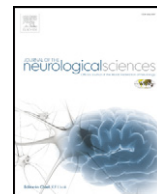




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Review article

Converging environmental and genetic pathways in the pathogenesis of Parkinson's disease

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ABSTRACT

As a prototypic neurodegenerative disorder Parkinson's disease (PD) is characterized by the progressive loss of specific neuronal subpopulations leading to a late-onset movement disorder. Based on familial forms of PD, to date a significant number of genes were identified that allowed first insight into the molecular pathogenesis of this common movement disorder. These pathways include impaired protein degradation and subsequent aggregation within neuronal cells and impaired mitochondrial function followed by energy depletion due to oxidative stress leading to cell death. The respective disease models were supported by pathoanatomical and biochemical findings in brains of sporadic PD patients without apparent genetic contribution to pathogenesis. Indeed recent genetic and epidemiological studies hint to a complex interplay of genetic susceptibility factors and environmental risk factors to converge to processes of pathological protein accumulation and mitochondrial damage that trigger neurodegeneration in PD. Therefore large-scale genoticoepidemiological studies combining genetic whole genome approaches with a detailed ascertainment of environmental exposures are expected to provide important clues to decipher the complexity of neurodegeneration of this most frequent neurodegenerative movement disorder.

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Contents

1. Introduction	0
2. Pathological protein aggregation	0
2.1. Genetic factors	0
2.2. Environmental factors	0
3. Mitochondrial dysfunction	0
3.1. Genetic factors	0
3.2. Environmental factors	0
4. Environmental factors	0
5. Conclusion	0
Acknowledgments	0
References	0

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and affects more than 3%

of the population over age 70. It is expected to rise in prevalence over the next decades and therefore represents one of the major burdens of the aging society [1]. The typical clinical symptoms that are slowly progressive during the course of the disease include slowing of movements, tremor, muscular rigidity and gait difficulties due to impaired balance. These motor symptoms are commonly treated with dopaminergic drugs, of whom the levodopa still represents the gold standard pointing to the loss of dopaminergic neurons in the substantia nigra pars compacta and subsequent reduced dopamine levels in the striatum as the primary cause of this movement disorder

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[2]. However, detailed pathoanatomical studies revealed a much more complex degeneration of specific neuronal populations in brains of PD patients that affect a variety of transmitter systems, i.e. noradrenergic (locus coeruleus), cholinergic (nucleus basalis Meynert) or serotonergic (raphe nuclei), and are supposed to contribute to non-motor symptoms of PD including depression, cognitive dysfunction, REM sleep behavior disorder, impaired olfaction and autonomic dysfunction [3–5].

The average age at disease onset is in the late 50s and based on extrapolations from pathoanatomical studies on different disease stages, it can be assumed that first motor symptoms occur, if already the majority of neurons of the substantia nigra are degenerated or at least dysfunctional [5]. Most of the patients present sporadic cases, meaning that no further affected family member is reported at diagnosis [6]. Thus PD has been regarded as a prototypic non-genetic disorder for a long time, which was also reflected by established diagnostic criteria, i.e. the United Kingdom Parkinson's disease Brain Bank (UKPDBB) criteria, that explicitly excluded a positive family history in typical, so-called idiopathic, PD [7]. At that time important efforts were undertaken to define environmental factors that contribute to PD and indeed, in the 1980s, first evidence for an exogenous toxin that caused Parkinsonian symptoms in humans was identified. After intravenous application of a synthetic drug that contained 1-methyl-4-tetrahydropyridine (MPTP), some of the drug addicts developed an akinetic-rigidic syndrome that responded to levodopa therapy [8]. Subsequent *in vitro* and *in vivo* experiments revealed that MPP⁺, as the active metabolite of MPTP, was generated due to monoamine B oxidase activity in glial cells and was sufficient to cause a selective damage of dopaminergic neurons upon uptake via the dopamine transporter [9]. Although additional cases of acute parkinsonism due to drug exposure or infection were reported, no major environmental factor that causes PD could be yet defined [10,11]. Nevertheless, epidemiologic studies provided sufficient evidence for an association of PD with traumatic brain injury [12], pesticide exposure [13] or heavy metal exposure [14] and showed that cigarette smoking and coffee or tea drinking may reduce the risk to develop PD [15,16].

