Chapter 1

General Introduction
Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting about 1% of the population of 60 years of age and older worldwide and with a prevalence increasing with age (1). The disease is clinically characterized by motor symptoms, such as resting tremor, bradykinesia, muscle rigidity and general postural instability (2). The motor manifestations of PD can be preceded by several non-motor symptoms, including hyposmia, sleep disorder, bladder dysfunction and depression (3). A clinical diagnosis of probable PD versus other parkinsonian syndromes currently relies upon the presence and progression of clinical features, following the criteria of the UK Parkinson's Society Brain Bank (4). The high variability in clinical phenotypes in early stage PD and considerable overlap between clinical features of PD and those of other PD-related disorders, as well as the lack of sensitive and specific biomarkers for early stage PD, represent a challenge for clinicians in diagnosing the disease (5). A definite diagnosis of PD can only be made after autopsy by studying the distribution of α-synuclein-immunoreactive intracytoplasmic protein aggregates and loss of catecholaminergic neurons in the substantia nigra (SN) and locus coerules (LC) microscopically.

Clinical phenotypes of Parkinson's disease

It is estimated that the average disease duration in PD is 15 years and the median age of onset is 60 years (4, 6). A prospective study in a population-based cohort in England has shown that within 10 years after the diagnosis 55% of the PD patients passed away, 68% developed postural instability and 46% suffered from dementia (7). The progression of the motor symptoms in PD can be divided in five stages by the clinical widely used rating scale Hoehn and Yahr (8). In stage 1, there are unilateral motor symptoms with minimal or no functional disability. In stage 2, the patient is bilaterally affected without impairment of balance. Stage 3 is defined by instability due to balance problems. In stage 4, the patients start to get dependent upon other people for daily activities, and they may have problems with speech and with autonomic functions. In the final stage 5, the patient is severely disabled and unable to care for him or herself. Pneumonia is the most common cause of death in PD (6).

The prevalence of non-motor manifestations ranges from 20-40% for hallucinations to 79% for nocturia in PD patients (9). In the advanced stage of PD, dementia is common and occurs in 40-80% of the patients. The prevalence of dementia increases with the duration of the disease and is associated with a reduced quality of life (10). Other non-motor symptoms, such as depression, fatigue, hyposmia and constipation, manifest throughout the progression of the disease, some of them occurring even before the onset of the motor symptoms (11, 12). The premotor phase of PD has been estimated to precede the motor phase by more than 20 years and those symptoms remain present or worsen throughout the disease (13, 14). An overview of the most frequently reported motor- and non-motor symptoms and their prevalence in several research cohorts is presented in table 1.

In PD, the following distinction in clinical subtypes has been made based on clinical appearance: early-disease onset, late-onset tremor dominant, late-onset non-tremor dominant and late-onset rapid disease progression without dementia (16). Early-onset PD is defined as starting before the age of 45 and is responsible for 10%-25% of all cases (4, 16). Specific genetic factors, such as mutations in α-synuclein or DJ-1, appear to play a role only in early-onset PD (17, 18). The late-onset subtypes of PD are patients diagnosed after the age of 45, and can be divided in two groups depending on a genetic predisposition or not: familial and sporadic. In familial late-onset PD, genetic factors are considered to be responsible for the disease, but for sporadic PD the cause is unknown. Clinical assessment of the subtypes showed that the early-onset group had the longest disease duration and the greatest delay to the manifestation of balance problems and cognitive decline. The non-tremor dominant group is
characterized by postural instability and gait disturbances, and displays cognitive impairment. Rapid disease progression is associated with older age, early depression and early midline motor symptoms affecting speech, swallowing, truncal mobility, gait or balance (16).

