



α -Synuclein in Parkinson's disease: causal or bystander?

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Abstract

Parkinson's disease (PD) comprises a spectrum of disorders with differing subtypes, the vast majority of which share Lewy bodies (LB) as a characteristic pathological hallmark. The process(es) underlying LB generation and its causal trigger molecules are not yet fully understood. α -Synuclein (α -syn) is a major component of LB and *SNCA* gene missense mutations or duplications/triplications are causal for rare hereditary forms of PD. As typical sporadic PD is associated with LB pathology, a factor of major importance is the study of the α -syn protein and its pathology. α -Syn pathology is, however, also evident in multiple system atrophy (MSA) and Lewy body disease (LBD), making it non-specific for PD. In addition, there is an overlap of these α -synucleinopathies with other protein-misfolding diseases. It has been proven that α -syn, phosphorylated tau protein ($p\tau$), amyloid beta ($A\beta$) and other proteins show synergistic effects in the underlying pathogenic mechanisms. Multiple cell death mechanisms can induce pathological protein-cascades, but this can also be a reverse process. This holds true for the early phases of the disease process and especially for the progression of PD. In conclusion, while rare *SNCA* gene mutations are causal for a minority of familial PD patients, in sporadic PD (where common *SNCA* polymorphisms are the most consistent genetic risk factor across populations worldwide, accounting for 95% of PD patients) α -syn pathology is an important feature. Conversely, with regard to the etiopathogenesis of α -synucleinopathies PD, MSA and LBD, α -syn is rather a bystander contributing to multiple neurodegenerative processes, which overlap in their composition and individual strength. Therapeutic developments aiming to impact on α -syn pathology should take this fact into consideration.

Keywords Parkinson's disease · α -Synuclein · Synucleinopathy · Gene expression · Protein interactions · Neuromelanin · *SNCA* gene · Neuroinflammation · Therapy · Autophagy · Proteasome · Lysosome

Introduction

Parkinson's disease (PD) is the second most frequent chronic neurodegenerative disease worldwide. PD is clinically characterized by bradykinesia and rigidity, often accompanied by postural instability and resting tremor. It is assumed that most cases have a multifactorial pathogenesis, and numerous genetic and environmental risk factors have been identified (Sian-Hulsmann et al. 2011; Nalls et al. 2014; Kalia and Lang 2015). Its incidence ranges from 8 to 18 per 100,000 persons each year. There are estimations which propose a twofold rise of PD prevalence within the next 20 years

(Dorsey et al. 2018). Onset of PD is rare under the age of 50 years and considerably rises after the age of 60 years. The exact cause of PD is not well understood. The term PD actually reflects a super ordinate concept for a disease entity consisting of different subtypes (Przuntek et al. 2004). A certain clinical and neuropathological overlap exists between each of them. However, not all these various PD forms share the neuropathological inaugurated concept of an increased presence of Lewy bodies (LB) in the substantia nigra (SN) as an essential pathological hallmark (Weiner 2008). Presumably, LB generation results from mutated α -synuclein (α -syn), elevated α -syn levels in the brain and/or post-translational modified α -syn, which becomes misfolded and subsequently forms protein aggregates such as oligomers and fibrils. These structures impair nerve cell communication and may spread to healthy neurons (Burke et al. 2008; Sian-Hulsmann et al. 2015). Consequences of LB formation

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(Fig. 1) are increased oxidative stress, disruption of axonal transport for instance of neurotransmitter vesicles, transcriptional dysregulation, protein sequestration, mitochondrial and synaptic dysfunction, inhibition of the ubiquitin/proteasome system and altered lysosomal clearance via autophagy (Foley and Riederer 1999; Burke et al. 2008; Sian-Hulsmann et al. 2015). Formation of LB is observed in the majority of PD patients, including more than 90% of sporadic PD cases. To date, it is far from clear whether these abnormalities represent a specific process responsible for triggering the early onset of PD. They may also result from an unspecific side reaction of the pathological event cascade responsible for cell death and neurodegeneration (Sian-Hulsmann et al. 2015). Neurochemically, motor symptoms of PD are mainly characterized by a loss of dopamine-synthesizing neurons as well as dopaminergic transporters, mainly in the SN (Uhl 1998). Additionally, chronic neuronal depletion also occurs in other neurotransmitter systems both in the periphery and the brain (Jellinger 2010). Thus, various neuronal cell types degenerate in an individually different and pronounced manner in various brain regions. This phenomenon contributes to the clinical heterogeneity of PD (Przuntek et al. 2004). It

is under debate whether this process of slow neuronal dying follows a certain pattern, for instance starting in the gastrointestinal tract, ascending over the brainstem with further spread to other regions of the brain (Brooks 2010; Braak and Del Tredici 2017; Rietdijk et al. 2017; Braak et al. 2003a, b; Jellinger 2010). Other authors meet this theory with criticism (Jellinger 2019).

Monogenic gene carriers for familial forms of PD have been identified. They only account for about 5–10% of PD patients. Up to date, a total of 23 loci and 19 causative genes have been associated with PD (Lunati et al. 2018; Del Rey et al. 2018). The corresponding genetic animal models of PD did not develop the typical motor PD symptoms of dopaminergic neurodegeneration and pathological SN protein aggregation during aging. Therefore, manifestation of PD probably results from so far unidentified environmental triggers acting on genetically predisposed individuals, possibly in combination with other predisposing and/or exacerbating risk factors, for example pesticide exposure with impaired detoxification mechanisms. Mostly, these investigations regarding environmental epigenetic influences, chronic exposure to toxins and perviously unknown

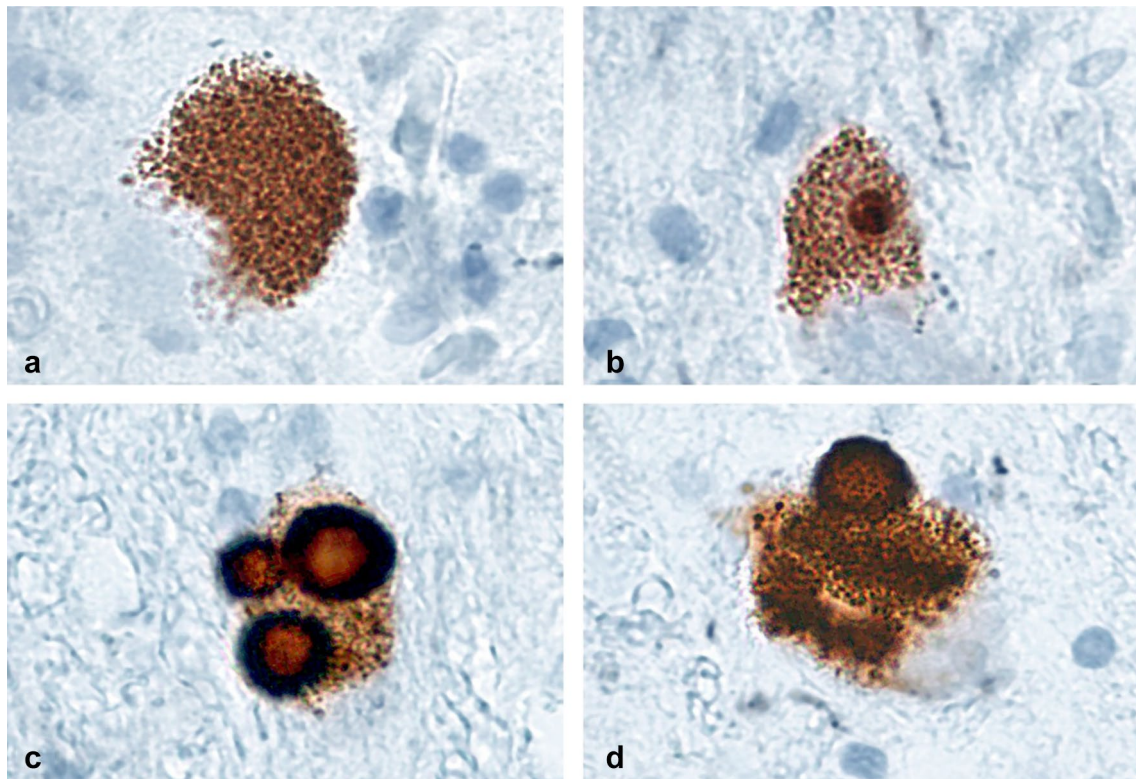


Fig. 1 **a** Normal neuromelanin-containing neuron of midbrain (substantia nigra). **b** Early stage of alpha-synuclein aggregation (Lewy body) in midbrain neuron. **c** Late stage of Lewy body with adhesion to the inner neuronal membrane (midbrain). **d** Lewy body already leaving the neuromelanin-containing neuron (midbrain) (in part from

Sian-Hulsmann et al. (2015)). Immunohistochemical staining with a monoclonal primary antibody directed against alpha-synuclein (Novocastra/NCL-ASYN, clone KM51, dilution 1:1000). Please note the intense staining of the Lewy body halo

infections did not take into consideration the various, yet not well-characterized subtypes of PD (Alonso-Navarro et al. 2014; Ahmed et al. 2017; Foley and Riederer 2000). Nevertheless animal models of PD, i.e., based on toxin exposure, were developed (Gotz et al. 2004; Jiang and Dickson 2018; Trigo-Damas et al. 2018; Bringmann et al. 1995). They show motoric symptoms of PD. However, the in vitro and most of the in vivo PD-mimicking models do not reflect the chronic progression of slow, sometimes intermittent neuronal death. In conclusion, the pathogenesis of sporadic PD forms is still not known. Manifestation of the disease is probably the consequence of several complementary mechanisms leading to onset of PD as a common end point. This individually different acceleration of neuronal dying is of multifactorial origin, its progression shows no uniform pattern and it can be discontinuous with relapses or constant. The majority of the at-risk population and PD patients themselves tend to avoid early genetic investigations or premotor diagnosis, since a disease-modifying therapeutic approach is not available.