Major advances in the understanding of molecular mechanisms involved in PD during the last decade were established by analyzing large families with classical Mendelian inheritance of the disease trait. The subsequent functional characterization of the identified genetic factors *in vitro* and *in vivo* hints to a critical role of pathological protein aggregation and/or mitochondrial dysfunction in neurodegeneration in PD.

Whereas the first genes identified to cause autosomal dominantly or autosomal recessively inherited forms of PD affect only a minor proportion of less than 1% of all PD patients, the identification of mutations in the *LRKK2* gene (*PARK8*; OMIM #607060) encoding the leucine-rich repeat kinase 2 protein unraveled the first genetic factor that relevantly contributes to the population level. The G2019S substitution is the most common mutation in the *LRKK2* gene with an elevated frequency of 40% among North African patients and 30% among Jewish patients with familial PD; patients from other populations can also be affected with less than 1% [17,18]. Up to date, *LRKK2* represents the most frequent genetic cause of PD. Although initially identified in large families with autosomal dominant inheritance of PD, more and more mutation carriers were identified among patients with the sporadic form of the disease [18–20].

Similarly also for other genes identified in familial PD, a role as a susceptibility factor also for the common sporadic form of the disease has emerged during the last years: (i) a promoter polymorphism and single nucleotide polymorphisms (SNPs) in the 3' region of the *alpha-synuclein* (*SNCA*) gene (*PARK1*; OMIM #168601) encoding the *alpha-synuclein* protein were identified as relevant risk factors for PD among different populations world-wide [21,22], moreover heterozygous point mutations in the genes encoding (ii) Parkin or (iii) PINK1 may

contribute to the common late-onset form of the disease [23,24]. However, these gene variants per se are not sufficient to cause the disease as mirrored by the reduced penetrance of genetic risk factors [25]. Genetic studies using a candidate-gene approach support the association between PD and the genes encoding *alpha-synuclein*, but also microtubule-associated protein tau (*MAPT*) with an increased risk for PD in carriers of the H1/H1 *MAPT* haplotype [26,27]. Subsequent unbiased genome-wide association studies (GWASs) finally confirmed the association between the *SNCA* gene and the *MAPT* locus as the main common contributors to PD genetic susceptibility in the Caucasian and Asian population [28–30].

Therefore current concepts on the etiology of PD hypothesize that for the majority of sporadic cases a concerted interplay of environmental risk factors and specific genetic susceptibility factors represents the basis of the chronic neurodegenerative process. In the present review, we delineate established genetic and environmental factors in the pathogenesis of PD with a special focus on their respective function in terms of molecular mechanisms leading to neuronal cell death and therefore allowing initial insight into converging mechanisms involved in pathological protein aggregation and mitochondrial dysfunction as pathological key features of neurodegeneration in PD.

2. Pathological protein aggregation

2.1. Genetic factors

The *SNCA* gene was the first gene identified as a cause of familial parkinsonism [31]. The encoded *alpha-synuclein* is predominantly localized to presynaptic nerve terminals, but can also be found in other compartments like cytosol and nucleus. Originally, it was identified in relation to the pathogenesis of Alzheimer's disease as the precursor protein for the non- β -amyloid component of amyloid plaques [32]. Although the physiological function of *alpha-synuclein* is still unknown, it may play a role in the integration of presynaptic signaling and neuronal plasticity, as deduced from *synelfin*, the homologue of *alpha-synuclein* in zebra finch [33].

Up to date, three missense mutations in the coding sequence of the *SNCA* gene are known to cause aggregation of *alpha-synuclein*, subsequently leading to a progressive loss of nigral dopaminergic neurons in autosomal dominantly inherited parkinsonism. The most prominent A53T mutation was discovered in a large Italian kindred and in five additional Greek families [31]. A second mutation (A30P) has been discovered in one German family [34] and a third one (E64K) was confirmed in one family from the Basque Country [35]. Mutation carriers of the *SNCA* gene show typical features of PD (rigidity, hypokinesia, postural instability, and resting tremor), however, some mutations display a more severe phenotype with early onset and rapid disease progression (A53T) and severe dementia (A53T, E46K), whereas carriers of the A30P mutation present a milder phenotype [35–37].