Table 1. Prevalence of motor and non-motor features in PD patients (8,9,15)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Prevalance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor symptoms (8, 15)</td>
<td></td>
</tr>
<tr>
<td>Tremor</td>
<td>70%</td>
</tr>
<tr>
<td>Rigidity</td>
<td>90%</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>100%</td>
</tr>
<tr>
<td>Postural instability</td>
<td>100%</td>
</tr>
<tr>
<td>Non-motor symptoms (9)</td>
<td></td>
</tr>
<tr>
<td>Sleep disorders</td>
<td>74-98%</td>
</tr>
<tr>
<td>Dementia</td>
<td>40-80%</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>18-35%</td>
</tr>
<tr>
<td>Hyposmia</td>
<td>68-85%</td>
</tr>
<tr>
<td>Anxiety</td>
<td>41-60%</td>
</tr>
<tr>
<td>Urine urgency</td>
<td>69-85%</td>
</tr>
<tr>
<td>Constipation</td>
<td>49%</td>
</tr>
<tr>
<td>Depression</td>
<td>50%</td>
</tr>
<tr>
<td>Nocturia</td>
<td>79%</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>20-40%</td>
</tr>
</tbody>
</table>

Clinical phenotypes of parkinsonian disorders

Parkinsonian disorders with α-synuclein aggregation, such as Progressive Supranuclear Palsy (PSP), dementia with Lewy bodies (DLB), Multiple System Atrophy (MSA), vascular parkinsonism and essential tremor, may resemble PD (19). Of these parkinsonian syndromes, MSA and DLB will be discussed below.

Dementia with Lewy Bodies

Clinically, DLB is characterized by dementia, fluctuating cognitive impairment, persistent visual hallucinations and parkinsonism. Although dementia is the most frequently presenting symptom, psychiatric symptoms or transient alterations of consciousness are other early features (19). Approximately 75% of patients with PD eventually develop dementia (20) and the differentiation of PD with dementia (PDD) from DLB is somewhat arbitrary. In PDD, dementia occurs in the setting of well-established parkinsonism, while in DLB dementia usually occurs concomitantly with or before the development of parkinsonian signs. Thus, patients are classified as having PD with dementia if parkinsonistic symptoms are present for more than one year before the onset of dementia (21).

Multiple system atrophy

MSA commonly presents with parkinsonism, but clinically patients also have varying degrees of dysautonomia, and cerebellar and pyramidal motor signs. Depending on the predominant symptoms, MSA is subdivided into MSA-C, i.e., patients with predominant degeneration in cerebellar circuitry and ataxia, and MSA-P, i.e., those with predominant degeneration of the basal ganglia with parkinsonism (22). The mean age of disease onset is 60 years with an average disease duration of 8 years (23). The
prominence of autonomic disorders and cerebellar involvement along with a symmetrical onset of motor symptoms, absence of tremor and poor response to levodopa suggest the diagnosis of MSA rather than PD (24). However, some cases of MSA may demonstrate responsiveness to levodopa, as well as its side-effects including motor fluctuations and dyskinesia in the early stages of the disease, and a declining benefit over time. Cognitive functioning in MSA tends to be relatively well preserved compared with PD and other parkinsonian syndromes, probably reflecting a lesser degree of cortical involvement (25).

Overall, it can be challenging to clinically distinguish the various parkinsonian syndromes from PD. Clinicopathological studies have identified several underlying neuropathological substrates for the observed phenotypes in parkinsonian disorders, and in different subtypes of PD (16, 25, 26). These neuropathological hallmarks will be discussed in the following paragraphs.

Neuropathology of PD and PD-related disorders

Neuropathology in early stage PD

The loss of dopaminergic neurons in the SN, leading to the cardinal motor symptoms, has long been considered the main pathological feature of sporadic PD. However, neuronal loss in other regions such as the LC, dorsal nucleus of the vagus (DMV), pedunculopontine nucleus and the raphe nuclei have also been observed in early stage PD patients and may contribute to the non-motor symptoms in PD (27, 28). The neuropathological hallmark of sporadic PD is the presence of intracytoplasmic and neuritic inclusions, which are termed Lewy bodies (LB) or Lewy neurites (LN), respectively. These inclusions consists of aberrant proteins, among misfolded α-synuclein (29). In addition, extracellular α-synuclein-immunoreactie aggregates have been observed.