In a clinical setting, heterogeneous onset and a combination of the main motoric symptoms of rigidity, akinesia and resting tremor are looked upon as the main diagnostic features. In clinical practice, a diagnosis is usually made when a substantial number of dopaminergic neurons and their terminals in the SN are lost and motoric symptoms appear. Axial symptoms such as gait (freezing) and balance problems mostly occur later in the course of PD. A wide array of initially unspecific non-motoric symptoms can precede the initially often temporary onset of motoric symptoms against the preexisting personality structure, which may influence the initial awareness (Poewe et al. 1990). Focus on initial non-motor signs of PD and initiatives for earlier detection of PD have gained significant interest to better characterize this so-called “prodromal” or “premotor” interval of PD. To date, no reliable and specific biomarker for an earlier detection of PD has been found. Notable techniques include functional imaging methods for visualization of the emerging dopaminergic deficit. Molecular probes to detect misfolded α -syn and the tracking of its accumulation for potential assessment of PD progression have not yet reached the clinical routine (Berg et al. 2013).

Basic pathology/neurochemistry

Intracellular inclusion bodies in post-mortem brain tissue from sporadic paralysis agitans (PD) patients were first described by Friedrich H. Lewy in 1912. In 1919, at the Salpêtrière, Constantin Trétiakoff reported pathological inclusions that he named ‘corps de Lewy’ in the SN in PD (Holdorff et al. 2013; Engelhardt 2017). Initial studies based on histochemical methods to visualize LB and later on

ultra-structural approaches revealed the presence of organized filaments within LB.

However, Lewy pathology is not restricted to the brain and can also be found in the spinal cord and peripheral nervous system. According to Braak's theory, Lewy pathology starts in the peripheral nervous system and progresses through six different stages within the central nervous system in a caudal-to-rostral direction, correlating with disease progression in PD (Braak et al. 2003a). In the Braak-staging classification, α -syn pathology is not found in the SN region of the midbrain until stage 3, suggesting that early pre-motoric symptoms in PD are caused by Lewy pathology mainly found in the enteric nervous system, olfactory system and brain stem including medulla and pons. However, further studies are needed to explicitly confirm Braak's staging classification as well as the α -syn spreading theory (Dickson 2012).

Another distinct characteristic of PD pathology is the specific loss of dopaminergic (DA) neurons within the SN pars compacta. It has been shown that the severity of PD motoric manifestations correlates with the reduction of DA neurons and thus striatal dopamine levels. Motoric symptoms in PD respond well to dopamine replacement therapy, which is still one of the standard therapies in PD (Fahn 2003). However, it is worth mentioning that by the time motoric symptoms are pronounced and PD is clinically diagnosed, already 50–60% of DA neurons are lost, resulting in an 80% reduction of striatal dopamine (Pavese and Brooks 2009; Bernheimer et al. 1973). These data have been replaced by the notion that at that time only about 30% of dopaminergic neurons but 50–60% of their terminals have been lost (Cheng et al. 2010). Furthermore, it must be kept in mind that the recent standard therapies—including L-dopa, dopamine agonists, monoamine oxidase-B-inhibitors and deep-brain stimulation—are not curative, since they do not stop the progressive loss of DA neurons. For the development of novel therapeutic targets, a comprehensive understanding of the molecular causes of PD is needed. Although α -syn aggregation has been proposed to have a causal role in the death of DA neurons, its exact relationship to neuronal loss has not been sufficiently elucidated to date. One important aspect in this regard is a better understanding of the impact of aggregated α -syn, which—under physiological conditions—is localized at the presynaptic terminal, promoting synaptic-vesicle fusion and trafficking on cellular pathways. Once formation of oligomers and fibrils result in insoluble forms comprising beta-sheets, an even faster aggregation can be triggered at a lower pH—as found in lysosomal compartments. However, it is not only an ineffective clearance by lysosomal or ubiquitin proteasome systems, but also oxidative stress, which is related to an increased tissue iron content in the SN in PD, further enhancing formation of α -syn aggregates. So far, the cause of the increased tissue iron content in PD is

not entirely understood, although it has been described that multiple iron pathways in the brain may play a role (Berg et al. 2001; Sian-Hulsmann et al. 2011; Zecca et al. 2004a; Ndayisaba et al. 2019; Foley and Riederer 2000; Wypijewska et al. 2010), with iron seeming to contribute to the aggregation of α -syn.

Large α -syn aggregates have been shown to interfere with synaptic vesicle motility and organelle axonal transport. Moreover, they disturb neurotransmitter release and contribute to mitochondrial, endoplasmic reticulum and Golgi dysfunction. Aggregated α -syn binds to histone complexes, reduces histone acetylation and causes mitochondrial and synaptic dysfunction (Lardenoije et al. 2015).

Importantly, the normal clearance mechanisms, including autophagy, which degrade damaged organelles and proteins, are also affected by α -syn overexpression and aggregates, likely resulting in a vicious circle perpetuating the toxic effects of the aggregates (Wong and Krainc 2017).

Underscoring these severe effects of α -syn aggregates on cellular function, further valuable information has recently come from genome sequencing as well as genome-wide association studies (GWAS) of PD patients. Sequencing results indicate that not only mutated or excess α -syn, but also genes related to mitochondrial oxidative stress, cellular trafficking and lysosomal function are involved in PD pathology (Chang et al. 2017).

Interestingly, loss-of-function mutations in one allele of the lysosomal enzyme β -glucocerebrosidase (GBA1) have been proven to be a significant risk factor in PD (Sidransky et al. 2009). Moreover, recent data indicate that the lysosomal link in PD extends beyond GBA1 with novel gene variants found in the autophagic-lysosomal pathways, including various lysosomal enzymes and lysosomal membrane proteins and transporters (Chang et al. 2017). Also, from a biochemical point of view, there is a strong relationship between lysosomal function and α -syn aggregation. Moreover, oligomeric α -syn conformers have been shown to negatively influence lysosomal pathways, for instance by blocking trafficking of lysosomal enzymes (e.g., GBA1), leading to a bidirectional pathogenic loop (Mazzulli et al. 2011). To fully understand underlying disease mechanisms in PD, molecular and structural details of α -syn aggregation have to be further deciphered. Typically, α -syn is a natively unfolded protein in aqueous solution, but is able to adopt oligomeric and fibrillar conformations under certain conditions [such as mutations in the α -syn gene (SNCA), lipid storage, oxidative stress and post-translational modifications]. Multiple studies have shown that glycosphingolipids metabolites, like the GBA1-substrate glucosylceramide (GluCer), can interact and induce α -syn aggregation. This is thought to potentially happen via a conversion of physiological α -syn oligomeric forms into neurotoxic oligomers, that, moreover, are able to seed amyloid fibril formation (Zunke et al. 2018) (Fig. 2).

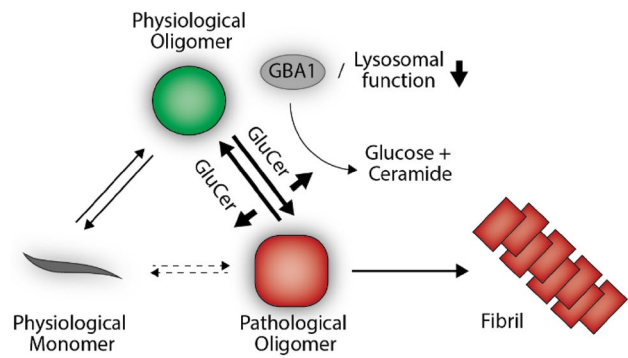


Fig. 2 The potential role of lysosomal function and metabolites on α -syn aggregation. Physiological α -syn can be found in monomeric as well as in oligomeric form in healthy human DA neurons. Lysosomal dysfunction and/or impairment in GBA1 activity leads to an increase in glucosylceramide (GluCer). Interaction of GluCer with α -syn oligomers leads to the formation of neurotoxic oligomers, which further accelerate fibril formation (Zunke et al. 2018)

Although genetic and mechanistic data strengthen the consequences of excess α -syn and its aggregation on a variety of cell functions in PD pathology, the full picture of molecular causes leading to the progressive loss of DA neurons is very complex and might be a reciprocal interplay of many different intra- and intercellular pathways.

Neuromelanin

Neuromelanin (NM) is an insoluble, granular pigment, chemically a complex organic polymer, which is responsible for giving certain parts of the brain a macroscopically dark appearance. It can be found in the brain in large concentrations principally in dopaminergic neurons of the SN and the ventral tegmental area, as well as in noradrenergic neurons of locus coeruleus, of the ventrolateral reticular formation and of the nucleus of the solitary tract in the medulla oblongata (Zecca et al. 2004a). Since the anatomical distribution of NM largely matches the pattern of neuronal degeneration in PD, it has long been hypothesized that it may be involved in the pathogenesis of PD. Surprisingly, the precise biochemical pathway leading to NM formation remains unknown. In dopamine (DA)-containing neurons, NM synthesis appears to result from iron-mediated oxidation of DA (Double et al. 2000; Jellinger and Paulus 1992; Gerlach et al. 2008), which leads to reaction with beta-sheet proteins (Zucca et al. 2017). It has been suggested that NM plays a role in the regulation of cytosolic dopamine by sequestering dopamine or its adducts in autophagic vacuoles/lysosomes (Fedorow et al. 2005).