All patients carrying mutations in the *SNCA* gene show protein aggregation and the formation of Lewy bodies according to the development of a severe PD phenotype [38,39] (Fig. 1). Strikingly, it was shown that *alpha-synuclein* was the major component of protein aggregates in a number of distinct brain regions that represent the pathological hallmark of PD [7]. Moreover *alpha-synuclein*-positive Lewy bodies and the Lewy neurites were not only identified in patients carrying point mutations in the *SNCA* gene, but also in patients presenting the most common sporadic form of the disease. This indicated a crucial role of *alpha-synuclein* also in the pathogenesis of typical sporadic PD.

Point mutations in the *SNCA* gene have been found to contribute to neurodegeneration in PD due to the increased tendency of the *alpha-synuclein* protein to form aggregates, if the peptide sequence is changed. The impairment of the ubiquitin-proteasome system (UPS)

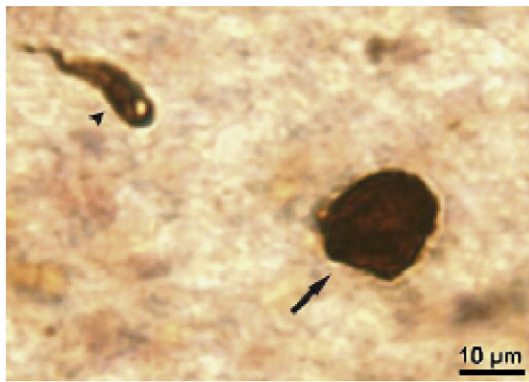


Fig. 1. Lewy bodies in the brain of the A30P mutation carrier in the *SNCA* gene. Immunohistochemical studies on the brain of the A30P mutation carrier using an antibody against alpha-synuclein reveal typical Lewy bodies (large arrows) and Lewy neurites (arrow heads) in the dorsal motor vagal nucleus.

results in protein misfolding and aggregation—the consequence of mutant alpha-synuclein. Although *SNCA* gene mutations are a rare event with less than 1% of PD cases [40], abnormal aggregation of alpha-synuclein was also observed in sporadic PD patients, indicating a role of wildtype alpha-synuclein in neurodegeneration.

Besides point mutations, it has been shown that duplications or triplications of the wildtype gene may also account for autosomal dominantly inherited PD with characteristic alpha-synuclein-positive Lewy bodies. This indicates that not only rare pathogenic mutations lead to a PD phenotype, but also abnormal levels of wildtype alpha-synuclein due to genomic multiplications contribute to neurodegeneration indicating a critical dose-effect [41–43]. Whereas duplications result in a typical PD phenotype, triplications cause a more severe phenotype with rapid disease progression, early dementia and reduced lifespan [41–43].

Apart from rare mutations in the *SNCA* gene causing familial PD, genetic variability in the non-coding regions of the gene was identified as a risk factor for the development of the common sporadic form of PD. A large-scale collaborative analysis shed light on a dinucleotide repeat polymorphism (REP1) within the promoter region of the *SNCA* gene and established it as a susceptibility factor for PD at the population level world-wide [21]. This polymorphism can increase *SNCA* gene expression in vitro and in vivo and therefore provides a similar pathogenic mechanism of gene overexpression as it was shown for multiplications of the *SNCA* gene [44,45]. Subsequent studies on polymorphic variants in multiple regions of the *SNCA* gene found an independent association between idiopathic PD and SNPs in the 3'UTR region of the *SNCA* gene [46–48]. Evidence that alpha-synuclein levels are influenced by genetic variability in the 3' region of the *SNCA* gene came from investigations of plasma as well as blood levels and human post mortem brain tissue [49,50]. Conclusively, it has been demonstrated that at least two separate loci in the untranslated regions of the *SNCA* gene contribute to PD susceptibility and are therefore of clinical importance for PD.

Indeed, recently, an unbiased genomic approach in a large number of PD patients confirmed the unequivocal role of common genetic variants in the *SNCA* gene as risk factors for sporadic PD and underscores the role of genetic factors with reduced penetrance in PD [28].

