.

Mitochondria are intracellular double membrane-bound structures (Fig. 1), and are partitioned in four main compartments: outer mitochondrial membrane (OMM), intermembrane space (IMS), inner mitochondrial membrane (IMM) and matrix. The five complexes – I [NADH (nicotinamide adenine dinucleotide, reduced form) ubiquinone oxidoreductase], II (succinate ubiquinone oxidoreductase), III (ubiquinone-cytochrome c reductase), IV (cytochrome oxidase) and V (ATP synthase) – of the mitochondrial respiratory chain, also called the electron transport chain, are all located in the IMM. Two mobile electron carriers, coenzyme Q (ubiquinone) and cytochrome c, are located in the IMM and IMS, respectively. The transport of electrons down the respiratory chain is energetically favourable. The released energy is used by complexes I, II and IV to transport protons from the matrix to the IMS, thus creating a proton and electrochemical gradient across the IMM. This gradient forms the basis of the inner mitochondrial transmembrane potential (ΔΨₘ) and is exploited by complex V of the respiratory chain to drive ATP synthesis.

The mitochondria contain their own genome. mtDNA is a multicopy, maternally inherited, 16.5-kb circular double stranded DNA molecule without any histone coating. mtDNA is extremely compact (93% coding sequence) and the majority of proteins required to build and maintain functional mitochondria is therefore encoded by nuclear DNA, synthesized in the cytosol and imported into mitochondria, where they are targeted to one of the four mitochondrial compartments.

**Oxidative stress**

Oxidative stress is the result of an imbalance between excessive production of reactive oxygen species (ROS) and limited antioxidant defences. Mitochondria generate most of the ROS as a byproduct of oxidative phosphorylation. An estimated 2% of the oxygen (O₂) consumed by mitochondria is converted to superoxide anion (O₂⁻) (Beal 2003), which is a precursor of most other ROS (Turrens 2003) (Fig. 2). These ROS can lead to oxidative damage of proteins, DNA and lipids (Raha & Robinson 2000).

Numerous studies indicated the involvement of ROS and oxidative stress in PD pathogenesis, including reduced amounts of the thiol-reducing agent glutathione (Pierce et al. 1997) and elevated concentrations of iron (Fe) (Kienzl et al. 1995) in SN of PD patients. Loss of neuromelanin-containing DAergic cells is characteristic for PD and the dark brown pigment neuromelanin attracted attention to the auto-oxidation of dopamine, as it consists primarily of products of dopamine redox chemistry (Wakamatsu et al. 2003). Normal
The metabolism of dopamine, partly accomplished by monoamine oxidases (MAO), produces hydrogen peroxide (H$_2$O$_2$) (Maker et al. 1981). From this reaction alone, DAergic neurons are exposed to oxidative stress. In addition, dopamine can be oxidized to a dopamine quinone. This oxidation occurs spontaneously, is accelerated by the presence of transition metal ions, or can be enzyme-catalysed. The resulting dopamine quinone covalently modifies cellular macromolecules, which may serve as a mechanism for dopamine induced neurotoxicity (Stokes et al. 1999).

**Apoptosis**

Apoptotic cell death is characterized by marked nuclear and cellular shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and the budding off of apoptotic bodies (Kerr et al. 1972). Apoptosis is triggered by a number of insults including e.g. misfolded proteins, ROS and mitochondrial complex inhibition (Bredesen et al. 2006), and is executed via two main pathways (Fig. 3), which eventually converge at the level of effector caspases activation and the subsequent cleavage of apoptotic substrates. Firstly, the death receptor (or extrinsic) pathway, which is initiated by activation of cell-surface death receptors (e.g. Fas), and secondly, the mitochondrial (or intrinsic) pathway, characterized by the release of mitochondrial pro-apoptotic proteins (e.g. cytochrome c) (Hengartner 2000).

The pivotal event in the mitochondrial pathway is mitochondrial outer membrane permeabilization (MOMP) that leads to the release of several IMS proteins, such as cytochrome c, apoptosis inducing factor (AIF), endonuclease G, second mitochondria-derived activator of caspases (Smac) and HTRA2 (van Loo et al. 2002). MOMP can occur via two mechanisms: the first one involves the opening of the permeability transition (PT) pore, a protein complex at the contact site between OMM and IMM (Zamzami & Kroemer 2001), whereas the second mechanism appears to be mediated by direct action of Bcl-2 family members on the OMM (Green & Kroemer 2004). Cytochrome c, released from mitochondria following MOMP, assists in the formation of the apoptosome (a complex consisting of cytochrome c, apoptosis protease-activating factor 1, procaspase 9 and ATP/deoxyATP). Apoptosome oligomerization activates caspase 9, which then triggers activation of caspase 3 and other caspases in an amplification cascade ultimately causing cell death (Li et al. 1997).

Inhibitors of apoptosis (IAPs) can still inhibit active caspases (Holley et al. 2002), but the IAP-mediated block may in turn be released by proteins as Smac (Du et al. 2000) or HTRA2 (Martins et al. 2002). Two other proteins released from mitochondrial IMS during MOMP (AIF and endonuclease G) translocate to the nucleus and induce chromatin condensation and DNA fragmentation, independent of caspase activation (Li et al. 2001; Susin et al. 1999).

The c-Jun N-terminal kinase (JNK) signal-transduction pathway is also important for the execution of apoptosis in response to different stress stimuli (Davis 2000). JNK is part of a sequential kinase-signalling cascade involving three kinases. Mitogen-activated protein kinase (MAPK) kinase activates JNK, whereas MAPK kinase activation is mediated by MAPK kinase kinases, including the mixed lineage kinase (MLK) family in neurons. The activation of the JNK pathway rapidly induces its downstream target activating protein-1 transcription factor c-Jun, which plays a major role in the

![Figure 2: ROS. These include (1) free radicals (containing highly reactive unpaired electrons), such as superoxide (O$_2^{-}$), nitric oxide (NO$^*$) and hydroxyl radical (OH$^*$); and (2) other molecular species, such as hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (ONOO$^*$). O$_2^{-}$ is converted to H$_2$O$_2$, either spontaneously or through a reaction catalysed by SOD (Fridovich 1995). H$_2$O$_2$ may in turn be fully reduced to water, by catalase or glutathione reductase/peroxidase, or partially reduced to hydroxyl radicals (OH$^*$). The latter reaction (Fenton reaction) occurs in the presence of reduced transition metals (e.g. Fe$^{2+}$), which may again be re-reduced by O$_2^{-}$, propagating the process (Lochev & Fridovich 1994). Alternatively O$_2^{-}$ can also react with NO radicals (NO$^*$; produced by nitric oxide synthase during conversion of arginine to citrulline) to form peroxynitrite (ONOO$^-$) (Beckman & Koppenol 1996).](image-url)
transcription of several pro-apoptotic genes (Ham et al. 2000). JNK acts as an effector of apoptosis through mitochondrial-dependent processes, as it catalyses the phosphorylation of antiapoptotic (Schroeter et al. 2003) and pro-apoptotic (Bhakar et al. 2003; Papadakis et al. 2006) Bcl-2 family members, induces release of cytochrome c (Schroeter et al. 2003) and Smac (Chauhan et al. 2003) and mediates a partial collapse of the mitochondrial membrane potential (Schroeter et al. 2003).

Another distinct pathway of apoptosis arises through increased expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its translocation from the cytoplasm to the nucleus (Ishitani et al. 1996; Sawa et al. 1997). In this pathway nitric oxide (NO) is the initial trigger to cell death (Hara et al. 2005).