NM has a strong affinity for transition metals such as iron (Zecca et al. 1994). Under physiological concentrations, iron may be cleaved in its redox-active form from the cytosol

(Gotz et al. 2004; Gerlach et al. 2008). In the presence of iron overload, however, iron may be bound by a low-affinity site of NM where it could promote harmful redox reactions. Iron released from NM increases oxidative stress in mitochondria (Shamoto-Nagai et al. 2006). NM itself also has antioxidant properties and can bind to organic molecules, including those that have been linked with PD etiology (Karlsson and Lindquist 2016).

Retention of potentially toxic agents may, on the one hand, protect NM-containing neurons. On the other hand, such intracellular depots may slowly release accumulated compounds and cause cellular toxicity. Degeneration of DA neurons in PD is accompanied by the release of NM into the extracellular compartment. Subsequent phagocytosis of NM by microglia gives rise to neuroinflammation. Along with concomitant release of previously cleaved metals and toxic compounds from NM, neuroinflammation may trigger a vicious cycle, resulting in accelerated neurodegeneration.

The issues discussed significantly point to NM as a potential contributor to the Parkinsonian pathogenesis. An intriguing question is whether and how pathogenic pathways connected to NM converge with other cardinal features of Parkinsonian pathogenesis. As α -syn is regarded at the top of LB pathology as the histological hallmark in sporadic as well as in some monogenetic form of Parkinsonism, the question of whether and how NM is connected with accumulation of misfolded α -syn is of special interest.

The interaction of NM and α -syn in PD has recently been reviewed by Xu and Chan (Xu and Chan 2015). α -Syn is expressed in the SN and especially in neurons containing NM (Purisasi et al. 2005). α -Syn increases with age (Xuan et al. 2011; Chu and Kordower 2007). This is associated with NM accumulation (Xuan et al. 2011) along with nigrostriatal dopamine depletion (Chu and Kordower 2007). Reasons for this might include cross-reactions of α -syn with NM (Fasano et al. 2003; Halliday et al. 2005). Here, NM is able to enhance the toxicity of α -syn (Li et al. 2012) as are early oxidation products of dopamine (Rekas et al. 2010; Bisaglia et al. 2007). Mechanisms of toxicity seem to include components of α -syn, NM, OS, iron and dopamine/dopamine oxidation products (Mandel et al. 2004).

Moreover, recent molecular genetic, biochemical and immunopathological studies in human specimens and animal models have implicated dysfunctional protein processing in the endosomal-lysosomal pathway as a unifying theme in the pathogenesis of PD (Perrett et al. 2015). Endosomes are critical hubs for the re-use, breakdown, trafficking of proteins and their extracellular release in exosomes. α -Syn overexpression and aggregation may disrupt endosomal and lysosomal function via multiple mechanisms (Perrett et al. 2015). NM granules are present in lysosomes of dopaminergic neurons (Tribl et al. 2006; Plum et al. 2016), and NM deposition is associated with α -syn accumulation in

aging neurons (Xuan et al. 2011). According to one hypothesis, NM deposition in dopaminergic neurons may lead to α -syn accumulation by enhancing their susceptibility to stochastic molecular defects in the endosomal-lysosomal pathway (Perrett et al. 2015). Lysosomal dysfunction may subsequently lead to deficits in mitophagy and the buildup of dysfunctional mitochondria (Gegg and Schapira 2016). Hence, NM may mediate neuronal vulnerability in PD via induction of α -syn expression and aggregation (Xu and Chan 2015). Conversely, α -syn also facilitates biosynthesis of NM, presumably by increasing the levels of cytosolic dopamine (Pan et al. 2012). Dopaminergic neurons derived from PD patients displayed mitochondrial oxidant stress that led to oxidized dopamine accumulation and ultimately resulted in lysosomal dysfunction and α -syn accumulation (Burbulla et al. 2017). According to this scenario, another vicious cycle between NM formation, α -syn aggregation, endo-lysosomal dysfunction and deficient mitophagy may develop. Higher concentrations of dopamine in human SN neurons, compared to other species, may explain these neurons' enhanced vulnerability as a consequence of enhanced NM formation (Zecca et al. 2008; Carballo-Carbajal et al. 2019; Aimi and McGeer 1996).

NM is composed of brown/black eumelanin and yellow/reddish pheomelanin that are arranged in a pheomelanin core and a eumelanin surface (Bush et al. 2006). Despite different biochemical synthesis, peripheral melanin contains the same two different melanin species as NM. The relationship of the two species appears to determine an individual's pigmentation trait of skin and hair, a known modulator of the risk of developing PD. Ultrasound sonography of the midbrain region in healthy individuals revealed that a lighter skin phototype was associated with a larger echogenic SN area and increased prevalence of an abnormally enlarged echogenic SN area (Rumpf et al. 2015). Since the iron-binding capacity of pheomelanin exceeds that of eumelanin, vulnerability of dopaminergic SN neurons toward oxidative stress may be enhanced with higher concentrations of pheomelanin in individuals with a lighter phototype. Although the substrate of an expanded SN echogenic area is not known, it is regarded as a biomarker of PD. Therefore, finding enhanced prevalence of enhanced SN echogenicity in light hair phototypes may provide evidence linking pigmentation to the pathogenesis of PD. However, the nature of this link and its relationship to other elements of the molecular pathogenesis of PD remains speculative (Rumpf et al. 2015).

Since NM is a scavenger of paramagnetic metals, regions rich in NM appear hyperintense in MR T1-fast spin echo weighted imaging. Using NM-sensitive sequences, topographically specific reductions of the NM-signal have been demonstrated in patients with Parkinsonian disorders (Pavese and Tai 2018). Interestingly, the NM MR signal seems to be distinct from that of iron in T2* relaxometry

of SN pars compacta, in that the former, but not the latter, is correlated to striatal dopamine function (Reimao et al. 2015). This finding suggests that both pathological pathways may be distinct at least at the time when the disease is clinically apparent.

Neuroinflammation

Another feature of PD pathology is neuroinflammation, which might also play a pivotal role in disease pathogenesis, since chronic microglial activation, potentially mediated by α -syn might contribute to the death of DA neurons (Sanchez-Guajardo et al. 2013).

In PD degeneration of dopaminergic neurons can be found, which is expedited by the risk factor of age and can be triggered by as yet unidentified factors. Until the first publication by McGeer et al. (1988) neurodegenerative disorders had rarely been associated with inflammatory processes in the brain (Eikelenboom and Stam 1982; Fischer 1907). In these seminal publications, an increase in the number of signs of neuroinflammation was described in the microglia of post-mortem brains. Neuroinflammatory processes contributing to the loss of dopaminergic neurons include activated microglia, reactive astrocytes and the release of cytokines and chemokines, component activation and reactive oxygen species. A number of authors were able to demonstrate that levels of pro-inflammatory mediators such as TNF- α , ILB, IL6, iNOS and COX2 in the SN are increased (Tansey et al. 2007; Mogi et al. 1994, 1995, 1996). It has been speculated that due to these neuroinflammatory processes the blood–brain barrier becomes permeable. Recently, substantial contribution has been added to our knowledge of neuroinflammatory changes in Parkinson patients (Hirsch et al. 1998, 2012). The relevance of T17 cells has gained special attention (Sommer et al. 2018).

In the meantime, we have sufficient evidence that there is a neuroinflammatory component in PD (Hirsch et al. 2012). However, neuroinflammation might be of low specificity since it has also been demonstrated in various psychiatric and neurological diseases such as for example Alzheimer's disease, depression, schizophrenia and, again, in PD. There is, however, still considerable debate on the relevance of these neuroinflammatory changes for necrosis in the dopaminergic neurons of the SN. Zecca et al. demonstrated that the injection of neuromelanin induced acute microglia activation (Zecca et al. 2008). But in spite of the many indications for neuroinflammatory changes in the brains of Parkinson's patients, the situation is similar to that of the status of α -syn in that it is still unresolved whether we have pathogenetic causation relevant for disease progression or rather a secondary alteration. One major concern here is that most of the data devolve from toxin-based animal models and

not from humans. Thus, there is debate on whether α -syn protein fragments induce T cell-mediated immune reactions or, vice versa, whether the development of α -syn is triggered by such processes. α -Syn can induce the formation of LB, which are assumed to have pro-inflammatory effects. Overexpression of α -syn in cell cultures leads to an increase of microglia-induced release of pro-inflammatory molecules (Su et al. 2008).

Genetic background

Two parallel developments led to the discovery that the protein α -syn was a major component of LB. The non-amyloid component of AD plaques (NACP) was identified and cloned, revealing a precursor of 140 amino acid residue protein in the brains of patients with dementia (Ueda et al. 1993). Second, Polymeropoulos and colleagues identified in 1997 the alanine to threonine change at residue 53 (A53T) of the α -syn gene (SNCA) in a large Italian kindred and two seemingly unrelated Greek families with an early-onset autosomal dominant PD (Golbe et al. 1996; Polymeropoulos et al. 1997; Puschmann 2013). This A53T mutation, the first mutation in α -syn to be linked to a monogenic PD, has been detected in about 70 cases and is thus the most frequent of the α -syn mutations.

