NeuroToxicology 32 (2011) 622–629

Contents lists available at ScienceDirect

NeuroToxicology

# α-Synuclein, leucine-rich repeat kinase-2, and manganese in the pathogenesis of parkinson disease

Jason P. Covy, Benoit I. Giasson *

*Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084, USA*

ARTICLE INFO

*Article history:*
Received 25 October 2010
Accepted 7 January 2011
Available online 14 January 2011

*Keywords:*
Parkinson disease
Mutation
α-Synuclein
LRRK2
Manganese
Toxicity

ABSTRACT

Parkinson disease (PD) is the most common movement disorder. It is characterized by bradykinesia, postural instability, resting tremor, and rigidity associated with the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Another pathological hallmark of PD is the presence of α-synuclein proteiniacous inclusions, known as Lewy bodies and Lewy neurites, in some of the remaining dopaminergic neurons. Mounting evidence indicates that both genetic and environmental factors contribute to the etiology of PD. For example, genetic mutations (duplications, triplications or missense mutations) in the α-synuclein gene can lead to PD, but even in these patients, age-dependent physiological changes or environmental exposures appear to be involved in disease presentation. Several additional alterations in many other genes have been established to either cause or increase the risk of parkinson disease. More specifically, autosomal dominant missense mutations in the gene for *leucine-rich repeat kinase 2* (*LRRK2/PARK8*) are the most common known cause of PD. Recently it was shown that G2019S, the most common diseasing-causing mutant of LRRK2, has dramatic effects on the kinase activity of LRRK2: while activity of wild-type LRRK2 is inhibited by manganese, the G2019S mutation abrogates this inhibition. Based on the in vitro kinetic properties of LRRK2 in the presence of manganese, we proposed that LRRK2 may be a sensor of cytoplasmic manganese levels and that the G2019S mutant has lost this function. This finding, alongside a growing number of studies demonstrating an interaction between PD-associated proteins and manganese, suggest that dysregulation of neuronal manganese homeostasis over a lifetime can play an important role in the etiology of PD.



## 1. Parkinson disease

Parkinson disease (PD) is the most common movement disorder, affecting over 6 million people worldwide. PD can present with a juvenile or early onset, but it predominantly afflicts individuals over the age of 55 and the incidence of disease sharply rises after the age of 65 (Gelb et al., 1999; Moghal et al., 1994; Simuni and Hurtig, 2000). The clinical features of PD include bradykinesia, postural instability, resting tremor, and rigidity which are predominantly associated with the progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta (Cronford et al., 1995; Forman et al., 2005; Forno, 1996). It is believed that during normal aging approximately 0.1–0.2% of the dopaminergic neurons in this area are lost per year, but this rate is greatly accelerated in patients with PD and symptoms manifests when ~70–80% of these neurons have been lost (Calne and Peppard, 1987; Damier et al., 1999; Pakkenberg et al., 1991; Uversky, 2004). Another pathological hallmark of PD is the presence of α-synuclein proteiniacous inclusions, known as Lewy bodies (LBs) and Lewy neurites (LNs), in some of the remaining dopaminergic neurons (Cronford et al., 1995; Forman et al., 2005; Forno, 1996). Importantly, the loss of neurons in PD is not exclusive to nigral dopaminergic neurons as several other neuronal populations are also affected (Braak et al., 2006; Cronford et al., 1995; Forman et al., 2005; Forno, 1996).

Although the majority of PD cases are idiopathic, ~10% of cases report with a family history, and a growing number of mutations have been associated with familial and sporadic forms of the disease (see Table 1) (Lesage and Brice, 2009; Westerlund et al., 2010). In addition to genetic defects, a range of environmental and occupational factors, such as pesticides like paraquat, to more ubiquitous metals such as manganese, have been implicated as risk factors in PD (Dick et al., 2007; Elbaz et al., 2009; Lai et al., 2002). While there are some cases where a single environmental or monogenetic factor may lead to PD (MPTP poisoning or triplication of α-synuclein, respectively) (Farrer et al., 2004; Przedborski et al., 2001; Singleton et al., 2003), it is more likely that a subtle yet complex interplay exists between genetic and environmental

* Corresponding author at: Department of Pharmacology, University of Pennsylvania School of Medicine, 125 John Morgan Building, 3620 Hamilton Walk, Philadelphia, PA 19104-6084, USA. Tel.: +1 215 573 6012; fax: +1 215 573 2236.
*E-mail address:* giassonb@mail.med.upenn.edu (B.I. Giasson).

0161-813X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2011.01.003

**Table 1**
Genes that can directly lead to parkinson disease. Genetic loci are listed alongside their chromosomal mapping, gene, protein product, putative protein function, and mode of genetic inheritance. AD: autosomal-dominant, AR: autosomal-recessive.