Although an increased immunoreactivity for effectors of the mitochondrial apoptotic pathway (e.g. caspase 3) (Tatton 2000), an increased activation of JNK downstream targets (Hunot et al. 2004), as well as nuclear accumulation of GAPDH (Tatton 2000) have been detected in post-mortem SN of PD patients, there is still no clear consensus concerning the contribution of apoptosis to loss of DAergic neurons in PD (Tatton et al. 2003b). Necrosis (or passive cell death) represents a form of non-apoptotic cell death, but is not likely to play a major role in PD (Kostrzewa 2000) because necrosis is associated with energy failure, cell swelling and rupture followed by an inflammatory response; features that are largely absent in PD (Dickson 2007). Another form of non-apoptotic cell death that has gained interest in recent years is autophagic cell death, resulting from excessive levels of cellular autophagy. Autophagy complements the UPS pathway as it degrades long-lived proteins, protein aggregates and organelles (e.g. damaged mitochondria) through a lysosomal degradation pathway (Rubinsztein 2006). Whereas the survival-promoting role of autophagy in nutrient starvation is well accepted, its role in cell death is more controversial (Eskelinen 2005). Cross-talk between apoptotic and autophagic pathways has been reported, but their molecular interdependence is not yet clear (Ferraro & Cecconi 2007). However,
mitochondria might represent the link at which apoptosis and autophagy interact, because mitochondria generate apoptotic signals, but are in turn removed by autophagy when damaged.

**Environmental exposures and PD**

The finding that humans intoxicated with MPTP develop a syndrome nearly identical to PD (Langston *et al.* 1983) lends support to the hypothesis that substances in the environment might contribute to PD aetiology. Indeed, epidemiological studies have implicated environmental risk factors such as rural living, consumption of well water and pesticide exposure in increased risk of PD (Priyadarshi *et al.* 2004; Ghezzi *et al.* 2003). Interestingly, recent research into the extent of pesticide exposure in increased risk of PD (Priyadarshi *et al.* 2004; Ghezzi *et al.* 2003) lend support to the hypothesis that substances in the environment might contribute to PD aetiology. Indeed, epidemiological studies have implicated environmental factors such as rural living, consumption of well water and pesticide exposure in increased risk of PD (Priyadarshi *et al.* 2004; Ghezzi *et al.* 2003). Conversely, cigarette smoking and coffee drinking are inversely associated with the risk for developing PD (Hancock *et al.* 2001; Hernan *et al.* 2007).

Cigarette smoking represents a good example of the relevance of an environmental toxin to mitochondrial function. Cigarette smoke is composed of more than 4000 compounds, but nicotine, the main alkaloid, seems to be particularly important. Nicotine reduces ROS generation from mitochondria (Cormier *et al.* 2003), and prevented neurotoxin-induced mitochondrial swelling and cytochrome c release *in vitro* through inhibition of the mitochondrial PT pore (Xie *et al.* 2005). The antioxidant properties of nicotine are likely important as well; e.g., nicotine may be protective against PD through complex formation with Fe²⁺, thus yielding Fenton-inactive Fe²⁺ (Fig. 2) and less oxidative stress (Linet *et al.* 1999). Chronic nicotine treatment was already shown to reduce paraquat-mediated nigrostriatal damage in a rodent model (Khwaaja *et al.* 2007).

**Genetics of PD**

**mtDNA genetics and PD**

Studies using PD cybrids (cytoplasmic hybrids, through transfer of mtDNA of PD patients into mtDNA depleted cells) showed loss of complex I activity, increased O₂ radical production, and increased susceptibility to MPTP-induced cell death (Swindlow *et al.* 1996), suggesting that mtDNA abnormalities may be crucial in the pathogenesis of sporadic PD. Several studies reported inherited mtDNA microdeletions or single nucleotide mutations resulting in parkinsonism, typically as one feature of a multisystemic disorder, e.g. the prominent parkinsonism associated with Leber’s hereditary optic neuropathy caused by a single nucleotide mutation in the complex I ND4 gene (Simon *et al.* 1999). There is, however, less evidence for mtDNA involvement in non-syndromic PD. Nevertheless, specific mtDNA haplotypes were proposed to be implicated in PD risk (Autere *et al.* 2004; Ghezzi *et al.* 2005; Pyle *et al.* 2005; van der Walt *et al.* 2003). Interestingly, recent research into the extent of mtDNA deletions in SN DAergic neurons showed increased levels of clonally expanded somatic mtDNA deletions with ageing (Bender *et al.* 2006b; Kraysberg *et al.* 2006), resulting in loss of mitochondrial function and cell death. The accumulation of mtDNA deletions was higher in PD patients as compared with age matched control individuals (Bender *et al.* 2006b).

**PD genetics of nuclear-encoded proteins**

Linkage analysis in extended families with highly penetrant Mendelian forms of PD has been successful in identifying specific disease-segregating mutations in previously unknown genes implicated in PD pathogenesis. Mutations in at least six genes were shown to cause familial parkinsonism: mutations in SNCA and LRRK2 account for autosomal dominant forms of PD, whereas mutations in parkin, PINK1, DJ-1 and ATP13A2 (Ramirez *et al.* 2006) show a recessive mode of inheritance (mutations in the latter are characteristic of Kufor-Rakeb syndrome, and will not be further discussed). In general autosomal dominant inherited mutations are gain-of-function mutations, while loss-of-function mutations are usually linked to recessive phenotypes. The use of animal models to evaluate gain- and loss-of-function mutations helped greatly in the illumination of the biological functions of the proteins encoded by these genes and yielded insights into their causal role in PD pathogenesis. Transgenic mice provide the ideal means to study possible disease-related gain-of-function mutations, knockout strategies in turn provide the opportunity to study diseases related to loss-of-function mutations.

The role of Mendelian genes in the common sporadic forms of PD is less known. On the other hand, association studies of candidate genes for PD try to define risk alleles that contribute to the sporadic forms of disease. Candidate gene studies evaluate genes based on their location in previously determined linkage regions (positional candidate genes), or based on a plausible biological function related to disease pathogenesis (functional candidate genes). Table 1 provides a general overview of the genetic causes of PD with mitochondrial involvement, whereas Fig. 4 depicts the proteins at their cellular action level.

**The inclusion protein SNCA**

SNCA is the major constituent of Lewy bodies, the neuropathological hallmark of PD (Spillantini *et al.* 1997). SNCA is expressed throughout the brain (Solano *et al.* 2000) and is particularly enriched in presynaptic nerve terminals (Goedert 2001). It has potential roles in synaptic plasticity (George *et al.* 1995), regulation of dopamine neurotransmission (Abeliovich *et al.* 2000) and turnover of synaptic vesicles (Lotharius & Brundin 2002). In spite of extensive studies, the exact cellular function of SNCA remains unclear.

Missense mutations and multiplications of SNCA (4q21) cause autosomal dominant PD (Chartier-Harlin *et al.* 2004; Ibanez *et al.* 2004; Kruger *et al.* 1998; Polymeropoulos *et al.* 1997; Singleton *et al.* 2003; Zarranz *et al.* 2004), whereas association studies showed that increased SNCA expression because of variation in the SNCA promoter region conferred risk for sporadic PD (Maraganore *et al.* 2006; Pals *et al.* 2004).