In the following years, α -syn mutations (A30P, E46K, Q51D and A53E) were discovered as additional PD-linked disease-causing mutations (Moore et al. 2005). More importantly, LB in brains of sporadic PD patients consisted of α -syn as their major component (Spillantini et al. 1997; Gai et al. 1998). Taken together, these neuropathological and genetic findings form the solid basis for the notion that neural LB are relevant for a number of diseases consequently named as synucleinopathies, such as sporadic PD, diffuse LB diseases and MSA. It should be noted that gene duplications/triplications have also been linked to PD, indicating that duplications/triplications of SNCA are implicated in an apparent gene-phenotype association (Singleton et al. 2003; Ibanez et al. 2004; Kalia and Lang 2015; Lee and Trojanowski 2006).

Structure and function of α -syn

Monomeric α -syn is a soluble, presynaptic, 140 amino acid long protein which is involved in normal mitochondrial and lysosomal function, synaptic transmission and neurotransmitter release (Bendor et al. 2013; Lee and Trojanowski 2006).

The SNCA gene is located on the long arm of chromosome 4 and comprises 6 exons out of which 5 are coding. α -Syn belongs to a highly conserved protein family and its

“brothers” are called beta and gamma synuclein (Bungeroth et al. 2014). The hydrophobic NAC domain (aa 61–95) is considered as the most important prerequisite for pathological α -syn assembly into fibrils involving a beta-sheet structure. α -Syn has multiple physiological functions in the CNS, which are not yet fully understood. A regular role in striatal dopamine release has been suggested using α -syn knock-out mice showing faster recovery from repetitive stimulation and partial striatal dopamine depletion (Abeliovich et al. 2000). Mostly observed within the presynaptic compartment, α -syn itself shows a SNARE-complex chaperone activity proposed to maintain neuronal synaptic homeostasis during aging. In general, α -syn is not restricted to the central nervous system, but interestingly had also been found to play an important role in membrane curvature and vesicular budding (Burre et al. 2010). In principle, α -syn is considered a natively unfolded protein with the potential to adopt alpha-helical but also beta-sheet structure, both tightly linked to its physiological or pathological properties. Finally, α -syn may occur physiologically as a helically folded tetramer, however, the aforementioned conformational flexibility of α -syn may be the underlying cause for its multi-functional properties (Bartels et al. 2010; Dettmer et al. 2015).

Although α -syn is predominantly expressed in the presynapse, it is released into the extracellular space facilitating the formation of LB and neuron-to-neuron transmission. Transsynaptic transmission of α -syn is believed to follow a stereotyped pattern of spread (Braak et al. 2003a). In consequence, α -syn can be found in the CSF (Shi et al. 2011). CSF α -syn levels have been investigated as potential biomarkers in patients and at-risk populations of PD (Sierks et al. 2011). In another clinical study, a panel of nine CSF biomarkers, including α -syn, was able to differentiate atypical Parkinson syndromes from patients with PD and dementia (Magdalinou et al. 2015). In addition, α -syn as a CSF biomarker may be useful to diagnose sporadic Creutzfeld–Jakob–Disease (Llorens et al. 2018). However, differences in analytical procedures yielded heterogeneous results with lower specificity.

While the causative role of pathologic SNCA mutations seems to be unquestionable in monogenic α -synopathies, the role of abnormal SNCA and α -syn aggregation in the pathogenesis of sporadic PD or other genetic variants of PD (e.g., those linked to *Parkin*, *PINK1* or *LRRK2* mutations) is less obvious. *Parkin* mutations cause autosomal recessive PD and heterozygous gene carriers have an increased risk for developing PD. The protein encoded in the *Parkin* gene is an ubiquitin ligase and its dysfunction might be associated with decreased autophagic degradation of α -syn aggregates (Tan et al. 2008). The *PINK1* gene encodes a kinase that is believed to protect against stress-induced mitochondrial dysfunction and apoptosis.

PINK1 (PARK 6)-induced autosomal recessive, early PD shows neuronal loss in SN but no LB pathology (Schneider

and Alcalay 2017). *LRRK2* (PARK 8)—most frequent late-onset PD—shows neuronal loss in SN and locus coeruleus but inconsistent LB pathology (Pont-Sunyer et al. 2017). The *LRRK2* gene is also associated with mitochondrial function and autophagy (Gomez-Suaga et al. 2012). While there is evidence for an association between these mutations and α -syn accumulation, the causal link is yet unclear. Here the notion is of importance, that there is no evidence for LB in postencephalitic parkinsonism (Jellinger 2009), MPTP-induced parkinsonism (Langston et al. 1999) and PD without nigral degeneration-SWEDD (Ling et al. 2016). In addition, lower brainstem pathology is not an obligatory trigger site of PD and in about 7–16% of PD the dorsal nucleus of the vagus is preserved as reviewed by Jellinger (2019). All this is indication to suggest that α -syn-related LB pathology is not necessarily a prerequisite of PD.

Dopaminergic terminal loss and its relationship to clinical symptoms depend on the type of monogenic disorder. Loss of dopaminergic terminals in relation to disease severity is much greater in *Parkin* carriers than in sporadic PD (Varone et al. 2004), while loss of striatal dopamine neurons as indexed with Dopamine Transporter SPECT is similar in sporadic PD and *LRRK2*-associated PD (Sierra et al. 2017; Wile et al. 2017). Asymmetry of dopaminergic terminal loss was greater in *LRRK2* carriers than in PD patients with *SNCA*, *PINK1* or *Parkin* mutations (McNeill et al. 2013). Taken together, the clinical consequences of the underlying pathology in *LRRK2*-associated PD resemble those in sporadic PD more than those in *SNCA*, *PINK1* or *Parkin*-associated forms.

Despite notable differences in dopamine transporter binding, the functional changes at a cortical network level in *Parkin*-associated PD and sporadic PD do not differ much (van Eimeren et al. 2010), and preclinical compensatory changes were found to be similar in *PINK1* and *Parkin* mutation carriers (van Nuenen et al. 2009). This evidence implies that functional endophenotypes in terms of cortical network function might be similar in monogenic and sporadic forms of PD, while the molecular synaptic endophenotype might be different.

Abnormalities of α -syn-related genes have rarely been detected in sporadic PD. While homozygous abnormalities in α -syn genes (*SNCA*=PARK1 or PARK4) cause monogenic PD as discussed, heterozygous mutations in α -syn-related genes are infrequently found in sporadic PD, even in patients with positive family history. The conception is that different levels of α -syn involvement or genetic abnormality could define different clinical subtypes of Parkinsonian syndromes, but data supporting this hypothesis are lacking. In general, earlier twin studies showed that the degree of heritability in PD is moderate (Tanner 2003).

It is currently consensus that a minor (non-pathologic) abnormality in *SNCA* alone is not sufficient to cause PD. A

second abnormality (“dual hit hypothesis”) is necessary for the defect in *SNCA* to become causative. Genome-wide association studies (GWAS) have revealed a number of candidate genes which could qualify as additional risk factors (Nalls et al. 2014). More recent meta-analysis of target genes of brain micro-RNA showed significant association of genetic variants in nine loci (Schulz et al. 2019). Post-translational modifications and other epigenetic factors are likely to play a significant role in this context (Lardenoije et al. 2015). Production and clearance of α -syn underlies an armada of enhancing, repressing and silencing gene-regulation mechanisms. The A53T *SNCA* mutation and hypomethylation of the *SNCA* gene can both cause increased transcription of *SNCA* mRNA and increase α -syn levels in the brain. One of six reported *SNCA* mutations, His50 Glu, was consistently identified in large population databases, but no enrichment was evident in PD cases compared to controls, thus showing insufficient evidence for pathogenicity (Blauwendraat et al. 2018). Other deregulations of *SNCA* gene transcription may shift the balance between α -syn production and clearance towards increased production (Miller et al. 2004). PD epigenetics is an evolving field which, however, has so far struggled to clearly identify disease-relevant epigenetic factors.

Despite this gain of knowledge, a number of controversies about α -syn genetics and function remain to be resolved. It has been difficult to clearly distinguish PD-related pathological processes from normal aging in the human brain. The initial trigger and sequence of pathological events which eventually result in the clinical manifestation of PD have been hard to identify. For example, the interaction of gut microbiota and α -syn dysfunction is a major focus of current research (Johnson et al. 2019). The roles of deleterious mitochondrial energy metabolism, cytosolic homeostasis, lysosomal dysfunction, oxidative stress and inflammation for aggregation of α -syn and manifestation of PD symptoms remain to be elucidated. The factors which lead to the clinically and pathologically distinct manifestations in PD, DLB or MSA are also unknown.

Alpha-synuclein physiology and the involvement of *SNCA* regulation in Parkinson's disease

Even though the exact function of α -syn is still unknown, the initial observation of α -syn in presynaptic terminals of the neurons suggested a physiological function of α -syn in neurotransmission. α -Syn was shown to modulate synaptic functions by facilitating vesicle clustering, recycling and docking to the cell membrane (Lashuel et al. 2013). As mentioned already, the unique character of α -syn in neurotransmission occurs via its chaperone activity, promoting SNARE-complex assembly and the rapid fusion of synaptic

vesicles. However, as α -syn is (1) not present in all presynaptic terminal buttons and (2) presents a weak membrane-interaction pattern to synaptic vesicles, α -syn was suggested to have a more global function (Huang et al. 2019).

Accordingly, α -syn interplays with multiple members of Rab GTPase family, a family of proteins involved in the regulation of intracellular trafficking processes (Miraglia et al. 2018). These interactions suggest that α -syn also plays a role in intracellular protein trafficking by promoting vesicular transport. Moreover, α -syn also contributes to axonal transport by regulating the nucleation and the growth velocity of microtubules in the assembly and in the remodeling of growth cone (Carnwath et al. 2018). Therefore, α -syn behaves as a soluble-interacting protein binding to diverse organelles and dispersing from membranes for the fine tuning of cytoskeleton plasticity.