Regarding the mechanisms involved in neurodegeneration caused by *SNCA* mutations, the fibrillization and aggregation of alpha-synuclein play a central role, either due to primary aberrations in the peptide sequence or due to increased levels of physiological alpha-synuclein possibly exceeding existing mechanisms of degradation. Under normal conditions, the UPS is essential to degrade intracellular proteins that are determined to be cleared from the cell [51]. Therefore, an increase in proteins that are determined to be degraded by the UPS or a primary impairment of the UPS function can contribute to proteolytic stress due to accumulation and aggregation of proteins in the cytosol. Moreover,

oxidative stress due to mitochondrial dysfunction may further increase misfolding of proteins and therefore contribute to aggregation of alpha-synuclein and subsequent death of dopaminergic neurons [52]. Indeed aggregation of alpha-synuclein itself may also lead to the increase of oxidative stress in the cell contributing to a vicious cycle [53] (Fig. 2).

The second gene identified in familial PD encodes Parkin that contributes to PD upon loss of function mutations in the *PARK2* gene [54]. Parkin is linked to pathological protein aggregation based on several experimental findings. (i) Parkin is an ubiquitin E3 ligase and therefore is an integral part of the ubiquitin-mediated proteasomal degradation pathway [55], (ii) Parkin itself is degraded by the proteasome after ubiquitination and (iii) recently, it was shown that Parkin modulates the activity of the 26S proteasome as the site of degradation of ubiquitinated proteins [56]. Interestingly, Parkin also integrates the proteasomal and lysosomal protein degradation pathway as it is able to link ubiquitin in both, position K48 and position K63 and therefore mediate 26S proteasomal degradation or formation of protein inclusion that can be cleared by autophagy [57]. In addition, it has been found that Parkin-mediated ubiquitination of proteins within Lewy-body-like inclusions formed by the co-expression of alpha-synuclein, Parkin and synphilin-1, an alpha-synuclein-interacting protein, occurs predominantly via Lys63 linkages [58]. Hence, PD-associated protein Parkin has an important role in pathological protein aggregation observed in PD.

Another protein comes into focus by summarizing alpha-synuclein and pathological aggregation in neurodegeneration in PD. Striking accumulation and aggregation of alpha-synuclein and ubiquitinated proteins also appears in aged *LRRK2* germ-line deletion mice with great loss of *LRRK2* [59]. Again, the autophagy–lysosomal pathway resulted to be impaired together with an increase of apoptotic cell death, inflammatory response and oxidative stress. Moreover, impaired mitochondrial function and an increased susceptibility towards apoptosis were described in ex vivo models based on fibroblasts from PD patients carrying the most common G2019S mutation in the *LRRK2* gene or based on induced pluripotent stem cells that carry the G2019S mutation [60,61]. Furthermore, dopaminergic neurons derived from G2019S-induced pluripotent stem cells showed an increase of key oxidative stress response genes and a pathogenic accumulation of the alpha-synuclein protein [61].

Together these studies support an involvement of *LRRK2* in mitochondrial dysfunction and impaired protein degradation pathways with alpha-synuclein accumulation over time.

2.2. Environmental factors

After the definition of a critical role of the UPS in the clearance of misfolded proteins and its potential impact on alpha-synuclein aggregation in PD pathogenesis, a search for environmental factors, believed to affect alpha-synuclein folding or UPS function, began. Indeed factors that accelerate alpha-synuclein aggregation including pesticides, heavy metals and toxins from plants were described that interfere with protein degradation and the formation of insoluble fibrils [62–65].

In brains of sporadic PD patients, evidence for proteasomal dysfunction was substantiated by the discovery of reduced levels of a subunit of the proteasome in dopaminergic neurons of the substantia nigra [66]. This leads to the conclusion that a failure or inhibition of the UPS in these patients occurred, followed by a disturbed clearance of abnormal proteins. Subsequent in vitro experiments in rodent cells revealed that the inhibition of the proteasomal function induces a concentration-dependent degeneration of dopaminergic neurons, alterations in protein handling and the formation of inclusion bodies that stain positive for alpha-synuclein [66,67].