Based upon an extensive survey of post-mortem humans brains of confirmed PD cases and brain donors without manifest PD, Braak and colleagues (34) were the first to suggest that the α-synuclein pathology progresses in a predictable manner throughout the brain. The earliest sites of α-synuclein pathology in the brain are the DMV in the medulla oblongata (MO) and the olfactory bulb (OB) (30). Photomicrographs of the α-synuclein pathology in MO and OB are displayed in figure 1. α-Synuclein aggregates have also been identified in the spinal cord (31) and peripheral nervous system (PNS) in the early stages of PD. In particular the sympathetic and parasympathetic α-synuclein pathology is well recognized in PD, affecting the vagal nerve, sympathetic chain and pelvic plexus (32). In the early phase of disease, deposits of misfolded α-synuclein have also been found in the wall of the colon (33) and the submandibular glands (34).

The brain areas that are affected in the premotor stages are hypothesized to be associated with clinical non-motor symptoms. For example, hyposmia may result of α-synuclein pathology in the OB (35). Likewise, α-synuclein pathology in the vagal nerve may be associated with cardiac dysfunction (36), and constipation and fecal incontinence to lesions observed in the pelvic plexus (37). Spinal cord lesions may contribute to clinical symptoms (e.g., pain, constipation, poor balance, lower urinary tract complaints, and sexual dysfunction) that occur during the premotor and motor phases of sporadic PD (31). Furthermore, an increased amount of depositions of α-synuclein in the cortical areas has been associated with dementia in PD patients (25).

Neuropathology during disease progression in PD

It has been postulated by Braak and colleagues that the distribution of α-synuclein pathology in the brain reflects the progression of the disease from the premotor phase to the early motor phase and, subsequently, the advanced phases of the disease with worsening of the motor symptoms and, in a considerable percentage of the patients, with dementia and/or depression (13, 30, 38). Braak et al.
proposed a neuropathological staging protocol for PD, where α-synuclein pathology progresses in a predictable topographical manner over the brain in six stages. The earliest sites of pathology in the brain are the anterior olfactory nucleus in the OB and/or the DMV and glossopharyngeal nerves in the brainstem forming stage 1. In stage 2, the α-synuclein aggregates are observed in the pontine tegmentum (LC, magnocellular nucleus of the reticular formation and lower raphe nuclei). In stage 3, the α-synuclein aggregates reach the pedunculopontine nucleus, the pars compacta of the SN and the cholinergic magnocellular nuclei of the basal forebrain. In stage 4, the hypothalamus, portions of the thalamus and, as the first cortical region, the anteromedial temporal mesocortex become involved. The latest stages are characterized by lesions in the neocortical high-order association areas (stage 5), followed by first-order association areas and primary cortical fields (stage 6) (figure 2).

The distribution pattern of α-synuclein pathology in PD patients and in aged individuals without a history of any clinical sign of PD, i.e. subjects with incidental Lewy body disease (iLBD), as described by Braak and colleagues (39), has been largely confirmed in other cohorts (40-43). Recent post-mortem studies have provided evidence for dopaminergic cell loss in the SN in iLBD (44-47). Possibly in line with this finding, a large clinicopathological study showed that iLBD subjects display moderate impairments in motor function compared to controls (48). This supports the idea that the presence of α-synuclein pathology is deleterious to the dopaminergic neurons and that iLBD may represent the premotor phase of PD.
Different studies have indicated that in 11-47% of the brain donors with α-synuclein pathology the pattern of this pathology deviated from the distribution described by Braak and colleagues (26, 43, 49). On the one hand, these donors were clinically-diagnosed PD cases with LB pathology in the upper brain stem or cerebral cortex without involvement of the DMV. On the other hand, it concerned neurologically normal elderly cases with an extensive amount and distribution of α-synuclein pathology throughout the brain without PD related symptoms. These findings suggest that there are alternative routes of progression of the pathology, perhaps due to concomitant diseases and genetic predisposition (50, 51).