Mechanisms involved in *SNCA* expression

Molecular mechanisms regulating gene expression are highly complex and interconnected. A simplistic approach to review and examine the relevance of these mechanisms concerning the expression of *SNCA* in PD is to find an association between mutations in *SNCA* and gene expression. Genome-wide association studies (GWAS) had already demonstrated that *SNCA* is one of the most common and consistent susceptible genes for the sporadic form of PD (Krüger et al. 1998).

Most significant associated variants are located in the 5', 3' and intronic regions, respectively, suggesting a critical role of non-coding regions of *SNCA* on its own expression. One important example is the presence of the binding sites of two microRNAs (miR-7 and miR-153) in the *SNCA* 3' untranslated region (UTR). As miR-7 was shown to decrease the expression of α -syn, variants in the binding site region affecting the affinity of binding may increase the expression of *SNCA* (Doxakis 2010). GWAS have highlighted an association of the promoter region of *SNCA* with PD. The microsatellite D4S3481, also termed REP1, is located about 10 kb upstream of the translational start of *SNCA*. GWAS had also contributed in the discovery of methylation-dependent putative promoter in *SNCA*, such as in the intron 1 in which the variant rs3756063 was associated with *SNCA* hypomethylation. Promoter hypomethylation is generally associated with increased expression, but the relevance of *SNCA* methylation on its expression has not been validated yet (Miranda-Morales et al. 2017) (see Fig. 3).

Multiple binding/enhancers/repressors were also identified in the genomic region surrounding *SNCA* but few were characterized in detail (Piper et al. 2018). For example, two enhancers located in intron 4 of the *SNCA* gene were identified using the Assay for Transposase-accessible Chromatin

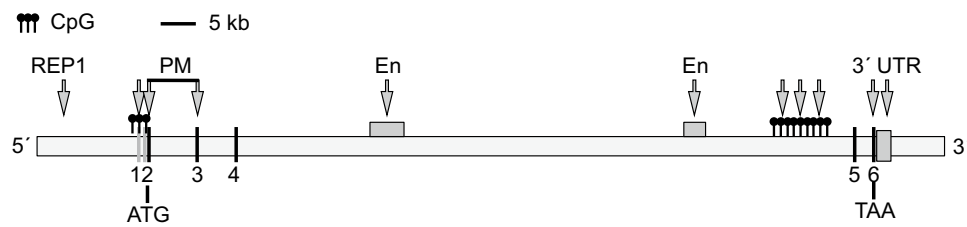


Fig. 3 Human *SNCA* regions in which main SNPs reviewed in the present study are located. Promoter (REP1 repeats), exon2 and exon 3 (PM: point mutation A30P, E46K, G51D, A53T), intron 4 (CpGs)

and 3' UTR (miRNA-binding sites). Exons are represented in the black, untranslated region in the gray and introns in the white colors. *En* enhancer, *PM* point mutation

Sequencing (ATAC-seq) and functionally evaluated using reporter assay and SNPs was pinpointed within these regions and correlated with PD. Similarly, multiple transcription factors were also reported to play a critical role in the expression of *SNCA*, such as PARP-1 which was shown to bind to the Rep-1 repeat by NACP-Rep1 and regulate activity of an artificial REP1/*SNCA* reporter construct. Another example is the ability of α-syn to bind to DNA, such as to the mitochondrial transcriptional co-activator gene *PPARGC1A*, and to RNA as α-syn was reported to binds its own mRNA (Surguchev and Surguchov 2017).

More recently, mosaicism and somatic mutations were also suspected to play an important role in neurodegeneration (Leija-Salazar et al. 2018). Mosaicism arising from early development or aging has been well characterized as a pathological mechanism associated with cancer due to the availability of the numerous sample biopsies. The limited availability of brain tissues is making the study of mosaicism in neurodegenerative diseases more challenging. Despite this limitation, aneuploidy and more precisely increased copies of *SNCA* were observed in the human brain as well. This phenomenon was reported to occur more frequently in PD dopaminergic neurons (Mokretar et al. 2018), nevertheless, the cause and consequence of this mechanism remain unknown.

In conclusion, large efforts were performed over the last 20 years to determine the exact function of α-syn. Diverse functions were highlighted over multiple and complex research fields demonstrating the need of a unified community to decipher the mechanism linking *SNCA* and PD.

α-Synuclein conformation: a crucial target for overexpression, fibrillation and spreading

As reviewed above, the major hypothesis for the pathogenesis of PD currently focuses on an overexpression of α-syn. However, there are other additional metabolic disturbances initiating or at least accompanying α-syn overexpression. Increased concentrations of α-syn might be due to a loss of metabolic capacity to degrade α-syn via a dysfunctional

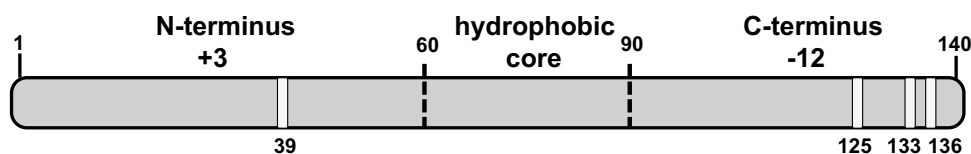
proteasomal and/or lysosomal-autophagy system. Loss of metabolic capacity may be due to changes in the proteins conformational state. Here, the role of oxidative stress (OS), nitration, phosphorylation of α-syn, composition of cytosol, excitotoxicity, calcium metabolism and energy metabolism comes into play. In addition, the solubility of proteins, e.g., in the cytosol, is dependent on the size of the molecule, the molecular structure, size and order of aminoacid (AA) side chains, pH-value, content of electrolytes as well as on the concentration of the protein of interest, e.g., α-syn. Beside these aspects of general protein chemistry, more recent publications on molecular biological aspects are in line with the suggestion that components of the cytosol interact with proteins at conditions when cells fluid deranges (Porcari et al. 2015; Paleologou and El-Agnaf 2012; Narayanan and Scarlata 2001; Mattson 2011; Ugalde et al. 2019; Bernstein et al. 2004; Iofrida et al. 2017; Kim et al. 2011; Batelli et al. 2008; Jungermann and Möhler 1980; Löffler et al. 1979). The time lag between initiation of fibril formation and aggregation is dependent on such parameters.

α-Syn oxidation

α-Syn is a 140 amino acid intrinsically disordered protein (Ruf et al. 2008) (see Fig. 4). Importantly, this protein contains four tyrosine (TYR) residues. As described by Olteanu and Pielak (2004), peroxidative aggregation of α-syn requires TYRs (Olteanu and Pielak 2004). The combination of cytochrome c with hydrogen peroxide causes TYR-dependent peroxidative aggregation of proteins. TYR-39 is essential for wild type-like covalent aggregation, when α-syn adopts a collapsed conformation (Ruf et al. 2008). The importance of TYRs is also demonstrated by experiments showing that fibril formation is absent if the three C-terminal TYR-residues (TYR 125, 133,136) are replaced by alanine (Ulrih et al. 2008) (Fig. 4).

The chemical reactivities of the TYRs in α-syn to form dityrosines have been studied by Ruf et al. (2008) using the reaction between cytochrome c and hydrogen peroxide. According to these studies by Ruf et al. (2008), TYR 133

Fig. 4 Location of tyrosine; this aminoacid is regarded as potential site of oxidation (modified from Ruf et al. 2008)



and 136 are the most reactive ones, while TYR 125 is less able to accept a radical from cytochrome *c*. Lack of α -dimer and large amounts of degradation indicate that TYR 39 is the least reactive (Ruf et al. 2008). Upon aggregation, α -syn adopts a beta-sheet structure (Vamvaca et al. 2009).

Therefore, it is not farfetched to assume that OS plays a role in α -syn pathology, especially as α -syn has been shown to be a “ferrireductase”, thus catalyzing ROS and exacerbating neurodegeneration (McDowall and Brown 2016) (Table 1).

Additionally, α -syn interacts with membrane lipids like polyunsaturated fatty acids (PUFA) to stabilize its three-dimensional structure. Peroxidation of PUFAs may modify α -syn and may reduce their affinity to α -syn (Shamoto-Nagai et al. 2006). 4-Hydroxy-2-nonenal, a degradation product of Hn = 6 PUFA, has been detected in post-mortem brain tissue from PD and has been shown to induce oligomerization and modification of α -syn (Shamoto-Nagai et al. 2006). Moreover, peroxidation products, the role of ROS and iron-induced generation of toxic hydroxyl radicals with and without participation of mitochondrial pathology including reduction of respiratory chain activity have been frequently reviewed in the past (Shamoto-Nagai et al. 2006; Sian-Hulsmann et al. 2011).

At present, however, it remains to be determined whether and which of these oxidative-driven post-translational modifications (PTMs) are disease causative or rather reflect a bystander role during disease progression. The precise pathways by which oxidative stress-associated α -syn modification mediate toxicity are also poorly understood at present. Moreover, PTMs of α -syn may not only be present within cells but may also occur in the extracellular space, leading to additional cellular damage.

This, however, is not a one-way pathology, as multifactorial players interact and finally contribute to disease onset and disease progression (Dickson 2007; Jellinger 2010,

2011; Sian-Hulsmann et al. 2011; Berg et al. 2001; Zecca et al. 2004b).

For example, pigmented neurons of the SN pars compacta (SNpc) and locus coeruleus (LC) are the most vulnerable ones in the pathology of PD (Hirsch et al. 1997).