In terms of involved environmental toxins, it was even shown that either naturally occurring or synthetic inhibitors of the proteasome are sufficient to cause neurodegeneration and a Parkinsonian phenotype in vivo. Treatment of adult rats over a period of two weeks caused

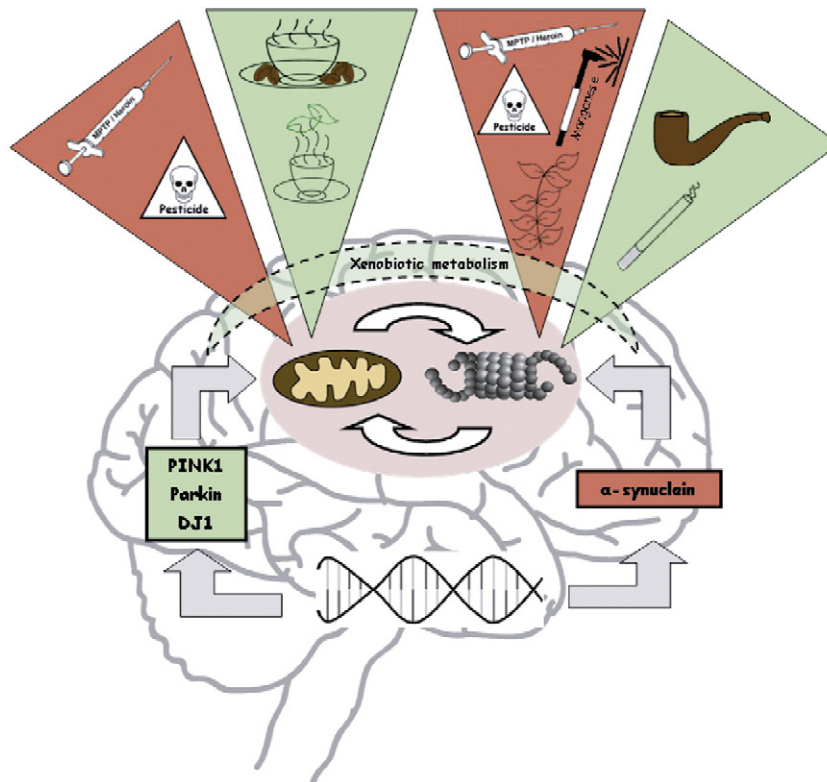


Fig. 2. Schematic view of the complex interplay of environmental and genetic factors in neurodegeneration in PD. Pathological protein aggregation due to impaired protein degradation and oxidative stress due to impaired mitochondrial function have been consistently shown to contribute to neurodegeneration in PD based on different genetic and toxic models. These pathways may be influenced by different protective (green) or toxic (red) environmental factors that themselves are modulated by the xenobiotic-detoxification machinery of the organism. Exposures that are known to inhibit the proteasome and propagate protein misfolding include MPTP, manganese, pesticides and certain naturally occurring plant extracts, i.e. Annonaceae. Possible protective factors in this regards include nicotine. Exposures which possibly lead to mitochondrial dysfunction are MPTP and pesticides, whereas green or black tea and caffeine may exert a protective effect. All environmental factors act on a specific genetic background that is determined by risk alleles in genes related to mitochondrial homeostasis or proteasomal function. In this context, mutations in the respective genes may be responsible for the loss of protective function (green) and cause mitochondrial impairment, whereas others are responsible for a toxic gain of function (red) that promotes proteasomal inhibition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

progressive parkinsonism with striatal dopamine depletion and dopaminergic cell death in the substantia nigra pars compacta [68]. In remaining neurons intracytoplasmic, alpha-synuclein/ubiquitin-containing inclusions were observed which resembled Lewy bodies and therefore reflected a key feature of PD. This clearly underscored that exogenous toxins were sufficient to cause parkinsonism.

Pesticides like paraquat and maneb were found to lead to proteasomal dysfunction and nitrative/oxidative damage causing an upregulation and subsequent fibrillization of recombinant alpha-synuclein [62,64]. MPTP, which is an established selective dopaminergic neurotoxin as described above, inhibits the mitochondrial complex I activity. This creates an environment of oxidative stress that drives to aggregation of alpha-synuclein and subsequent cell death of dopaminergic neurons [53]. It has been shown that mice lacking the gene for *SNCA* are resistant to the toxic effect of MPTP, hence alpha-synuclein may play a critical role in the pathogenesis of toxin-induced dopaminergic neuron injury [69].