Inducible expression of human p.Ala30Pro SNCA in PC12 cells (rat pheochromocytoma cells) decreased proteasome activity and increased sensitivity to mitochondria-dependent apoptosis (Tanaka *et al.* 2001). More recently human
Table 1: Nuclear genes linked to autosomal inherited PD with mitochondrial involvement

| Protein                  | Function                          | Cellular localization | PD locus | Chromosomal location | Gene | Inheritance | Type of mutations                                                                 | Key references                                                                 |
|--------------------------|-----------------------------------|-----------------------|----------|----------------------|------|-------------|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| α-synuclein              | Synaptic vesicle formation        | Mainly cytoplasm      | PARK1    | 4q21                 | SNCA | Dominant    | Missense mutations (p.Ala30Pro, p.Glu46Lys, p.Ala53Thr), duplication, triplication| Chartier-Harlin et al. 2004; Ibanez et al. 2004; Kruger et al. 1998; Polymeropoulos et al. 1997; Singleton et al. 2003; Zarranz et al. 2004 |
| Parkin                   | Ubiquitin E3 ligase               | Mainly cytoplasm, OMM | PARK2    | 6q25.2–q27           | parkin | Recessive   | Missense, nonsense and frameshift mutations, intronic deletions or insertions, promoter and exon deletions, exon multiplications | Hedrich et al. 2004b; Kitada et al. 1998; Mata et al. 2004 |
| Phosphatase and tensin homologue (PTEN)-induced kinase 1 | Mitochondrial kinase           | Mainly IMM, occasionally OMM | PARK6    | 1p36                 | PINK1 | Recessive   | Missense, nonsense and frameshift mutations, exon deletions                   | Li et al. 2005; Marongiu et al. 2007; Rogaeva et al. 2004; Valente et al. 2004 |
| DJ-1                     | Redox sensor, mitochondrial antioxidant | Cytoplasm, IMS and matrix | PARK7    | 1p36.33–p36.12       | DJ-1  | Recessive   | Missense and frameshift mutations, insertions and exon deletions               | Abou-Sleiman et al. 2003; Bonifati et al. 2003; Hugue et al. 2003 |
| Leucine-rich repeat kinase 2 | Multifunctional kinase/GTPase    | Cytoplasm, OMM        | PARK8    | 12q12                | LRRK2 | Dominant    | Missense mutations                                                             | Berg et al. 2005; Paisan-Ruiz et al. 2004; Zimprich et al. 2004 |
| High temperature requirement A2 | Pro-apoptotic protease, important for mitochondrial homeostasis | IMS                   | PARK13   | 2p12                 | HTRA2 | Dominant*  | Missense mutations                                                             | Strauss et al. 2005 |

*In contrast with the other genes in the table, HTRA2 was not identified through linkage studies, but was associated with PD using a candidate gene approach.
SNCA overexpressing transgenic mice were reported to develop intraneuronal inclusions, as well as mtDNA damage and degeneration (Martin et al. 2006). Pathogenic p.Ala30Pro and p.Ala53Thr SNCA was also shown to be poorly degraded by chaperone-mediated autophagy (CMA) (Cuervo et al. 2004). Although these mutants bound with high affinity to the lysosomal membrane, they were not translocated into the lysosome. In addition to inhibiting their own degradation, they also blocked degradation of other CMA substrates; which is consistent with a toxic gain-of-function of these mutants.

On the other hand, loss of SNCA appears to have minimal effects because SNCA knockout mice were viable and fertile. Despite the lack of morphological abnormalities, these mice displayed a modest decrease in total striatal DA levels (Abeliovich et al. 2000), and a reduction in the reserve pool of synaptic vesicles in the hippocampus (Cabin et al. 2002).

The ubiquitin E3 ligase parkin Ubiquitously expressed parkin encodes an ubiquitin E3 ligase (Shimura et al. 2000), which is a component of the UPS (Ciechanover 1998). Parkin was localized in mitochondria of proliferating cells and was also shown to play a role in mitochondrial biogenesis by regulating both transcription and replication of mtDNA (Kuroda et al. 2006). Loss-of-function mutations in parkin cause autosomal recessive juvenile parkinsonism (Kitada et al. 1998). Parkin mutations may explain up to half of the patients with early onset PD and a family history compatible with recessive inheritance (Dekker et al. 2003). Mounting evidence suggests that heterozygous parkin mutations may increase susceptibility to late onset PD (Foroud et al. 2003; Oliveira et al. 2003; Sun et al. 2006; West et al. 2002). A promoter deletion in a family with a previously described heterozygous parkin exon 3-deletion recently extended the parkin mutation spectrum, as this compound heterozygous mutation was reported to result in complete absence of parkin expression (Lesage et al. 2007).

Drosophila parkin knockout mutants exhibited reduced lifespan, locomotor defects resulting from apoptotic muscle degeneration and male sterility. Mitochondrial structural alterations were prominent features of both muscle and germline pathology (Greene et al. 2003). Proteomics of midbrain from parkin knockout mice showed a decreased abundance of proteins involved in mitochondrial respiratory chain activity and protection against oxidative stress (Palacino et al. 2004).

Parkin overexpression models further support the role of parkin in mitochondria. Parkin overexpression in PC12 cells protected against ceramide-mediated cell death by delaying mitochondrial swelling, subsequent cytochrome c release and caspase 3 activation, and this protective effect was abrogated by parkin mutations (Darios et al. 2003). In human DAergic neuroblastoma cells (SH-SY5Y) parkin overexpression
not only protected against apoptosis, but also decreased cellular ROS levels (Jiang et al. 2004).

On the other hand, mitochondrial dysfunction and oxidative stress can in turn affect parkin function, e.g. S-nitrosylation of parkin diminishes its ubiquitin E3 ligase activity and compromises its protective function (Chung et al. 2005). Such nitrosative stress combined with a single parkin mutation could lead to haploinsufficiency and might be particularly relevant for heterozygous parkin mutations associated with sporadic PD.

The mitochondrial kinase PINK1
The ubiquitously expressed PINK1 consists of an N-terminal mitochondrial targeting motif, a highly conserved serine–threonine kinase domain and a C-terminal autoregulatory domain (Silvestri et al. 2005). The PINK1 kinase is localized to the mitochondrial membranes (Gandhi et al. 2006).

Homozogous PINK1 mutations were first described in three consanguineous families (Valente et al. 2004). PINK1 loss-of-function mutations are estimated to account for 1–7% of early onset PD (Tan et al. 2006). Heterozygous PINK1 mutations were also reported, and were threefold enriched in sporadic PD patients compared with control individuals (Abou-Sleiman et al. 2006). It is hypothesized that heterozygous PINK1 mutations are a risk factor for the development of late onset PD (Khan et al. 2002).

Downregulation of PINK1 expression by small interfering RNA (siRNA) decreased SH-SY5Y cell viability and increased apoptosis (Deng et al. 2005). RNA interference-mediated inactivation of the PINK1 homologue in Drosophila resulted in progressive loss of DAergic neurons. This neurodegeneration was suppressed by human antioxidant superoxide dismutase (SOD) 1, which suggests that PINK1 inactivation can induce neuronal death via an oxidative stress pathway (Wang et al. 2006). Notably, PINK1 inactivation in Drosophila leads to a phenotype that shares marked similarity with that of Drosophila parkin knockout mutants, including shortened lifespan, apoptotic muscle degeneration, male sterility and defects in mitochondrial morphology. Transgenic expression of parkin markedly ameliorated all PINK1 loss-of-function phenotypes, but not vice versa, suggesting that PINK1 and parkin function, at least in part, in the same pathway, with PINK1 functioning upstream of parkin (Clark et al. 2006; Park et al. 2006; Yang et al. 2006).