| Locus (gene) | Map position | Protein | Putative function(s) | Inheritance pattern |
|---|---|---|---|---|
| PARK1/PARK4 (SNCA) | 4q21 | α-Synuclein | Presynaptic protein, neurotransmission, chaperone vesicle recycling | AD |
| PARK2 | 6q25–q27 | Parkin | Ubiquitin E3 ligase, mitophagy, neuroprotective | AR/juvenile and early onset |
| PARK5 | 4p14 | Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1) | Ubiquitin hydrolase | AD |
| PARK6 | 1p35–p36 | PTEN-induced putative kinase 1(PINK-1) | Mitochondrial S/T-protein kinase, mitophagy neuroprotective | AR/early onset |
| PARK7 | 1p36 | DJ-1 | Chaperone, antioxidant, RNA binding, neuroprotective | AR/early onset |
| PARK8 | 12q12 | Leucine-rich repeat kinase 2 (LRRK2) | Protein kinase | AD |
| PARK9 | 1p36 | ATP13A2 | Lysosomal ATPase probable cation-transporting | AR/juvenile and early onset |
| PARK14 | 22q13.1 | PLA2G6/85 kDa calcium-independent phospholipase A2 | Catalyzes fatty acids release from phospholipids | AR/juvenile onet |

factors in the etiology of disease. For example, the autosomal dominant G2019S mutation in leucine-rich repeat kinase 2 (LRRK2) is the most common known cause of familial and sporadic patients with PD, yet its penetrance is age-dependent, and some individuals may never be afflicted (Goldwurm et al., 2007; Kachergus et al., 2005). The manifestation of such a predominant mutation as a late onset disorder, where it is still not fully penetrant, suggests that genetic defects may serve to predispose individuals to certain environmental challenges.

## 2. The role of α-synuclein in PD

Although LBs and LNs were originally observed ~100 years ago in the brain of PD patients, it was not until 1997 following the discovery of a PD kindred with a point mutation in the gene for α-synuclein (SNCA) that its presence in these proteinaceous inclusions was examined (Polymeropoulos et al., 1997; Spillantini et al., 1997). It is now accepted that α-synuclein filaments are the major ultrastructural component of these cytoplasmic pathological inclusions, which can be observed in a spectrum of neurodegenerative diseases termed "synucleinopathies" (Forman et al., 2005; Goedert, 2001; Spillantini et al., 1997).

α-Synuclein is a highly charged 140-amino acid heat stable protein that is soluble and natively "unfolded" (Davidson et al., 1998; El-Agnaf et al., 1998; Weinreb et al., 1996). It is predominantly expressed in neurons of the central nervous system (CNS), where it localizes to presynaptic terminals in close proximity to synaptic vesicles (George et al., 1995; Jakes et al., 1994; Withers et al., 1997). Although the function of α-synuclein is still poorly understood, several studies suggest that it is involved in modulating synaptic transmission and neuronal plasticity (Abeliovich et al., 2000; Cabin et al., 2002; Iwai et al., 1995; Murphy et al., 2000; Withers et al., 1997; Greten-Harrison et al., 2010), as well as providing support in the assembly and folding/refolding of SNARE proteins critical for neurotransmitter release, vesicle recycling, and synaptic integrity (Chandra et al., 2005; Burre et al., 2010).

In addition to the first missense mutation (A53T) that was identified in α-synuclein, two additional disease-causing missense mutations were found: an A30P mutation in a German family (Kruger et al., 1998), and an E46K mutation in a Spanish family (Zarranz et al., 2004). In vitro, α-synuclein can readily form fibrils similar to those seen in LBs (Conway et al., 1998; Giasson et al., 1999; Wood et al., 1999) and the A53T and the E46K mutations can both increase this rate of fibril formation (Conway et al., 1998; Giasson et al., 1999; Greenbaum et al., 2005) suggesting a link between α-synuclein aggregation and disease. This link was strengthened with the identification of several PD kindreds with triplication (Farrer et al., 2004; Singleton et al., 2003) or duplication of the α-synuclein gene (Chartier-Harlin et al., 2004). In vitro, increased concentrations of α-synuclein have been shown to promote the polymerization of α-synuclein into fibrils (Wood et al., 1999), and patients with a gene triplication have greater disease severity and younger age of onset than those with gene duplication, suggesting a possible "*SNCA* gene dosage effect" leading to PD (Singleton and Gwinn-Hardy, 2004). Furthermore, these findings indicate that a 50% increase in the expression of α-synuclein due to gene duplication is sufficient to cause disease and is consistent with the aggregation of α-synuclein contributing to disease.

The polymerization of α-synuclein from unstructured monomer to mature amyloid fibrils proceeds through the formation of partially folded intermediates and several altered-sized oligomers (Conway et al., 2000; Uversky et al., 2001a). Several of these intermediates (as well as products that may not culminant into fibrils) have been described as spheres (2–6 nm in size), chains of spheres (also termed protofibrils), and rings resembling circular protofibrils (also termed annular protofibrils) (Conway et al., 2000; Ding et al., 2002; Goldberg and Lansbury, 2000). Some investigations suggest that protofibrils or some form of α-synuclein oligomers may increase membrane permeability leading directly to cell death [reviewed in (Waxman and Giasson, 2009)], or indirectly injuring cells by α-synuclein extracellular activation of microglia, and subsequent induction of proinflammatory responses and generation of ROS (Gao et al., 2008; Lee et al., 2010b; Su et al., 2009; Theodore et al., 2008). However, there is also substantial evidence, especially using transgenic α-synuclein mice, supporting the toxic nature of mature α-synuclein inclusions that can behave as "sieves", which can trap other macromolecules and perturb cellular homeostasis, axonal transport, and synaptic transmission [reviewed in (Waxman and Giasson, 2009)]. Aside from the sequestering of other proteins, the loss of synuclein function in and of itself may have relevance towards disease progression, as studies of genetically altered mice null for α-, β, and γ-synuclein demonstrated age-dependent neuronal dysfunction and alterations in synaptic structure and transmission, and revealed that continuous presynaptic SNARE-complex assembly required a nonclassical chaperone activity mediated by synuclein proteins (Burre et al., 2010; Greten-Harrison et al., 2010). Importantly, the proposed mechanisms of α-synuclein toxicity are not mutually exclusive, and several forms of aberrant α-synuclein aggregates may lead to neuronal demise.