Within PD, different rates of disease progression have been observed in the different clinical subtypes and these have been linked to concomitant pathology or phenotypic variability of α-synuclein pathology (16, 25, 52, 53). The presence of cerebral amyloid angiopathy lesions and Alzheimer’s disease lesions are thought to be associated with the cognitive decline and progression of the neurodegenerative process (52, 53). The early-onset and the tremor-dominant subtypes demonstrate similar distribution patterns and severity of α-synuclein pathology throughout the brain (16). Patients with non-tremor dominant group display significantly more amyloid plaques and cortical Lewy bodies compared to the early-onset and the tremor-dominant group (16, 26). The late-onset patients (> 70 years old) demonstrated cortical depositions of amyloid pathology (16).

The progression of nigral neuronal loss is thought to occur in a non-linear manner after the clinical diagnosis of PD: 30-60% loss of neurons has been observed in the first decade after the diagnosis with a stabilization of the loss in the second decade of PD (54, 55). In addition, many other non-dopaminergic nuclei degenerate during the progression of PD. The loss of noradrenergic cells in the LC has been well documented by various neuropathological studies (56-60), although the role of noradrenergic loss in the clinical progression of PD is still under debate (61, 62). Degeneration of the LC might contribute to the tremor and cognitive dysfunction in PD (63, 64). It has been further hypothesized that noradrenalin might be neuroprotective and that the loss of LC neurons contributes to the progressive loss of dopaminergic neurons in the SN (65). Also, loss of cholinergic neurons have been observed in PD patients, including the nucleus basalis of Meynert, which supplies the majority of the cholinergic input to the cerebral cortex, and the pedunculopontine nucleus, providing many

Figure 2. Progression of pathology in PD. A. Distribution of α-synuclein pathology throughout the 6 Braak stages and pre- and motor stages. B. Four main structures affected by α-synuclein pathology in the premotor stage of PD.
subcortical structures with acetylcholine. Both these output structures undergo degeneration in PD, with more severe loss associated with cognitive impairment (66).

**Pathology in other Parkinsonian syndromes**

*Multiple system atrophy*

Macroscopically, MSA-P patients reveal atrophy and brownish discoloration of the posterolateral putamen post-mortem. In cases with significant cerebellar signs, there is also atrophy of the pontine base and atrophy and gray discoloration of the cerebellar white matter. More subtle atrophy is noted in the medulla (e.g., inferior olive) and the cerebellar cortex (67). Microscopically, MSA is characterized by α-synuclein-immunopositive glial cytoplasmic and neuronal inclusions in some or all of the following structures: inferior olives, pontine nuclei, cerebellar Purkinje cells, putamen, caudate nucleus, globus pallidus, SN, LC, autonomic nuclei of brainstem, intermediolateral cell columns of the spinal cord, and Onuf’s nucleus in the sacral cord (68). In PD, no α-synuclein aggregates have been observed in pontocerebellar and olivocerebellar fibers. Noteworthy, neurons in MSA may also have α-synuclein inclusions within their cell nuclei (69), a feature not seen in affected neurons in PD. A staging scheme for MSA exists that scores severity of striatonigral degeneration (SND) and olivopontocerebellar atrophy (OPCA), each on a three-point scale. The final classification is indicated by an OPCA + SND score (e.g., OPCA 1 + SND 3 for a typical MSA-P case and OPCA 3 + SND 1 for a typical MSA-C case) (25). Detection of MSA in neurologically healthy individuals (“incidental MSA”) is extremely uncommon (70), and large numbers of such cases would be needed to determine the earliest sites of involvement to develop a staging scheme for MSA analogous to the Braak staging scheme for PD.

*Dementia with Lewy Bodies*

The pathological progression of DLB is thought to occur in a similar fashion as in PD. Unlike PD, DLB is characterized by large numbers of Lewy bodies in cerebral cortical areas, such as the entorhinal and cingulate cortices (71). A pathological distinction between DLB and PDD is therefore yet not possible, and differentiation of these syndromes is based on the presence of clinical symptoms and disease progression, as described before.

To summarize, the clinical syndrome of PD has multiple pathological substrates. This not only hampers an early and accurate diagnosis in clinical practice, but it also poses significant problems with respect to an adequate therapy. An important factor in developing new therapeutic strategies is formed by a better understanding of the pathogenetic mechanisms involved in early stage PD.