Overexpression of wild type PINK1 in SH-SY5Y cells attenuated neuronal apoptosis by reducing the release of cytochrome c and subsequent activation of caspases under basal and apoptotic stress conditions. However, PD-related mutations and an artificial kinase-dead mutant abolished this protective effect (Petit et al. 2005). Most likely, the PINK1 kinase exerts its neuroprotective effect by phosphorylating specific mitochondrial proteins and in turn modulating their functions. However, the physiological substrates of PINK1 remain unknown.

The antioxidative DJ-1
DJ-1 is expressed in a variety of tissues, including brain, and is partially localized to the mitochondrial matrix and IMS (Zhang et al. 2005a). DJ-1 is a H2O2 responsive protein, suggesting a function as antioxidant (Mitsumoto & Nakagawa 2001). DJ-1 acts as a redox sensor within cells and the acidification of a key Cys-residue (Cys106) seems to have an important signalling function. The same Cys-residue is also important for relocalization of DJ-1 to mitochondria (Canet-Aviles et al. 2004).

Recessively inherited DJ-1 missense and exonic deletion mutations were first identified in two European early onset PD families (Bonifati et al. 2003). Additional loss-of-function mutations have since been identified (Abou-Sleiman et al. 2003, 2004; Hague et al. 2003; Hedrich et al. 2004a). Loss-of-function mutations in DJ-1 are rare and are estimated to account for 1% of early onset PD (Lockhart et al. 2004). Single heterozygous DJ-1 mutations were also found (Clark et al. 2004; Hedrich et al. 2004a), but as for heterozygous mutations in parkin and PINK1, their effect remains elusive.

DJ-1 downregulation by siRNA in neuronal cell lines enhanced cell death by oxidative stress (Yokota et al. 2003). In Drosophila inhibition of DJ-1, a Drosophila homologue of the human DJ-1, resulted in cellular accumulation of ROS, hypersensitivity to oxidative stress as well as dysfunction and degeneration of DAergic neurons (Yang et al. 2005). Knockout flies of DJ-1α and DJ-1β, the two DJ-1 homologues in Drosophila, displayed a selective sensitivity to environmental toxins such as paraquat and rotenone (Meulener et al. 2005). DJ-1α and DJ-1β are prominently localized to enlarged and swollen mitochondria, implicating that the localization of DJ-1 to mitochondria is a protection mechanism against oxidative stress (Park et al. 2005). DJ-1 deficient mice show no gross anatomical or neuronal abnormalities and have normal numbers of SN DAergic neurons (Chen et al. 2005; Goldberg et al. 2005; Kim et al. 2005), however, their nigrostriatal pathway is dysfunctional (Chen et al. 2005; Goldberg et al. 2005), leading to higher dopamine concentrations and possibly more cellular oxidative stress.

The multidomain and multifunctional LRRK2
LRRK2, also known as dardarin, is expressed at low levels in most tissues (Paisan-Ruiz et al. 2004) and shows an expression pattern in brain that directly relates to the nigrostriatal dopaminergic system. It is expressed in the dopamine target areas, striatum and frontal cortex, whereas the dopamine neurons themselves are devoid of LRRK2 messenger RNA (Galter et al. 2006). On a cellular level LRRK2 resides diffusely throughout the cytosol, but also associates with the OMM (West et al. 2005). Sequence analysis indicates that LRRK2 comprises several domains including a leucine-rich repeat domain, a GTPase (guanosine triphosphatase) domain Ras of complex proteins (Roc) followed by its associated C-terminal of Roc domain, a MLK-like domain and a C-terminal WD40 domain (Paisan-Ruiz et al. 2004; Zimprich et al. 2004). The kinase activity may be the link between LRRK2 and its role in PD pathogenesis.

Mutations in LRRK2 cause autosomal dominant late onset PD (Paisan-Ruiz et al. 2004; Zimprich et al. 2004). LRRK2 missense mutations reported so far are distributed along the protein (Berg et al. 2005; Khan et al. 2005; Paisan-Ruiz et al. 2004; Zimprich et al. 2004). p.Gly2019Ser is the most common pathogenic LRRK2 mutation in Caucasians and
accounts for approximately 5% of familial PD and 1.5% of sporadic PD (Di Fonzo et al. 2005; Gilks et al. 2005; Nichols et al. 2005). We recently detected a founder effect of p.Arg1441Cys, the second most frequent pathogenic LRRK2 mutation, in the Belgian population (Nuytemans et al. in preparation).

p.Arg1441Cys in the GTPase domain and p.Gly2019Ser in the MLK-like domain were shown to result in increased kinase activity in vitro (West et al. 2005), a similar increase was seen for p.Ile2020Thr (Gloeckner et al. 2006). These findings are in favour of a gain-of-function mechanism for LRRK2-linked disease. Expression of p.Arg1441Cys and p.Gly2019Ser LRRK2 caused neuronal degeneration in both SH-SY5Y cells and mouse primary neurons (Smith et al. 2005). Overexpression of p.Gly2019Ser LRRK2 in rats did not alter normal appearance of DAergic neurons, but significantly increased apoptosis (MacLeod et al. 2006). Extensive in vivo validation of these data is, however, required.

**The mitochondrial serine protease HTRA2**

HTRA2 is ubiquitously expressed (Faccio et al. 2000) and nuclear encoded but carries a mitochondrial targeting sequence at its N-terminus. HTRA2 has an N-terminal IAP-binding motif, a serine protease domain and, at its C-terminus, a PDZ domain, which restricts access to the active site of the serine protease (Li et al. 2002; Verhagen et al. 2002). HTRA2 is localized to the IMS, but is released into the cytosol following apoptotic stimuli. In the cytosol it can induce apoptosis in a caspase-dependent manner by antagonizing caspase–IAP interaction, or in a caspase-independent manner relying on its serine protease activity (Hegde et al. 2002).

Transient HTRA2 overexpression in HEK293 cells (human embryonic kidney cells) induced apoptosis even in the absence of an apoptotic insult, whereas artificial protease-inactive mutant HTRA2 (Li et al. 2002) had lower death-inducing ability (Martins et al. 2002). In non-apoptotic conditions the proteolytic activity of HTRA2 is involved in maintaining mitochondrial homeostasis, as was first shown in the mnd2 mouse for motor neuron degeneration 2 (Jones et al. 2003) and later also in HTRA2 knockout mice (Martins et al. 2004). Both mnd2 and HTRA2 knockout mice died prematurely because of loss of striatal neurons. The identification of a p.Gly399Ser mutation in the HTRA2 PDZ domain in patients with sporadic PD (Strauss et al. 2005) was of direct relevance to PD. At the cellular level p.Gly399Ser HTRA2 impaired the regulation of proteolytic activity, caused mitochondrial swelling, decreased mitochondrial membrane potential and increased staurosporine-induced cell death. We recently identified another PD-specific HTRA2 mutation in the PDZ domain, p.Arg404Trp, in an extensive mutation analysis of over 250 Belgian PD patients (Bogaerts et al. unpublished data).