This vulnerability (Surmeier et al. 2017; Surmeier 2018) has been associated with the regionally characteristic pigment NM. The earliest intra-cellular changes have been attributed to an increase of NM density in A9 neurons of normal morphological appearance and no characteristic pathology in PD and has been associated with oxidation and iron load (Halliday et al. 2005). Interestingly, it has been found that even in this early stage, α -syn can concentrate in the lipid component of the pigment (Halliday et al. 2005).

Other reactions of α -syn also may be of importance for changes in α -syn conformation including nitration (Uversky et al. 2005), glycation (Munch et al. 2000; Vicente Miranda et al. 2017), polyamine complexes (Fernandez et al. 2004) and phosphorylation (Pinho et al. 2019; Mbefo et al. 2010; Paleologou et al. 2010, 2008). Phosphorylation of α -syn along with copper also affects the protein aggregation process as described by Castillo-Gonzalez et al. (2017). It is, therefore, not surprising that there is an interaction of α -syn with divalent metal ions as reviewed recently (Binolfi et al. 2006, 2008, 2011; Miotto et al. 2014a, b, 2017; Gentile et al. 2018; Rasia et al. 2005). Metals such as iron and copper have been associated with oxidative stress and generation of ROS (Sian-Hulsmann et al. 2011; Zecca et al. 2004a; Gotz et al. 2004; Double et al. 2003).

While most of these interactions of α -syn have been described in models of α -syn conformation and fibrillation, verification in PD is lacking. The reasons for this are manifold: first, there is limited availability of post-mortem (p.m.) brain tissue. Second, isolation and purification of α -syn from such tissue requires pooling of p.m. tissue due to the low concentration of the various expected α -syn species. Third,

Table 1 Parameters of α -synuclein pathology

α -Syn overexpression due to genetic failure(s) of its synthesis
α -Syn overexpression due to genetic mutations
α -Syn overexpression due to loss of degradation capacity
α -Syn overexpression due to changes of concentration in cytosolic parameters
α -Syn as stressor of mitochondrial-, ER-, lysosomal-autophagy-, and cellular dynamics
α -Syn structural modification by OS, nitration, phosphorylation, glycation, complex build-up, binding to lipids, interaction with NM
α -Syn as a “ferrireductase”

highly sensitive analytical methods are required to detect changes in α -syn molecular structure. The methodological armamentarium includes NMR studies (Li et al. 2009; Salmon et al. 2010; Cho et al. 2009; Schwalbe et al. 2014) and highly sophisticated methodology like N–H spin–spin couplings (Xiang et al. 2013), a six-dimensional alpha-proton detection-based automated projection spectroscopy (APSY) (Yao et al. 2014) or neutron reflectometry and fluorescence spectroscopy (Jiang et al. 2015). For the determination of protein-bound 3,4-dihydroxy-phenylalanine as a marker for post-translational protein hydroxylation of tyrosine in human tissues *ex vivo*, a HPLC-method equipped with an electrochemical detector resp. fluorescence detector has been described by Harth et al. (Harth et al. 2001a, b).

An intermediate conclusion from these facts is that the pathology of α -syn is multifactorial and based on both genetic and non-genetic disturbances/dysregulations.

Hierarchical spreading of Lewy body pathology

An important feature of α -syn pathology is its propensity to form aggregates upon missense mutations causing misfolded α -syn or (relative) overexpression of the wild-type protein, the latter being represented by either multiplications of the α -syn gene locus (SNCA; duplications, triplications) or by common genetic variants (SNPs) in the SNCA gene that alter the expression levels of physiological α -syn *in vitro* and *in vivo* (Fuchs et al. 2008; Cronin et al. 2009). This explains how mutations in the SNCA gene contribute to either monogenic forms of PD with nearly 100% penetrance of the autosomal dominantly inherited mutations, or to the common sporadic form of the disease with SNPs acting as a genetic modifier of PD susceptibility.

The observation that regional differences of α -syn aggregation, in form of LB and Lewy neurites, occur in the brain of patients with PD led to the hypothesis of an intercellular spread of α -syn pathology along neuroanatomical structures (Braak et al. 2003a). As the toxic α -syn deposition in human brains occurs primarily in distal axons and synapses, this confers to the distal accentuation of α -syn pathology with primary loss of function reflected by impaired synaptic contacts before neurodegeneration occurs. In fact, axonopathy in presymptomatic PD is followed by neuronal degeneration (Longhena et al. 2017; Bridi and Hirth 2018). α -Syn is a naturally unfolded soluble protein that can randomly adopt a beta-sheet structure and subsequently polymerize into larger units of protofibrillar structures which finally form insoluble fibrils that coalesce to form the pathognomonic intraneuronal protein inclusions in PD. This process seems to be stochastically favored by increased concentrations of physiological α -syn protein or misfolded, more aggregation prone,

mutant α -syn. Indeed, it was shown that synthetic preformed fibrillary α -syn precursors are able to seed the aggregation of α -syn by recruiting physiologically unfolded α -syn into amyloid-like protein inclusions, reviewed in Uchiyama and Giasson (2016).

Here, it has been postulated that the conformational change of α -syn acts as a template for itself to form aggregates which leads to a spread of pathology as in prion diseases. In line with this hypothesis of a prion-like mechanism of α -syn spreading and neurodegeneration, a post-mortem study of patients with implantation of fetal human midbrain neurons revealed the development of LB pathology in the transplanted cells (Kordower et al. 2008). Using animal models, the injection of α -syn aggregates from brains of patients with PD or dementia with LB resulted in the formation of inclusions in wild-type mice and monkeys even in distant brain regions, further supporting the concept of prion-like spreading of α -syn pathology (reviewed in Goedert et al. 2017). As described in detail by Niu et al. (2018), the spread of misfolded α -syn starts in the olfactory bulb (OB) and the gut with a progressive invasion of the posterior part of the brain. Indeed human mutant α -syn applied to the OB induces pathological changes in sensitive brain areas (Niu et al. 2018). However, caveats have been published by Gelpi and Colom-Cadena as well as by Masaracchia et al. indicating that the prion hypothesis of human synucleinopathies has to be reconsidered (Gelpi and Colom-Cadena 2019; Masaracchia et al. 2018).

As pathological α -syn aggregates were not only observed in the central nervous system, but may occur early in the enteric nervous system, a retrograde spreading of the pathology from the gut to the brain via the vagal nerve was considered. Indeed, the dorsal motor nucleus of the vagal nerve is, together with the olfactory bulb, among the first anatomical structures in the brain related to LB formation in PD (Braak et al. 2003a).

Interaction of α -synuclein and gut microbioma

Although there is still some discussion, experimental and epidemiological data provide evidence that vagotomy might be protective against the development of PD. Scandinavian retrospective registry studies show that truncal but not selective vagotomy reduces the risk of later developing PD, an effect which seems to be time dependent and only observed after a long follow-up period of > 5–20 years (Liu et al. 2017; Svensson et al. 2015). Similar evidence is provided by Pan-Montojo and colleagues in a mouse model of PD, by showing that the spread of α -syn pathology from the gut into the brain is inhibited by vagotomy (Pan-Montojo et al. 2010, 2012). Moreover, vagotomy completely blocks

the dopaminergic degeneration within the SN after enteral treatment with the PD-inducing compound rotenone in this PD mouse model. Additionally, recent epidemiological data showed an association between appendectomy and PD risk, with a lower risk for the development of PD after appendectomy (Killinger et al. 2018). This might be of great interest since the appendix is known to harbor particularly strong α -syn immunoreactivity in conjunction with neural structures and receives substantial input from the vagal nerve. These data suggest the vagal nerve as a highway facilitating the spread of α -syn aggregates from the gut to the brain and supports the hypothesis that pathological α -syn aggregation could be initiated in the gut. However, other factors might be involved in the peripheral and central distribution patterns of α -syn and its pathological aggregates.

What are the triggers of α -syn pathology in the gastrointestinal tract as a potential initiation event of subsequent central PD pathology? A first potential trigger is the presence of infection or inflammatory disease in the gastrointestinal tract, since α -syn expression in this region might be a mechanism of immune response against infections and other inflammatory events (Stolzenberg et al. 2017). Indeed, a vicious cycle with mutual potentiation of inflammation and α -syn deposition was described as a mechanism contributing to the chronic progressive neurodegeneration in a mouse model of PD (Gao et al. 2011). Another potential factor contributing to the initiation of α -syn pathology in the gut and subsequently in the brain might be dysbiosis of the gut microbiota. Indeed, constipation as one clinical presentation of dysbiota is an early premotor symptom of PD, and the gut microbiome that was found to be altered in PD patients compared to healthy controls showed a characteristic over- and underrepresentation of distinct bacterial species (Hill-Burns et al. 2017; Petrov et al. 2017; Schepers et al. 2015). Interestingly, some of the dysregulated gut microbes were found to interfere with the intestinal mucosal barrier and, therefore, might elicit neuroinflammatory responses (Hill-Burns et al. 2017; Petrov et al. 2017) including OS (Sian-Hulsmann et al. 2015). In an elegant set of experiments, Sampson and colleagues were able to show that signals from microbes are able to promote α -syn-mediated motor dysfunction and brain pathology in a mouse model of PD, most likely via microglia activation (Sampson et al. 2016). Interestingly, even rotenone toxicity in PD mouse models might be mediated through gastro-intestinal dysbiota (Yang et al. 2017). Indeed, human gut microbiota from PD patients induced enhanced motor dysfunction in this PD mouse model with overexpression of human α -syn (Sampson et al. 2016). Other experimental/toxic PD models affect mitochondrial function and ROS production (Jiang and Dickson 2018; Bringmann et al. 1995).

