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Invited critical review

# Candidate genes for Parkinson disease: Lessons from pathogenesis

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ABSTRACT

Parkinson disease (PD) is a multifactorial neurodegenerative disease characterized by the progressive loss of specific neuronal populations and accumulation of Lewy bodies in the brain, leading to motor and non-motor symptoms. In a small subset of patients, PD is dominantly or recessively inherited, while a number of susceptibility genetic loci have been identified through genome wide association studies. The discovery of genes mutated in PD and functional studies on their protein products have provided new insights into the molecular events leading to neurodegeneration, suggesting that few interconnected molecular pathways may be deranged in all forms of PD, triggering neuronal loss. Here, we summarize the most relevant findings implicating the main PD-related proteins in biological processes such as mitochondrial dysfunction, misfolded protein damage, alteration of cellular clearance systems, abnormal calcium handling and altered inflammatory response, which represent key targets for neuroprotection.



**Contents**



*Abbreviations:* α-syn, alpha-synuclein; ATF, Activating Transcription Factor; Atg9, Autophagy Related Protein 9; ATP13A2, ATPase Type 13A2; BIM, Binding Immunoglobulin Protein; Bip, Binding Immunoglobulin Protein; CHOP, CCAAT-enhancer-binding protein homologous protein; CMA, Chaperone-Mediated Autophagy; DRP1, Dynamin-Related Protein1; eIF2a, Eukaryotic translation Initiation Factor 2A; ER, Endoplasmic Reticulum; ERK, Extracellular signal-Regulated Kinase; Fbxo7, F-Box Only Protein 7; Fis1, Mitochondrial Fission 1 protein; GBA, Glucosidase Beta Acid; GBA, β-glucocerebrosidase; GRP, Glucose-Regulated Protein; GWA, genome wide association; HSC70, Heat-Shock Cognate 70; IL-1, Interleukin; iNOS, inducible Nitric Oxide Synthase; iPS, induced Pluripotent stem Cell; IP3R, Inositol 1,4,5-Trisphosphate Receptor; IRE1, Inositol-Requiring Enzyme 1; LAMP-2, Lysosome-Associated Membrane Protein 2; LBs, Lewy bodies; LC3, microtubule-associated protein Light Chain 3; LPS, Lipopolysaccharide; LRRK2, Leucine-rich repeat kinase 2; MAM, Mitochondria Associate Membranes; MFN1, Mitofusin 1; MFN2, Mitofusin 2; Miro, Mitochondrial Rho-GTPase; MPTP, 1-Methyl-4-Phenyl-1,2,3,6-Tetra hydro Pyridine; mtDNA, mitochondrial DNA; mTORC1, mammalian Target Of Rapamycin Complex 1; NF-kB, Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells; NGS, next generation sequencing; OMM, Outer Mitochondrial Membrane; OPA1, Optic Atrophy 1; Pael-R, Parkin-associated endothelin receptor-like Receptor; PARIS, Parkin-Interacting Substrate; PD, Parkinson disease; PERK, Protein kinase RNA-like Endoplasmic Reticulum Kinase; PGC-1α, Peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha; PINK1, PTEN-induced putative kinase 1; PUMA, p53-Up-regulated Modulator of Apoptosis; Rab, Ras-related protein; SCF, SKP1-Cullin-F-box; SNCA, alpha-synuclein gene; TNFα, Tumor Necrosis Factor α; Tom20, Translocase of Outer Membrane; TRAF6, TNF Receptor-Associated Factor 6; Ub, ubiquitin molecules; UCH-L1, Ubiquitin Carboxy-terminal Hydrolase L1; ULK1, Unc-51 Like autophagy activating Kinase 1; UPR, Unfolded Protein Response; UPS, ubiquitin–proteasome system; VDAC, Voltage-Dependent Anion Channels; VPS35, Vacuolar Protein Sorting-associated protein 35; XBP1, X-box binding protein 1.

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## 1. Introduction

Parkinson disease (PD) is one of the commonest neurodegenerative disorders, with prevalence over 1% in the seventh decade of life [1,2]. The phenotype of bradykinesia, resting tremor, rigidity and postural instability is mainly caused by the massive death of dopaminergic neurons in the substantia nigra pars compacta. Surviving neurons show the Lewy bodies (LBs), typical cytoplasmic inclusions containing ubiquitin, alpha-synuclein (α-syn) and other proteins. Despite familial aggregation had long been recognized as a common feature in PD, only in the past fifteen years the contribution of genetics has been deeply explored, with the identification of few genes clearly responsible for Mendelian forms of the disease, either with autosomal dominant (SNCA, LRRK2) or recessive (PARK2/Parkin, PINK1, DJ-1, ATP13A2) inheritance. Besides these, some other genes have been found mutated only in rare families, and their actual contribution to the disease remains to be confirmed [3].

However, it was recently estimated that mutations in known genes can only explain about 10% of familial PD, leaving a large proportion of cases unaccounted [4]. It is conceivable that in the vast majority of these patients, as well as in those with negative family history, the disease underlies a multifactorial inheritance, with several common and rare genetic variants interplaying among them and with environmental factors to reach a threshold of disease. In fact, heterozygous mutations in the GBA gene were found to represent a strong genetic risk factor for development of PD, with an average relative risk of about 5, and several polymorphisms (mostly residing within the same genes mutated in Mendelian forms of PD) were identified as PD susceptibility factors by large whole genome association (GWA) studies [3,4].