**Other genes relevant to mitochondrial function and oxidative stress**

Several polymorphisms in functional candidate genes related to mitochondrial function and oxidative stress have been studied for their possible association with PD. MAO, a principal enzyme of dopamine metabolism that has two isoforms: MAO-A and MAO-B, was tested for its supposed role in PD pathogenesis: a polymorphism in intron 13 of MAO-B was significantly associated with increased risk of PD (Kang et al. 2006; Tan et al. 2000b). Another polymorphism in exon 22 of inducible nitric oxide synthase (encoded by NOS2A), an enzyme that produces NO (Fig. 2), showed an inverse association with PD (Hague et al. 2004; Levecque et al. 2003). Although NO is a biological messenger, NO overproduction contributes to oxidative stress, impairment of mitochondria and overload of degradation pathways. Interestingly NOS2A polymorphisms were also reported to interact with smoking (Hancock et al. 2006). Glutathione S-transferase (GST) variants have been extensively studied in PD because of their ability to detoxify exogenous and endogenous toxic substances. Moreover, polymorphisms in GST Omega-1 and -2 were shown to modify age at onset of PD (Li et al. 2006). These genes represent only a handful of the list of functional candidate genes for PD, but their implication in PD at least supports the pathophysiological significance of mitochondrial dysfunction to PD.

**The relevance of genetic findings in PD**

The future of drug therapies depends on the development of animal models that strongly recapitulate the disease, not only to discover the mechanisms of disease but also to identify new neuroprotective drugs and to test their efficacy. The genetic findings described above provide a direct mechanistic link with PD and therefore animal models expressing the discovered PD-causing mutations probably mimic human PD better than neurotoxic animal models. The identified PD genes themselves represent direct targets for therapeutic application, e.g. by RNA interference. In addition, evidence from genetic studies indirectly suggests that mitochondrial dysfunction plays an integrative role in PD pathogenesis, and therefore mitochondrial function and mechanisms to cell death represent valid targets for potential neuroprotective therapies.

**Direct relevance of genetic research to treatment**

**Transgenic mouse models of PD**

The identification of causal PD genes in rare Mendelian forms of PD triggered the development of transgenic mouse models. The underlying hypothesis is that mutations causing Mendelian forms of PD highlight pathways, relevant to sporadic PD (Melrose et al. 2006). However all of these mouse models had shortcomings, of which the most notable is failure to exhibit degeneration of the nigrostriatal system, a key pathological feature of PD (Manning-Bog & Langston 2007). Efforts to create genetic models of PD in simple systems, e.g. *Drosophila* and yeast, have resulted in robust phenotypes. However, the principal value of these simple models of PD is perhaps that results in these systems might be used to improve current mouse models of PD (Whitworth et al. 2006).
Given its clear link with PD, a variety of SNCA transgenic mice have been generated, varying in tissue specificity, mutation and expression level (for a review see Fernagut & Chesseau 2004). The developed SNCA transgenic models proved to be valuable in partially recapitulating the predisposition of certain neuronal populations to SNCA aggregation. Parkin knockout mice had normal numbers of DAergic neurons, but displayed subtle deficits in behaviour and DAergic neurotransmission (Goldberg et al. 2003; Itier et al. 2003). Similar to parkin knockouts, loss of DJ-1 did not result in loss of SN DAergic neurons (Chen et al. 2005; Goldberg et al. 2005, Kim et al. 2005), but again subtle behavioural deficits and increased striatal DA reuptake were reported. So far PINK1 knockout mice have not been reported, but silencing of PINK1 expression in mice by use of RNA interference did not cause a loss of DAergic neurons either (Zhou et al. 2006; Zimprich et al. 2004).

Yet no perfect animal model for PD exists; most transgenic mouse lines do not show damage to the nigrostriatal system, but rather have subtle changes. Opposed to this, neurotoxic animal models do show degeneration of SN DAergic neurons. Combining these animal models might thus be very fruitful to develop new and better PD models. Such fused genetic and neurotoxic models might better capture the full spectrum of PD features, and provide excellent means to study gene–environment interactions (Manning-Bog & Langston 2007). Nevertheless, genetic mouse models have at least illustrated that neuronal dysfunction in PD most likely occurs long before cell loss. Current transgenic or knockout mouse models might indeed provide insights into the early stages of PD (Melrose et al. 2006). However, considerable variability in pathological phenotype was already documented for several pathogenic LRRK2 mutations (Rajput et al. 2006; Zimprich et al. 2004).

Gene-based therapy
A straightforward approach, supported by genetic findings, is the possibility to lower SNCA levels. Indeed, given the gain-of-function mechanism of PD-linked SNCA mutations, silencing expression of mutant SNCA or reducing overexpression of wild-type SNCA is an attractive target for therapeutic RNA interference (Gonzalez-Alegre 2007). Allele-specific silencing of mutant SNCA was already shown in vitro, as well as suppression of overexpressed human SNCA in vivo in rat brain (Sapru et al. 2006). However, these studies need replication in different animal models of PD. A vaccination approach was also tested: immunization of human SNCA transgenic mice with recombinant human SNCA led to decreased SNCA accumulation in neuronal cell bodies and synapses, as well as reduced neurodegeneration (Masliah et al. 2005). Human trials on the proposed vaccine are currently underway (Singh et al. 2007). For parkin, PINK1 and DJ-1, where PD is caused by loss-of-function mechanisms, increasing expression is likely to ameliorate the phenotype (Farrer 2006). For example, parkin gene therapy was already shown to be effective against SNCA overexpression in rats (Mochizuki et al. 2006). Last but not least, the identification of LRRK2 mutations as a frequent cause of familial and sporadic PD was a landmark discovery in PD research. The increased kinase activity observed for mutant LRRK2 (West et al. 2005) may, if proved in vivo, translate relatively quickly into a novel treatment option involving kinase inhibition (Litvan et al. 2007). However, the physiological targets for LRRK2 activity are not yet known, hence uncertainty remains whether we can target this kinase activity without detrimental effects.

Drugs targeting mitochondrial pathways to PD
A scientific rationale, evidence of blood brain barrier (BBB) penetration, adequate safety data, efficacy in animal models and/or preliminary efficacy data in humans are requirements to identify promising neuroprotective drugs in PD (Ravina et al. 2003). In contrast with symptomatic treatments, neuroprotective drugs aim to slow or halt the progression of PD by a direct effect on the vulnerable cells (Olanow & Jankovic 2005).

The neuroprotective drugs include dopamine agonists and MAO-B inhibitors, which are already used for years to improve symptoms in the early stages of PD. Based on our current understanding of PD pathogenesis and mitochondria-related mechanisms of cell death, neuroprotective drugs can be divided into drugs that (1) attenuate mitochondrial apoptosis, (2) reduce oxidative stress in mitochondria or (3) directly target mitochondrial function (Fig. 5).

Antiapoptotic
Dopamine agonists
Dopamine agonists have proven efficacy in the early stages of PD. Dopamine agonists directly stimulate dopamine receptors and unlike levodopa their symptomatic effect does not depend on the presence of dopamine producing neurons (Junghanns et al. 2004). The neuroprotective effect of dopamine agonists may be the result of different mechanisms, including a ‘levodopa sparing’ effect (delaying the need for levodopa therapy) (Holloway et al. 2000; Rascol et al. 2000), antioxidative properties by scavenging free radicals (Iida et al. 1999), as well as inhibition of apoptosis (Nair et al. 2003). Pramipexole is perhaps the best-studied dopamine agonist. Pramipexole decreased apoptotic cell death in SH-SY5Y cells exposed to MPP+ and rotenone (Kitamura et al. 1998; Schapira et al. 2002) and was shown to inhibit opening of the PT pore, reduce release of cytochrome c and decrease activation of caspase 3 (Cassarino et al. 1998; Gu et al. 2004; King et al. 2001). In vivo studies showed that marmosets (non-human primates) pretreated with pramipexole had significantly greater numbers of surviving SNpc neurons upon MPTP treatment (Irvani et al. 2006). The need for pretreatment, which is in agreement with earlier observations in SH-SY5Y cells (Gu et al. 2004; Kitamura et al. 1998), does not preclude clinical application of pramipexole, but rather emphasizes the importance of pramipexole administration early
in the disease process to protect the remaining SN neurons from future dysfunction or degeneration.