While most researchers are in favor of the gut-brain axis pathology in PD, recent evidence from time-dependent

analyses following 6-hydroxydopamine-induced degeneration of the nigro-striatal tract in animal models suggest peripheral nervous system denervation, including peripheral inflammation, respectively, immunity (Armentero et al. 2006; Engler et al. 2009; Ambrosi et al. 2017), respiratory disturbances (Oliveira et al. 2019), cardiac sympathetic denervation (Jones et al. 2014) and gastrointestinal disturbances (Blandini et al. 2009). These data suggest that degenerative processes leading to SN degeneration and PD might start from both poles, e.g., gut, respectively, other peripheral sites as well as from the SN, respectively, brain stem nuclei depending on the various triggers of PD and the patients individual genetic vulnerability (Fig. 5).

α -Synuclein and its interactions with proteins involved in neuronal pathology

There are many more questions remaining to be solved, all of which could not be discussed in this review. For example, (1) why is there frequently a generation of more than one LB in a SN dopaminergic neuron and (2) is α -syn the key dominator of LB generation? If so it should be detected in the core of LBs rather than in the halo (Fig. 1). This, however, seems not to be the case. According to Kurt Jellinger (pers. communication), LB have a distinct central Parkin- and ubiquitin-positive domain while α -syn is localized in the halo of the LB. This should suggest a primary pathological process focusing on the proteasomal pathway of protein degradation and would be in line with the fact that α -syn interacts with other key players of neurodegenerative diseases, such as $A\beta$, Tau and TDP 43.

These interactions may result in a synergism of toxic events underlying synucleinopathies such as PD, MSA

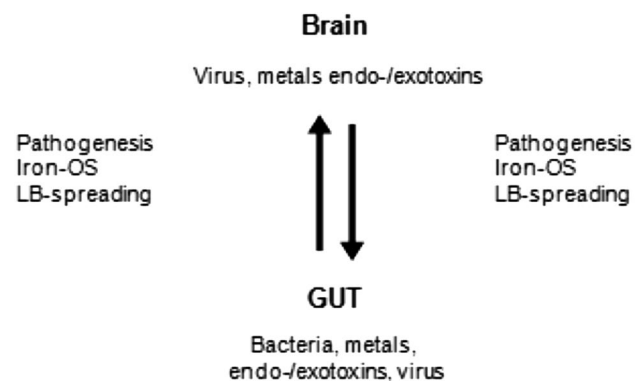


Fig. 5 Schematic presentation of pathways underlying PD pathology: gut-brain axis (Braak et al. 2003a, b; Pan-Montojo et al. 2010, 2012; Killinger et al. 2018; Machado et al. 2011), brain-periphery axis (Armentero et al. 2006; Engler et al. 2009; Blandini et al. 2009; Ambrosi et al. 2017; Oliveira et al. 2019)

and LBD, depending on their appearance in specific brain regions and relative proteins with pathological contributions, thus contributing to the discrimination potential between these disorders (Tables 2 and 3).

α-Synuclein and proteasomal degradation

The fidelity of cellular machineries implicated in the removal of damaged and dysfunctional proteins, such as the ubiquitin–proteasome system (UPS) or the autophagy–lysosome pathway (ALP), plays a crucial role in maintaining protein homeostasis (proteostasis). A decrease in the efficiency of these systems paralleled by an increase in the abundance of misfolded proteins occurs during aging and is a common denominator of neurodegenerative diseases (reviewed in Vilchez et al. 2014; Hipp et al. 2019).

α-Syn protein levels have been reported to increase in the SN with aging (Li et al. 2004; Chu and Kordower 2007), suggesting an inefficient clearance of α-syn. This may particularly pertain to misfolded, aggregated and oxidatively modified α-syn species. Both the UPS and the ALP have been implicated in the degradation of α-syn, and there is an ongoing discussion on the preferences of these degrading systems regarding the type of α-syn species.

The 26S proteasome holoenzyme is composed of the 20S barrel-shaped core complex and the 19S regulatory particle that is attached to one or both ends of the 20S core (rev. in Finley and Prado 2019). The 20S core complex harbors caspase-like, trypsin-like, and chymotrypsin-like activities inside the barrel, whereas the 19S cap recognizes, unfolds, deubiquitinates and translocates substrates into the proteasomal core. In fact, substrate ubiquitination is usually a prerequisite for degradation via the proteasome.

LB found in synucleinopathies stain positive for ubiquitin and co-localize with proteasomal subunits. Although the proteasome is probably not able to deal with large aggregates, it can handle monomeric or oligomeric α-syn extracted from LB with the help of the segregase VCP (valosin-containing protein)/p97. Several recent publications add evidence for a role of the UPS in clearing α-syn. Based on overexpression approaches in cellular models, RER1 (retention in endoplasmic reticulum 1) was reported to promote α-syn degradation by the proteasome (Park et al. 2017). RER1 localizes to early compartments of the secretory pathway and cycles between the cis-Golgi and the ER. It may promote retrieval of membrane-bound α-syn to the ER and subsequent proteasomal degradation, although an effect of endogenous RER1 has not yet been demonstrated in this context.

An interesting link between the transcriptional regulation of α-syn and proteasomal degradation was provided in a study that identified ZSCAN21 (zink finger and SCAN domain containing 21) as a transcription factor driving the expression of α-syn (Lassot et al. 2018; Clough et al. 2009; Dermentzaki et al. 2016). Under normal conditions, ZSCAN21 levels are controlled by TRIM41-mediated ubiquitination and subsequent proteasomal degradation. Cellular stress, for example induced by MPTP treatment, induces up-regulation of TRIM17 that inhibits TRIM41 and thus causes stabilization of ZSCAN21 and enhanced α-syn expression. In support of a disease-relevant role of this pathway, genetic variants of *TRIM41* and *ZSCAN21* co-segregate with PD and result in stabilization of ZSCAN21 protein.

A recent study indicated that the proteasome has an ATP-dependent, ubiquitin- and proteolysis-independent fragmentation function in vitro that disassembles large tau and α-syn fibrils into smaller aggregates (Cliffe et al. 2019). These smaller aggregates were more toxic than large fibrils and

Table 2 α-Synuclein interactions (Jellinger 2011)

There is an overlap between α-synucleinopathies and other protein-misfolding diseases, especially tauopathies
Inclusions are not specific for a neurodegenerative disease, suggesting interactions of pathological proteins at downstream pathways
There are synergistic effects between α-syn, pτ, Aβ and other proteins and the underlying pathogenic mechanisms including induction and spread of protein aggregates
There are multiple neurodegenerative processes which can overlap
The etiology of most processes is still unknown

Table 3 Combined protein toxicity

There is a role of pτ in the pathogenesis of PD aside from α-syn
Alzheimer's disease (AD) and LBD: overlap of pτ, α-syn and TDP-43 pathology
MSA: interaction of α-syn and pτ
PD and AD: progressive accumulation of pτ, GSK-3β and α-syn
Intracellular accumulation of pτ and α-syn promoted by Aβ deposition as a common pathway of an activated caspase and caspase-cleft tau
Combined analysis of cerebrospinal fluid, α-syn, pτ and Aβ-differential patterns of these neurodegenerative disorders

caused cell lysis when added exogenously to HEK293 cells. Whether this mechanism plays a role *in vivo* remains to be determined. For the proteasomal degradation, the N-terminal part of α -syn is essential. Experimental work demonstrates that aggregated α -syn is also degraded by the proteasome, although with a reduced rate due to Met 1/5 oxidation (Alvarez-Castelao et al. 2014). These authors also report that even mild oxidation inhibits the proteasome, especially when hydrogen peroxide is used as source of oxidation. The result is an accumulation of oxidatively damaged α -syn and initiation of PD (Alvarez-Castelao et al. 2014). Vice versa, Melo et al. (2018) and Pinho et al. (2019) demonstrate that α -syn dysfunction as a cellular stressor impairs mitochondria, endoplasmic reticulum, autophagy and cellular dynamics causing dopamine depletion, LB formation and PD.

In fact, a controversial issue has been the question whether misfolded α -syn species can impair proteasomal function. Conflicting results have been reported depending on the approach (*in vitro* versus *in vivo*), cellular models (neuronal versus non-neuronal) and type of α -syn species used (Lindersson et al. 2004; Snyder et al. 2003; Petrucelli et al. 2002; Bence et al. 2001; Tanaka et al. 2001; Zhang et al. 2008; Tofaris et al. 2003). It appears that cell type- and context-specific features account for these discrepancies and that an inhibitory effect of α -syn on proteasomal activities can contribute to the selective vulnerability of dopaminergic neurons in PD (Zondler et al. 2017). In conclusion, α -syn is not only degraded by the proteasome, pathogenic α -syn species obviously have the propensity to compromise the UPS, indicating a pathologically relevant reciprocal interaction.