In some industries, exposure to toxic fumes occurs, which can cause neurotoxicity and therefore seems to play a role in the development of a neurologic syndrome that resembles PD [65]. Workers, who were exposed to welding fumes containing manganese, a paramagnetic heavy metal that is widely distributed in the environment, have a higher risk to develop parkinsonism [70]. There is increasing evidence that manganese damages different areas of the brain, especially the basal ganglia including the globus pallidus and striatum, and leads to pathological features comparable with typical for PD. Most interestingly manganese-induced neurotoxicity was recently linked to molecular pathways

involved in pathological alpha-synuclein aggregation and therefore provides further evidence for the convergence of environmental and genetic factors in the pathogenesis of PD [71].

Other environmental toxins occur naturally in plants and may be responsible, under certain circumstances, for neurodegenerative syndromes. Such compounds include plants from the Annonaceae family that are ingested by isolated populations and were recently identified as responsible for neurodegeneration causing a Parkinsonian phenotype [63].

On the other hand, some environmental substances were found to decrease the incidence of PD, such as nicotine and hydroquinone. Although smoking is an established risk factor in terms of cancerogenesis, especially nicotine was reported to protect from PD [15]. Recently, a potential mechanism for the neuroprotective role of nicotine and hydroquinone by inhibiting the fibrillization of alpha-synuclein and stabilizing oligomeric structures has been described *in vitro* [72,73].

These examples show that certain environmental exposures in combination with genetic risk factors may enhance abnormal aggregation of alpha-synuclein and therefore may contribute to neurodegeneration in PD.

3. Mitochondrial dysfunction

3.1. Genetic factors

Some genes are known to cause an autosomal recessive form of PD due to loss of the physiological function of the respective encoded

proteins. There is increasing evidence for a crucial role of these genes (i.e. *PINK1*, *Parkin* and *DJ-1*) for mitochondrial homeostasis [74–78].

PINK1 (*PARK6*; OMIM #605909) is a putative serine/threonine kinase, which is expressed ubiquitously. Due to its N-terminal mitochondrial targeting sequence *PINK1* is localized to the mitochondrial intermembrane space and bound to mitochondrial membranes. As *PINK1* was also identified as a component of Lewy bodies in patients with sporadic PD [79], its final destination within mitochondria is not entirely known.

The suggested physiological role of *PINK1* in mitochondria comprises the phosphorylation of mitochondrial proteins in response to cellular stress and the protection against mitochondrial dysfunction.

Up to date, homozygous missense and nonsense mutations affecting the kinase domain as well as insertions and deletions leading to frameshifts and truncation of the peptide were observed, all of them putatively impairing the kinase function [80,81]. There is evidence that wildtype *PINK1* in contrast to mutant *PINK1* may protect neurons from stress-induced mitochondrial dysfunction and apoptosis [75]. Loss of *PINK1* function in human cell lines caused morphological abnormalities of mitochondria and impaired energy metabolism indicated by a reduced mitochondrial membrane potential [80]. Interestingly, this phenotype could be rescued by wild-type *Parkin*, another PD-related protein. This provided first evidence for a potential common signaling pathway formed by mitochondrially targeted proteins in the pathogenesis of PD [82].

The *Parkin* gene (*PARK2*; OMIM #602544) encodes an E3 ubiquitin protein ligase, whose activity seems to be compromised by pathogenic mutations [83]. Homozygous or compound heterozygous mutations in the *PARK2* gene are responsible for the majority of autosomal recessive early-onset PD cases [54,84]. Homozygous deletions as well as duplications, insertions, frameshifts and missense mutations in the *PARK2* gene are known to cause a loss of protein function leading to impaired mitochondrial integrity. It has been shown that *PARK2* knockout mice exhibit decreased level of proteins involved in protection from oxidative stress and deficits in mitochondrial function [76]. Studies in *Drosophila melanogaster* pointed out that *Parkin* mutants display impaired mitochondrial integrity due to interference with mitochondrial dynamics [85,86]. The loss of *Parkin* led to impaired mitochondrial clearance and accumulation of dysfunctional mitochondria that contribute to cell death [87,88].