Two prospective randomized double-blind clinical trials in early PD patients (diagnosed within 5 years) supported the neuroprotective effect of dopamine agonists (Schapira 2003), but further trials are needed to confirm their neuroprotective potential.

**MAO-B inhibitors and propargylamines**

MAO-A and MAO-B are tightly associated with the OMM and oxidatively deaminate monoamine neurotransmitters (e.g. dopamine), as well as exogenous monoamines (e.g. tyramine). MAO inhibitors were considered in PD therapy as dopamine sparing drugs, however, the first generation of non-selective MAO inhibitors had a major side-effect, known as the ‘cheese reaction’ (hypertensive crisis because of potentiation of the sympathomimetic activity of tyramine by peripheral inhibition of its MAO metabolism). Selective MAO-B inhibitors, such as the propargylamine-related L-deprenyl (Birkmayer et al. 1975) and the more potent rasagiline (Siderowf & Stern 2006), do not have such effect (Youdim & Weinstock 2004). Neuroprotection by L-deprenyl (Birkmayer et al. 1985) and other propargylamines is most likely because of interference with apoptotic signalling pathways rather than by MAO-B inhibition itself (Tatton et al. 2003a). In addition, L-deprenyl was shown to inhibit the formation of SNCA fibrils and to destabilize preformed SNCA fibrils (Ono et al. 2007).

In SH-SY5Y cells rasagiline suppressed apoptosis by preventing the fall in $\Delta \Psi_{m}$ and opening of the mitochondrial PT pore, preventing nuclear translocation of GAPDH (Youdim & Weinstock 2001) and increasing expression of antiapoptotic Bcl-2 family members (Akao et al. 2002). In rats and marmosets L-deprenyl and rasagiline attenuated the neurotoxic effects of MPTP (Kupsch et al. 2001; Wu et al. 2000).
The neuroprotective effect of L-deprenyl and rasagiline was tested in prospective randomized multicentre placebo-controlled studies: the DATATOP (Deprenyl and Tochopherol Antioxidative Therapy for Parkinson’s disease) (Shoulson et al. 1993) and TEMPO study (TVP-1012 (rasagiline mesylate) in Early Monotherapy for Parkinson’s disease Outpatients) (Parkinson Study Group 2004a). The TEMPO study showed that PD patients treated with rasagiline for 12 months showed less functional decline than patients whose treatment was delayed for 6 months. Such difference cannot be simply explained by a symptomatic effect, which was a confounder in studies of L-deprenyl (Olanow et al. 1995; Shoulson et al. 2002), and is consistent with rasagiline having a neuroprotective effect. A larger double-blind delayed-startstudy is under way to confirm these data.

Another promising drug with potential neuroprotective effect is TCH346 (also known as CGP3466). TCH346 incorporates a propargyl ring within its molecular structure and resembles L-deprenyl, but does not inhibit MAO-B and was therefore not anticipated to have confounding symptomatic effects (Olanow et al. 2006).

TCH346 prevented nuclear translocation of GAPDH in a cellular system (Hara et al. 2006), and in bilaterally MPTP-treated monkeys TCH346 prevented death of SN DAgic neurons (Andringa et al. 2003). Preclinical safety studies and small trials in healthy volunteers showed that TCH346 was well tolerated and free of clinically significant adverse effects. A highly anticipated first double-blind placebo-controlled clinical trial was already completed (Olanow et al. 2006). Unfortunately, no significant differences were found between any of the tested doses and placebo with respect to the primary outcome (time to disability requiring DAgic treatment) or secondary outcome measures [e.g. changes in Unified Parkinson’s Disease Rating Scale (UPDRS)] in patients with early untreated PD.

Nevertheless, the evidence for neuroprotection by L-deprenyl and rasagiline still deserves further study. Both propargylamines differ from TCH346 by their additional MAO-B inhibitory effects, which might not be redundant to explain the apparent differences in their potential neuroprotective effects.

**JNK inhibitors**

The first successful compound targeting the JNK signalling pathway was CEP-1347 (Maroney et al. 1998). CEP-1347 does not directly inhibit JNK, but reduces neuronal cell death by MLK inhibition (Maroney et al. 2001). JNK activation is relevant to PD pathogenesis, as activated JNK was associated with SNCA pathology in SNCA p.Ala30Pro transgenic mice (Frasier et al. 2005) and loss of parkin function was already shown to activate the JNK pathway in Drosophila (Cha et al. 2005).

CEP-1347 attenuated the neurotoxic effects of MPP+ in differentiated SH-SY5Y cells (Mathiasen et al. 2004), and reduced MPTP-mediated nigrostriatal DAgic neuron loss in vivo in mice (Saporito et al. 1999). A placebo-controlled pilot study showed that CEP-1347 was safe and well tolerated by PD patients (Parkinson Study Group 2004b), and this supported the initiation of a large trial of longer duration with CEP-1347: the PRECEPT study (Parkinson Research Examination of CEP-1347 Trial).

The PRECEPT study was, however, prematurely abrogated when an interim analysis showed that it would be futile to continue experimental treatment. In all tested CEP-1347 dose regimens more patients had reached the primary and secondary end-points compared with patients randomized to placebo (Shoulson et al. 2006). Again these findings contrasted with research in preclinical models that predicted favourable effects of CEP-1347 on the progression of PD.

**Minocycline**

Minocycline, a second-generation tetracycline, has a proven safety record in humans. Among several activities minocycline impairs microglial activation, neuroinflammation and apoptosis (Yong et al. 2004). In midbrain cultures of parkin knockout mice minocycline neuroprotection was related to inactivation of microglial cells and protection against neuronal apoptotic cell death (Casarejos et al. 2006). Increasing evidence suggests that its antiapoptotic effect is achieved through mechanisms acting at the level of mitochondria.

Minocycline inhibited mitochondrial PT mediated cytochrome c release in isolated brain mitochondria (Zhu et al. 2002), and in kidney cells minocycline not only induced antiapoptotic Bcl-2, but also antagonized pro-apoptotic Bcl-2 family members (Wang et al. 2004). Furthermore, minocycline was shown to prevent nigrostriatal DAgic neurodegeneration in the MPTP mouse model of PD (Du et al. 2001; Wu et al. 2002). In contrast with numerous reports of beneficial effects of minocycline, some studies reported significant exacerbation of MPTP-induced damage by minocycline in mice (Yang et al. 2003) and monkeys (Diguet et al. 2004). It remains, however, plausible that minocycline has different, sometimes even deleterious, effects according to its mode of administration and dose. A recent study suggested that the mitochondrial effects of minocycline contribute to toxicity rather than tissue protection at high dosing in animals and humans (Mansson et al. 2007). The possible harmful effects of minocycline must thus be ceaselessly kept in mind when considering minocycline use in clinical PD settings.

A randomized, double-blind, futility clinical trial of minocycline in early PD patients could not reject minocycline as futile (Ravina et al. 2006). Such futility trials are intended to determine if treatments are worthy of larger and longer term studies, or if they should be abandoned (Elm et al. 2005). Although the results of this futility study do not provide evidence for clinical use of minocycline in the treatment of PD, they indicate that minocycline at least merits further consideration for larger clinical trials.