Mitochondrial functioning in Parkinson's disease

Reactive oxygen species (ROS) are permanently produced during metabolic intraneural processes and contribute in particular to important cellular maintenance functions. The imbalance in the level of ROS and their degradation by antioxidative mechanisms is referred to as “oxidative stress”. To maintain ROS levels, mitochondrial function is the crucial intracellular organelle, in particular in neurons. The fact that neurons have high energy requirements and functionally depend on fine-tuned levels of calcium helps to explain the role of mitochondria for the functionality of the nervous system, in particular the tight link of mitochondrial impairment to neurodegenerative diseases. Genetic evidence links important genes such as *Parkin* and the mitochondrial kinase *PINK1* to genetic forms of PD, in particular regulating the elimination of damaged mitochondria ($\hat{=}$ mitophagy) by Parkin signaling downstream to PINK1 (Trempe and Fon 2013; Baker et al. 2011). However, the first evidence connecting mitochondria to PD was

derived from the observation of severe Parkinsonism and cognitive dysfunction in individuals using “synthetic heroin”, primarily consisting of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in a region of Northern California (Langston et al. 1983). Consequently, it became evident that in particular the MPTP' metabolite MPP⁺ produced by oxidation via the monoamine oxidase (MAO-B) was toxic. MPP⁺ is selectively taken up by dopaminergic neurons within the SN, leading to severe dopamine depletion. Further evidence supporting the important role of mitochondrial damage is that specifically decreased catalytic activity of complex 1 has been described in the SN and frontal cortex of PD patients. In addition, exposure to specific pesticides such as rotenone with its potential to inhibit complex 1 catalytic activity results in an increased susceptibility for these exposed individuals to develop PD later in life. Furthermore, the accumulation of α -syn may impair mitochondrial homeostasis, i.e., by decreasing the activity of mitochondrial complex 1 (Devi et al. 2008). In general, the kidney bean-shaped mitochondria form a highly dynamic reticular network controlled by a fusion and fission machinery enabling mitochondria to constantly fuse and to divide. There is a plethora of mitochondrial quality pathways, within a given cell governing molecular quality control by antioxidative chaperones and protease activity, but additionally an organelle quality control by removing dysfunctional mitochondria via mitophagy (Baker et al. 2011).

Interaction of ubiquitin proteasome and lysosomal functioning in Parkinson's disease

Protein degradation via the ubiquitin-proteasome system and the autophagy-lysosome pathway regulates both the intracellularly produced and ingested protein cargo. To maintain protein homeostasis, the proteasomal function within the SN is crucial, since evidence suggests that in particular α -syn is ubiquitinated in LB (Furukawa et al. 2002). In general, the autophagosom-lysosomal pathway (ALP) involves three major modes, namely microautophagy, chaperone-mediated autophagy and macroautophagy (Deter and De Duve 1967). By definition, microautophagy is the non-selective engulfment of small cytoplasmic portions by lysosomes (Mijaljica et al. 2011). In contrast, chaperone-mediated autophagy is a very selective process by which proteins possessing a distinct motif (KFERQ) like α -syn are recognized and guided to the lysosomal membrane transporter LAMP2A thereby entering the lysosome (Cuervo and Wong 2014).

Autophagy

More recent work demonstrates that autophagy is eventually controlled by GTP-ase-p38 MAPK signaling (Obersteiger et al. 2018), a pathway which might be disturbed in PD. It has been shown that modulators of autophagy such as FOXO 1, SESN 3 and TSC 2 are present in LB and that TSC 2 increases after α -syn overexpression (Miki et al. 2018). On the other hand, impaired formation of autophagosomes, which under physiological conditions reduce the intracellular burden of α -syn, increases the exosomal formation of α -syn (Fussi et al. 2018).

Macroautophagy refers to the process of degradation of large portions of cytoplasm, including protein aggregates and organelles (i.e., mitochondria), via inclusion into double-membrane lipid structures called “autophagosomes” and finally fusing into lysosomes (Takeshige et al. 1992; Tsukada and Ohsumi 1993). Currently, in the light of recent ultra-structural evidence showing that LB are composed of proteins, lipids and increased mitochondria, autophagosomes and lysosomes suggest that the degradation machinery is severely impaired and thereby providing a direct link between ALP-impairment and LB formation (Shahmoradian et al. 2019).

Therapy

The heterogeneity of PD symptoms calls for an individually adapted therapeutic regime. It aims to improve these individual combinations of motoric and non-motoric symptoms (Lee and Koh 2015). Their onset and severity are related to one another to a certain extent, based on the considerable impact of applied therapies (Muller et al. 2017). A diverse drug portfolio is employed for amelioration of PD symptoms with individually balanced combination of various drugs being applied. Careful and slow drug titration with continuous attention to the tolerability, safety and the needs of patients and their caregivers is essential for a successful treatment of PD in the long term. An optimized therapeutic regime can prevent an adjustment of the human body to features of PD, as unconscious learning processes may aggravate certain typical symptoms such as bound posture or walking with small steps. Currently, the best pharmacological treatment options exist for balancing the dopamine deficit in PD (Muller 2012). Generally, the focus of early PD treatment is on alleviating mild motoric symptoms. As PD progresses, L-dopa is used to mitigate more severe motoric symptoms. Subsequently, L-dopa is employed alongside other therapies, such as monoamine oxidase-B (MAO-B) inhibitors, dopamine

agonists, NMDA antagonists or catechol-*O*-methyltransferase inhibitors (COMT-I). These substances aim to minimize L-dopa-associated complications of motoric response. Deep-brain stimulation (DBS) or pump systems with application of apomorphine or L-dopa intestinal gel (LCIG) are mostly used as a final therapeutic approach in more advanced PD patients.

A definite cure for PD is still not available. Cumbersome transplantation trials, gene therapies or disease course-modifying treatments have not provided a significant promising breakthrough in the past years. The resurgence of complex stem cell implantation in the basal ganglia as a dopamine substitution method still has to prove its superiority over simple drug intake (Cyranoski 2018). Effects of drugs on specific non-motoric symptoms or balance problems are still somehow neglected.

Future therapeutic concepts

New treatment concepts are underway, most of them focusing on α -syn pathology. A better understanding of α -syn pathophysiology will be key for developing new therapeutic strategies. Such strategies could include a reduction of α -syn production by modification of SNCA gene transcription or RNA interference, inhibition of α -syn aggregation by insertion of “intrabodies” or viral-vector therapies, and clearance of α -syn aggregates by targeted immunotherapy or promoting autophagy of α -syn oligomers (Lardenoije et al. 2015; Brundin et al. 2017). Vaccination or application of antibodies has also been tested (Wang et al. 2019; Vaikath et al. 2019; Shen et al. 2019; Wood 2014). It is far from clear whether neuropathological findings, such as LB accumulation or misfolded protein enrichment, definitely play an active role in the ongoing chronic disease process itself. They could also merely reflect well-wrapped protein waste produced by disease-affected neurons. In general, chronic neurodegenerative processes result from several different metabolic cascades. It is well known that they ultimately result in cell death via well-described mechanisms. These processes induce individually different clinical signs and symptoms. Driven by experimental research, many hypotheses support the concepts of protein misfolding. However, occurrence of protein misfolding is a first-line defence process. It involves protein refolding mediated by chaperone proteins. A failure of these processes can cause protein degradation and/or accumulation. If this refolding/degradation machinery cannot prevent misfolded proteins, a stress response will be activated involving up-regulation of refolding and degradation processes. If stress levels induced by protein misfolding become too severe, cell death programs are activated. Accordingly, potential therapeutic strategies currently being tested in experimental settings include

reduction of protein misfolding, repair of misfolded proteins and facilitation of degradation of proteins, particularly when they are damaged beyond repair. There is a certain capacity of the human brain to compensate these initial events for considerable intervals before the clinical onset of initially mild and unspecific symptoms of neurodegenerative disease. Thus, initiation and rate of disease progression varies individually. Preclinical and experimental researchers still primarily focus on these processes, although animal models can only partially reflect the variability of PD symptoms, disease progress and severity as observed in clinical practice. A similar development is known from Alzheimer's disease, with all β -amyloid trials having failed to date. Future trials on disease modification will also test the usefulness of wearable, non-invasive body worn companion devices that collect movement-related measurements to assess patients' daily activities in relation to disease progression and symptomatic drug effects (Schlachetzki et al. 2017; Klucken et al. 2018). They will also further estimate the close relationship between the presence of motoric and non-motoric features in PD, i.e., the close association between motion, apathy and mood (Muller et al. 2017).

Based on this outlook, there are potential therapeutic approaches which could develop into viable treatment options and influence disease progression. Very new strategies are also being applied to influence the effects of micro-RNA molecules which, as non-coding RNA, are decisive in regulating gene expression. The objective here is (1) to decrease both pro-inflammatory reactions in microglia and MHC II-expression or (2) to modulate the inflammasome, for example with tyrosine kinase-inhibitors. Corresponding studies have been and are at present being conducted (Lawana et al. 2017; Thome et al. 2016; Brahmachari et al. 2017).

Altogether these approaches in the area of microglia are at present highly promising, due to the fact that medications already in use can be "repurposed" and because a re-orientation from a purely anti-inflammatory strategy to active immunomodulation can be pursued (Abushouk et al. 2018; Pagan et al. 2016; Pena-Altamira et al. 2016).

What we have learned about α -syn knock-out in mice, however, is a somewhat disconcerting factor because these mice showed an increase in microglia-induced inflammation (Austin et al. 2011). This could entail considerable risk for patients if modern therapeutic strategies, which are used to reduce abnormal α -syn, could conversely induce a reactive inflammation by completely depleting α -syn. Further studies are needed to reveal whether such a therapeutic approach is feasible.

Generally, one has to accept that treatment of chronic neurodegenerative disorders such as PD necessitates a combination of therapeutic options which are individually adapted to disease process and progression. The individual

patient, the caregiver and the treating physician must determine the value of a patient tailored therapy in the long run. The interaction between the physician, the caregiver and the patient decide and determine the feasibility of a treatment approach in PD. PD asks for a personalized medicine concept, which adds or switches treatments to best manage the symptoms of PD.

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