Another important role in coping with oxidative stress and mitochondrial toxins has been attributed to *DJ-1* (*PARK7*; OMIM #606324), a multifunctional protein with antioxidant and transcription modulatory activity [89]. Upon basal conditions, *DJ-1* is mostly localized to cytosol, but under stress conditions this shifts towards mitochondria and nucleus [90]. Wildtype *DJ-1* has a role in the antioxidative stress reaction, whereas deletions and point mutations in the *DJ-1* gene cause a loss of its physiological function and therefore lead to neurodegeneration in rare families with autosomal recessive PD [91–93]. It was shown that *DJ-1* protects from mitochondrial damage and therefore acts as an oxidative stress sensor further conferring neuroprotection [77,78]. Very recently, a direct link between loss of *DJ-1*, an impaired mitochondrial stress response and a reduced clearance of mitochondria by lysosomal degradation was described. An accumulation of fragmented and dysfunctional mitochondria upon reduced basal autophagy contributes to the loss of function phenotype in cells from knockout mouse and human carriers of the E64D mutation in the *DJ-1* gene [94].

The characterization of different in vitro and in vivo loss of function models of the described PD-associated proteins *Parkin*, *PINK1* and *DJ-1* substantiated a critical role of impaired mitochondrial dynamics in PD by either shifting the dynamic system towards fusion or fission events (reviewed in [95]). Alterations of mitochondrial dynamics are closely linked to mitochondrial dysfunction and finally to the onset of autophagic or even apoptotic signaling cascades. For alpha-synuclein it has been shown by in vitro experiments that it has

an inhibitory function on membrane fusion due to its unique membrane interaction, hence shifting the dynamic morphologic equilibrium of mitochondria towards increased mitochondrial fragmentation [96]. Interestingly, this phenotype could be rescued by co-expression of physiological *PINK1*, *Parkin* or *DJ-1*, but not PD-associated mutants of these proteins.

Besides the established role of homozygous mutations in the *PARK2* or *PINK1* gene as a cause of autosomal recessively inherited early-onset PD, a role of heterozygous *PINK1* or *PARK2* mutations as a possible cause of the common sporadic form of PD is currently under debate [97–99]. Indeed genetic association studies suggested that heterozygous mutations in recessive genes like *PINK1* or *PARK2* may act as the risk factor for the development of late-onset PD [100]. Indeed asymptomatic mutation carriers already showed signs of dopaminergic dysfunction leading to the hypothesis of the mutation pathogenicity via haploinsufficiency [100]. This led to the conclusions that heterozygous loss-of-function mutations may act as genetic risk factors with reduced penetrance and therefore contribute to the risk of developing PD.

In summary, insight from autosomal-recessively inherited genes in PD revealed disturbed mitochondrial function and dynamics due to a loss of mitochondrial proteins resulting in cell death in affected brain regions of PD patients (Fig. 2). Abnormal function of the mitochondria results in an imbalance of fusion and fission processes, normally implicated in cell death regulation. Interestingly this affects the second common intracellular degradation pathway, macroautophagy, which like the UPS is not only able to degrade aggregated proteins, but also clears dysfunctional mitochondria from the cell. Impaired autophagy therefore seems to contribute to neurodegeneration observed in these recessive forms of PD.

4. Environmental factors

Mitochondrial dysfunction, which leads to oxidative stress and subsequent damage of biological molecules (lipids, DNA, and proteins), is involved in the onset of neurodegeneration. It was shown that exposure to environmental agents, such as pesticides and toxins may increase the risk to develop PD. Some of these environmental factors are supposed to act via disturbed mitochondrial complex I activity of the mitochondrial respiratory chain and therefore contribute to the death of dopaminergic neurons (Fig. 2).

In this context MPTP, a toxin that induces parkinsonism and shares structural similarities to the pesticide paraquat, is the best studied exposure in terms of selective dopaminergic nerve cell death. MPTP occurred during the incomplete synthesis of the drug MPPP and was intravenously taken by drug addicts causing severe and acute parkinsonism [8]. Based on subsequent in vitro and in vivo studies an inhibition of the mitochondrial complex I activity due to the active metabolite MPP⁺ was established that propagates the aggregation of alpha-synuclein and subsequent cell death of dopaminergic neurons by increased levels of oxidative stress [69].

A similar pathogenic mechanism was suggested for rotenone, a common naturally occurring botanical pesticide, which also acts as an inhibitor of mitochondrial complex I and leads to a failure of the mitochondrial energy supply of the cell. It was shown that chronic treatment with rotenone was sufficient to cause typical characteristics of PD including loss of dopaminergic neurons and alpha-synuclein-positive inclusions in rodents. These animals displayed motor impairments, i.e. hypokinesia and muscular rigidity that were typically observed in PD [101,102]. The involvement of oxidative damage caused by mitochondrial dysfunction after rotenone treatment was demonstrated in different in vitro models clearly indicating specific dopaminergic neuronal death by rotenone [103].