**Oestrogen analogues**

Postmenopausal oestrogen replacement therapy in women with early PD was positively associated with lower symptom severity (Saunders-Pullman et al. 1999) and suggested a potential neuroprotective effect of oestrogens. Indeed, numerous in vitro and in vivo studies established that oestrogens act as neuroprotectants when challenged by various toxic stresses.
Several effects might explain the beneficial actions of oestrogens on brain; including anti-inflammatory activity and protection against apoptosis (Maggi et al. 2004). These effects appear to be primarily mediated through activation of intracellular oestrogen receptors (ER) and result in modulated transcription of oestrogen target genes, e.g. upregulated expression of antiapoptotic Bcl-2 (Garcia-Segura et al. 1998). Neuroprotective effects of oestrogens might also result from ER-independent mechanisms, including antioxidative effects (Moosmann & Behl 1999) related to their basic chemical properties as hydrophobic phenolic molecules. Mechanistic and structure–activity studies with oestrogens yielded a model in which they intercalate into cell membranes where they block lipid peroxidation reactions, and thereby stabilize membrane structure (Behl et al. 1997), which is especially critical to mitochondrial function.

Both the naturally occurring 17β-oestradiol (17β-E2) and its 17α-oestradiol (17α-E2) isomer, which is at least 200-fold less active as transactivating hormone, protected DAergic neurons from MPP+-induced neuronal death (Sawada et al. 2002). 17β-E2 showed beneficial effects in animal models of PD using MPTP as lesioning agent (Caliler et al. 2000; Ramirez et al. 2003), and 17α-E2 treatment in 6-hydroxydopamine injected rats later showed significantly improved behavioural outcomes (Dykens et al. 2005b).

A first small double-blind placebo-controlled clinical trial of 17α-E2 was completed including eight healthy postmenopausal women; the tested doses of 17α-E2 were well tolerated and no adverse events were reported (Dykens et al. 2005a). However, the neuroprotective, or better mitoprotective, effects of 17α-E2 remain to be tested in PD patients.

**Antioxidants**

**Iron chelators**

Alterations in cellular iron metabolism and iron-induced oxidative stress are important factors in the pathogenesis of PD (Bharath et al. 2002; Riederer et al. 1989; Sofic et al. 1991; Youdim et al. 1993). Moreover, iron was shown to accelerate the aggregation of SNCA (Kalivendi et al. 2004) and to induce alterations of parkin solubility, with depletion of soluble functional parkin leading to reduced proteasomal activities and increased cell death (Wang et al. 2005). Reducing the SN iron content through iron chelation might thus be a straightforward therapeutic approach to the treatment of PD (Zhang et al. 2005b).

Desferrioxamine, the prototype of iron chelators (Jiang et al. 2006), blocked iron-induced oxidative damage in SK-N-SH neuroblastoma cells (DAergic origin) (Sangchot et al. 2002). In rat brain mitochondria desferrioxamine also protected mitochondrial complex I activity against inhibition by 6-hydroxydopamine, possibly through a combined neuroprotective effect of iron chelation and NADH dehydrogenase activation (Glinka et al. 1996, 1998). The neuroprotective effect of preinjected desferrioxamine was later confirmed in rat (Ben Shachar et al. 1991; Youdim et al. 2004) and mice models (Lan & Jiang 1997).

As desferrioxamine has poor BBB penetration, other lipophilic chelators with enhanced BBB penetration have been synthesized; e.g. VK-28 (Ben Shachar et al. 2004). Novel iron chelators were further developed based on VK-28, that combine its iron chelating potency with a MAO inhibitory propargylamine moiety (Youdim et al. 2005), e.g. the multifunctional M30. M30 exhibited neuroprotective activity upon in vitro and in vivo testing (Gal et al. 2005; Zheng et al. 2005), but its effect in PD patients remains to be evaluated. Nevertheless, as multifunctional compounds possess various targets within the central nervous system, they may provide better efficacy and utility as potential disease-modifying drugs (Youdim & Buccafusco 2005).

**Free radical scavengers**

Under physiological conditions several cellular mechanisms are able to neutralize a possible excess of free radicals and thereby prevent oxidative stress. These comprise free radical scavengers, which are either endogenously produced molecules or nutrients, and scavenger enzymes such as SOD, glutathione peroxidase and catalase (Fig. 2). The free radical scavengers include vitamins A, C and E, as well as glutathione in its reduced form, and melatonin (Contestabile 2001). RNA interference-mediated inactivation of the Drosophila PINK1 homologue already showed that addition of SOD1 and vitamin E to the flies’ diet inhibited degeneration of photoreceptor neurons (Wang et al. 2006).

The lipid-soluble vitamin E received much attention as a chain-breaking antioxidant that prevents propagation of the radical chain (Burton et al. 1983). In vitro studies indicated oxidation of vitamin E upon incubation with free radicals in rat brain mitochondria (Vatassery et al. 1995) and protection against H2O2-induced oxidative stress in a murine hippocampal-derived cell line (Behl 2000). However in vivo studies did not consistently find positive effects of vitamin E administration (Gong et al. 1991; Roghani & Behzadi 2001).

Already in 1987 α-tocopherol, the biologically most active form of vitamin E, was selected for the first clinical trial of neuroprotection in PD: the DATATOP study (Shoulson et al. 1993). This study did not show beneficial effects of supplemental α-tocopherol intake in PD patients, but a more recent meta-analysis suggested that diets rich in vitamin E might exert a protective effect against the development of PD (Etminan et al. 2005). There is, however, some concern that high-dosage vitamin E supplementation may be harmful (Miller et al. 2005).

Use of reduced glutathione as a free radical scavenger is hampered by a poor BBB permeability (Zeewalk et al. 2007). On the other hand, the pineal hormone melatonin is a highly lipophilic free radical scavenger (Reiter et al. 1993) and antioxidant (Tan et al. 2000a). Some of the melatonin metabolites resulting from scavenging actions are also efficient free radical scavengers (Lopez-Burillo et al. 2003). The action of melatonin as a free radical scavenger is thus a sequence of scavenging reactions, resulting in a cascade of protective reactions.

Pharmacological doses of melatonin, which greatly exceed endogenous levels, were shown to be neuroprotective in different neurotoxic models of PD (Joo et al. 1998; Thomas & Mohanakumar 2004), but to our knowledge melatonin has not yet been tested for the treatment of PD in clinical trials.
Targeting mitochondrial function

Coenzyme Q<sub>10</sub>

Coenzyme Q shuttles electrons from complexes I/II to complex III of the electron transport chain, and the predominant form of coenzyme Q in humans is coenzyme Q<sub>10</sub>. In addition, ubiquinol (its reduced form) functions as antioxidant, prevents lipid peroxidation in most subcellular membranes and also protects mitochondrial membrane proteins and DNA from ROS-induced oxidative damage (Ernster & Dallner 1995).

Coenzyme Q<sub>10</sub> was shown to prevent in vitro mitochondrial PT pore opening upon apoptotic stimuli (Papucci et al. 2003). In aged mice coenzyme Q<sub>10</sub> attenuated MPTP-induced loss of striatal dopamine and DAergic axons (Beal et al. 1998). In addition, PD patients showed lower levels of coenzyme Q<sub>10</sub> in platelet mitochondria (Shults et al. 1997), as well as elevated levels of oxidized coenzyme Q<sub>10</sub> (Sohmiya et al. 2004) compared with control individuals.

A pilot study in PD patients showed that oral administration of coenzyme Q<sub>10</sub> was well tolerated and caused a trend towards increased complex I activity (Shults et al. 1998). A later study also showed reduced functional decline in PD patients taking coenzyme Q<sub>10</sub> (Shults et al. 2002). More recently, a randomized, double-blind, futility clinical trial of coenzyme Q<sub>10</sub> in early PD could not reject coenzyme Q<sub>10</sub> as futile (The NINDS NET-PD Investigators 2007). However, large trials are still needed to evaluate the long term effects of coenzyme Q<sub>10</sub> intake and to assess its disease-modifying potential in the treatment of PD patients.