In line with this hypothesis, the advent of next generation sequencing (NGS) technologies, which have impressively enhanced the identification of novel causative genes in most genetic disorders, has not brought the expected revolution in the field of PD genetics. Indeed, despite NGS has been available for over 6 years, only few rarely mutated genes have been found in isolated families, and a single study has been published reporting whole exome sequencing in 100 Sardinian patients with PD, which failed to identify robust novel PD candidates [5].

While it can be safely expected that the huge amount of genetic data generated through NGS and GWA strategies contains a wealth of new relevant information to better understand the complex genetic basis of PD, nevertheless it is undoubted that the meaningful analysis of such data to extrapolate novel PD candidate genes represents a truly challenging task. A possible lead to get oriented in the maze of common and rare genetic variants emerging from genome-wide studies may derive from the analysis of pathogenic mechanisms of neurodegeneration. Intriguingly, it is clearly emerging that neuronal death results as a consequence of the derangement of very few essential and highly interconnected cellular pathways, and that many if not all genetic factors implicated in PD pathogenesis converge on, and affect, the same cellular processes, including mitochondrial dysfunction, misfolded protein damage, impairment of clearance systems, abnormal calcium handling and enhanced pro-inflammatory responses [6].

Here we will briefly review the involvement of various PD-related proteins in each of these pathways, which to date represent the key molecular targets for the development of neuroprotective and therapeutic strategies [7]. A schematic representation of some of these pathways is presented in Fig. 1.

## 2. Impairment of cellular clearance systems

In long-living cells such as neurons, the efficient functioning of intracellular clearance systems is crucial for an efficient and timely removal of both misfolded proteins and damaged organelles, before they could trigger noxious pathways eventually leading to apoptotic death. Two main quality control systems act in synergy: the ubiquitin–proteasome system (UPS), mainly aimed at removing misfolded proteins, and the autophagy pathways, which target both misfolded proteins and aggregates (through macroautophagy and chaperone-mediated autophagy), as well as dysfunctional organelles (through highly specialized pathways such as mitophagy). Here we will briefly discuss these systems, to demonstrate how PD-related proteins have been deeply involved in the correct functioning of both UPS and autophagy.

### 2.1. The ubiquitin–proteasome pathway

UPS is a proteolytic system in which the conjugation of polyubiquitin chains to specific substrates induces their selective degradation by the proteasome in an ATP-dependent manner. The ubiquitination steps are highly regulated and allow the binding of one or more ubiquitin molecules (Ub) to a lysine residue of the target protein, through the subsequent action of Ub activating enzymes (E1), Ub conjugating enzymes (E2), and substrate-specific Ub ligases (E3). Ubiquitinated proteins are then delivered by Ub receptors to the proteasome, where the substrate is de-ubiquitinated, unfolded, and degraded [8]. A malfunctioning of the UPS leads to accumulation of protein oligomers and aggregates, which play a well-established role in PD pathogenesis. This was first shown by experiments of proteasomal inhibition using both in vitro and in vivo models, which resulted in the formation of α-syn positive inclusions and neurodegeneration [9,10]. Moreover, proteasomal impairment was observed in the substantia nigra of brains from patients with PD [11]. Further substantiating a role for UPS in PD pathogenesis, several genes mutated in familiar PD have been implicated in this pathway, first of all α-syn. This is one of the most abundant brain proteins, which has the propensity to misfold and generate oligomeric and protofibrillar intermediates, causing widespread neuronal damage. Genetic mutations of the SNCA gene, overexpression of wild type protein due to gene multiplication, as well as post-translational modifications and oxidative stress, are all known to enhance misfolding and aggregation of the protein [12,13]. Indeed, α-syn is one of the main components of LBs, typical cytoplasmic inclusions found in the neurons of patients with both familial and sporadic PD, which have been suggested to represent a cytoprotective response to isolate toxic elements and reduce cellular damage [14].

Another relevant gene for UPS functioning is Parkin, the first gene to be identified as causative of autosomal recessive early onset PD, which encodes for an E3 ubiquitin-ligase [15]. Several putative substrates of Parkin have been identified, including proteins with toxic function for neuronal cells. For instance, Parkin was found to mediate the proteasomal degradation of Pael-R, a putative G-protein-coupled transmembrane protein known to induce ER stress and cell death, and PD-causative Parkin mutations led to accumulation of unfolded Pael-R, both in vitro and in the patients' brains [16,17]. Another relevant substrate of Parkin is PARIS, a transcriptional repressor of the mitochondrial co-activator PGC-1α which also accumulates in tissues from Parkin-mutated or sporadic PD patients, Parkin-knockout mice and MPTP intoxicated mice [18]. Mutations of Parkin cause an increase in PARIS levels, leading to mitochondrial dysfunction and progressive degeneration of dopaminergic neurons, a phenotype that can be reverted by PARIS silencing [18]. Last but not the least, α-syn was found to be a