Further support of a role of exogenous toxins in the pathogenesis of PD came from epidemiological association studies in professionally exposed persons suggesting a dose-effective involvement of pesticides

in the pathogenesis of PD [13]. These data were supported by large cohort studies that established another link between pesticide exposures and the incidence of PD [104,105].

This association between environmental exposures and PD seems to be modulated by genetic factors, i.e. not all drug addicts that were exposed to MPTP developed a Parkinsonian syndrome indicating that the genetic background may be critical to cause neurodegeneration and express clinical symptoms. In this context the cellular detoxification machinery that is responsible for the metabolism of xenobiotics like pesticides came into focus. For example genetic variations in the debrisoquine hydroxylase in cytochrome P450 (CYP2D6) may be attributed to different metabolic activities, including poor metabolizers, which present with an undetectable activity of the enzyme [106]. Indeed the latter were found to have a two-fold increased risk to develop PD upon occupational exposure to pesticides compared to individuals presenting with normal enzymatic activity. Consistently, individuals carrying the poor metabolizer variant of CYP2D6 did not show increased PD risk in the absence of pesticide exposure [106].

In contrast to disease-promoting environmental factors, a number of natural antioxidants are thought to have a protective effect in terms of neurodegeneration [107]. Recent studies on coffee consumption revealed an inverse association between coffee drinking and PD. In this context the protective effect was supposed to be linked to the inhibition of adenosine A2 receptors by caffeine and correlated with total caffeine intake [108]. It has been further suggested that caffeine provides an anti-apoptotic function by activation of the phosphatidylinositol-3-kinase (PI-3K)–Akt pathway [109]. Concerning the potential protective role of black or green tea in the pathogenesis of PD, there is still some inconsistency among existing studies [107,110,111]. However, studies that suggest a beneficial effect of green tea are supported by *in vitro* studies providing evidence that particular components of green tea are associated with a dose-dependent reduction of the risk to develop PD [111,112]. This protective function implies cellular mechanisms including iron-chelation, scavenging of reactive oxygen species and activation of pro-survival signaling pathways. The activation of intracellular signaling pathways, such as MAPK, PKC and PI-3K pathways, has been described to play a central function in neuronal protection against a variety of extracellular insults [112].

5. Conclusion

Recent evidence from genetic whole genome association studies in PD underscored the role of genetic risk factors in the pathogenesis of the common sporadic forms of PD [28,113]. However, even these large-scale approaches that confirmed a role of *SNCA* gene as a risk factor for PD in the Asian and Caucasian population and pointed to a role of tau and *LRRK2* as additional risk factors in sporadic PD, did not define genetic causes for the majority of PD cases. This indicates that PD is a more complex multifactorial disorder and that future studies need to combine genetic whole genome approaches and detailed ascertainment of environmental factors to allow for the complexity of pathways leading to neurodegeneration in PD. Recent studies of genetic models of PD and mutation screenings in index patients with familial PD provide first insight into the complexity of converging mechanisms leading to protection from intrinsic and extrinsic stress. Based on a genetic model of *PINK1*- and *Parkin*-mediated neurodegeneration a critical role of the mammalian stress response by transcriptional regulation was assessed by the identification of 4E-BP as a critical modulator of neuronal cell death [114]. Interestingly the translation inhibitor 4E-BP was also identified as a substrate of mutant *LRRK2*, the most common genetic cause of PD [114]. Activity of 4E-BP is critically involved in the immediate response of cells to a wide variety of stress including oxidative stress and protein misfolding, both implicated in PD pathogenesis based on genetic and toxic insults as detailed above. The relevance of this pathway is further supported by the identification *eIF4G1* as a potential novel disease gene in PD

[115]. *eIF4G1* is involved in the regulation of stress response and loss of *eIF4G1* function has been observed to cause mitochondrial dysfunction and increased autophagy. Therefore a common point of convergence of environmental exposures (extrinsic) and genetic susceptibility factors (intrinsic) may emerge from these recent findings and further substantiate the relevance of large-scale studies combining genetic approaches with a detailed ascertainment of environmental exposures to provide insight into the complexity of neurodegeneration in most common sporadic form of PD.

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