Creatine

The endogenous guanidine-derived creatine is a substrate for cytosolic and mitochondrial creatine kinases (CK). Aerobic glycolysis, coupled to mitochondrial ATP synthesis via oxidative phosphorylation, is the primary pathway of ATP synthesis in brain, but in addition the CK/phosphocreatine system provides a rapid alternative source for ATP synthesis (Wallimann et al. 1992). Mitochondrial CK together with high cytoplasmic creatine levels also inhibits mitochondrial PT and the consequent triggering of apoptosis (Andres et al. 2005).

In primary neuronal cultures creatine exerted neuroprotective effects against neurotoxic insults as MPP<sup>+</sup> exposure (Andres et al. 2005). Oral supplementation with creatine protected against MPTP-induced dopamine depletion in mice and reduced damage to the nigrostriatal DAergic system (Matthews et al. 1999).

A double-blind placebo-controlled pilot study on the effect of oral creatine supplementation on PD progression did not find a treatment effect on UPDRS scores, although a significantly smaller dose increase of DAergic therapy in creatine-treated patients indicated a potential neuroprotective effect (Bender et al. 2006a). A randomized, double-blind, futility clinical trial of creatine in early PD could not reject creatine as futile (Ravina et al. 2006). Creatine thus remains a promising neuroprotective drug for PD, but its neuroprotective potential may only be shown by large multicentre studies over an extended observation period.

Conclusion

Molecular genetic studies identified key proteins involved in PD pathogenesis. Mutations or polymorphisms in mtDNA, but especially mutations in the nuclear-encoded SNCA, parkin, PINK1, DJ-1, LRRK2 and HTRA2 provided direct or indirect links between mitochondria and PD pathogenesis. Other chromosomal loci still await refinement and characterization (Bogaerts et al. 2007; Gasser et al. 1998; Hicks et al. 2002; Pankratz et al. 2003) and will further increase our knowledge of the basic mechanisms underlying PD pathogenesis.

Perhaps the most relevant question for research into the causes of PD is: how can we exploit the gained knowledge on mitochondrial involvement in PD to develop better therapies? Neuroprotective therapies that slow or halt disease progression remain an unmet need. Our insights into the pathogenic mechanisms of cell death in PD led to several potential neuroprotective drugs, which have actions as attenuating mitochondrial apoptosis and reducing oxidative stress in mitochondria, or directly target mitochondrial function. The selection of drugs covered in this review all exert at least one of the three above mentioned actions, along with possible involvements in other non-mitochondrial pathways to PD.

However, no drug has yet been shown to be neuroprotective in PD patients, although several were tested in clinical trials. This discrepancy, reported for several potential neuroprotective drugs targeting different elements of the mitochondrial pathway, raises serious concerns as to the suitability of currently available models (Waldmeier et al. 2006). Particularly, the currently used animal models, typically acute or subacute neurotoxic models, might not adequately recapitulate the pathogenesis of the slower progressive neurodegenerative process in humans. Therefore, genetic models of PD are highly desirable because they produce a delayed onset and a neurodegenerative process that probably mimics human PD better than neurotoxic models.

On the other hand, the clinical end-points used to assess neuroprotective drugs in PD, such as need for symptomatic treatment or change in UPDRS score, may be confounded by symptomatic effects, or may simply be too insensitive. True biomarkers that accurately reflect disease progression remain an urgent priority. An additional concern is that DA neurons may already be beyond rescue when clinical features appear. This reinforces the need for biomarkers to identify patients who are in the presymptomatic or early stages of PD. Ideally, neuroprotective drugs should be administered during this early period (Halbig et al. 2004).

Last but not least a very important questions remains: do we target the right pathway? Moreover, how can we fit all the cellular processes uncovered by genes involved in familial forms of PD in the mitochondrial pathway? And is this mitochondrial pathway also relevant to sporadic forms of PD? Association studies in sporadic PD patients, showing modest effects for candidate genes involved in mitochondrial function and oxidative stress, support this contention. On the other hand, monogenic PD explains only 20% of early onset PD and less than 3% of late onset PD at best (Klein 2006). In most PD patients, the loss of DAergic neurons results from a combination of exogenous stresses and a genetic
Genetic findings in Parkinson’s disease and translation into treatment

predisposition. Given this complexity, it is unlikely that any neuroprotective drug will be applicable to the full spectrum of PD patients. However, it is more plausible that mixtures of therapies targeting several different processes converging in a common pathway may prove useful in PD neuroprotection. The recent development of multifunctional compounds represents a move into that direction.

As the repeated failure of neuroprotective drugs in clinical trials has unfortunately shown, we must acknowledge that at present we merely have a rough sketch of the mitochondrial pathway to neurodegeneration in PD. This sketch turns out inadequate to predict fruitful neuroprotective approaches and further research is definitely needed. Nevertheless, the future looks bright. Our understanding of PD pathogenesis is rapidly evolving, boosted by a massive amount of research into the genetic causes of PD, the influence of genetic and environmental susceptibility factors on disease penetrance and manifestation, and the development of better models to test potential neuroprotective therapies. New insights from these future studies will redirect our efforts and eventually will provide us with neuroprotective therapies that might really make the change in the treatment of PD.

Glossary

Association study: Study of the correlation between a genetic variant in a population and a disease. If a correlation is observed, then there is said to be an association between the variant and the disease.

Candidate gene: Gene with evidence for a possible role in the disease under study.

DAergic: Related to or activated by dopamine.

Early PD: Early stages of PD with subtle impairment. In clinical trials, early PD patients have Hoehn and Yahr stage I (unilateral disease) or stage II (bilateral disease with preservation of postural reflexes) PD for less than 5 years. The Hoehn and Yahr rating scale is a simple and popular scale to establish the severity of PD with stages ranging from I to V (wheelchair-bound or walking only with assistance).

Early onset PD: Having an onset at or before 45 years of age.

Haploinsufficiency: Situation in which half of the normal level of gene expression is not sufficient to permit cells to function normally.

Haplotype: Sequential set of alleles present on the same chromosome.

Linkage analysis: Mapping loci by genotyping genetic markers in families to identify chromosome regions implicated in disease. Such linked regions are of great help in the identification of genetic variants causing disease.

Meta-analysis: A statistical analysis combining the results of several independent studies.

Mitochondrial respiratory chain (electron transport chain): Collective term used to describe the peptides, organized in five enzymatic mitochondrial complexes (I–V), and the electron shuttle molecules that are needed to produce ATP.

Oxidative phosphorylation: Phosphorylation of adenosine 5’-diphosphate to ATP driven by the transfer of electrons to oxygen.

SH-SY5Y: Human neuroblastoma subclonal cell line of the neuroepithelioma cell line SK-N-SH. These cells are widely used in studies addressing the molecular mechanisms of neurodegenerative disease.

UPS: Cellular system responsible for the degradation of damaged or misfolded proteins. Ubiquitin molecules are attached to lysine residues of a given protein and target the protein for destruction by the multienzyme proteasome.

UPDRS: Unified Parkinson’s Disease Rating Scale used to monitor the progression of PD. It is the most widely used clinical rating scale for PD and consists of a four-part evaluation: I non-motor experiences of daily living, II motor experiences of daily living, III motor examination and IV motor complications.

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