**Genetics**

About 10% of the familial PD patients have been linked to a genetic cause (72), with a prevalence which is highly dependent upon the population (73). Only during the past decade, the role of genetic factors in PD development has firmly been established with the identification of 17 ‘PARK’ loci and multiple genetic associations, found in several cohorts through three main approaches: 1) linkage studies in families affected by PD, 2) genome sequencing, or 3) genome wide association (GWAS) studies (72). Several mutations or duplications in genes have been implicated in juvenile and early-onset PD. These include PTEN-induced kinase 1 (PINK1), DJ-1, Parkin, duplication variants of α-synuclein (SNCA), nuclear receptor related 1 protein (NR4A2), ubiquitin carboxy-terminal hydrolase L1 (UCHL-1), eukaryotic translation initiation factor 4 gamma 1 (EIF4G1), 85 kDa calcium-independent phospholipase A2 (PLA2G6), probable cation-transporting ATPase 13A2 (ATP13A2) and F-box
only protein 7 (FBXO7) (17, 74-82). By contrast, mutations in leucine rich repeat kinase 2 (LRRK2), vacuolar sorting protein 35 (VSP35) and point mutations in SNCA have been identified in late-onset PD patients (83-85). The gene mutations α-synuclein, VSP35 and LRRK2 mutations have shown to be inherited in an autosomal dominant manner (75, 77, 83). Autosomal recessive inheritance is known for mutations in DJ-1, parkin, PINK1, ATP13A2 and FBXO7 (17, 76, 78). In addition, GWAS studies have provided several loci in the general population as risk factor for developing PD (86-90). An overview of all identified mutations and risk loci is displayed in table 2.

Table 2: Genes associated with genetic forms of PD, inheritance pattern and main clinical phenotype.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chr.</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Main clinical phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1/4</td>
<td>4q21</td>
<td>α-synuclein</td>
<td>AD</td>
<td>Early- or late-onset parkinsonism parkinsonism ± dementia</td>
<td>(18, 85, 89, 91, 92)</td>
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<tr>
<td>PARK2</td>
<td>6q25.2-q27</td>
<td>Parkin</td>
<td>AR</td>
<td>Early-onset parkinsonism</td>
<td>(93, 94)</td>
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<td>PARK3</td>
<td>2p13</td>
<td>n/a</td>
<td>AD</td>
<td>Early- or late-onset parkinsonism</td>
<td>(95)</td>
</tr>
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<td>PARK5</td>
<td>4p14</td>
<td>UCHL1</td>
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<td>(96)</td>
</tr>
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<td>PARK6</td>
<td>1p36</td>
<td>PINK1</td>
<td>AR</td>
<td>Early-onset parkinsonism</td>
<td>(97, 98)</td>
</tr>
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<td>PARK7</td>
<td>1p36</td>
<td>DJ-1</td>
<td>AR</td>
<td>Early-onset parkinsonism</td>
<td>(17)</td>
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<td>PARK8</td>
<td>12q12</td>
<td>LRRK2</td>
<td>AD</td>
<td>Late-onset parkinsonism</td>
<td>(83, 87, 89, 99)</td>
</tr>
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<td>PARK9</td>
<td>1p36</td>
<td>ATP13A2</td>
<td>AR</td>
<td>Juvenile parkinsonism, pyramidal signs, dementia</td>
<td>(81)</td>
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<td>PARK13</td>
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<td>PARK14</td>
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<td>PLA2G6</td>
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<td>Early-onset dystonia-parkinsonism</td>
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<td>PARK15</td>
<td>22q12-q13</td>
<td>FBXO7</td>
<td>AR</td>
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<td>(82, 103)</td>
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<td>PARK16</td>
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<td>PARK17</td>
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<td>4p15.32</td>
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<td>1p31.3</td>
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<td>17q21.1</td>
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<td>6q21.3</td>
<td>HLA-DRA</td>
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<td>Late-onset parkinsonism</td>
<td>(86, 88)</td>
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<td>11p15.4</td>
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<td>n/a</td>
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<td>Inheritance</td>
<td>Main clinical phenotype</td>
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<td>7p15.3</td>
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<td>GPNMB</td>
<td>n/a</td>
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<td>(90)</td>
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</table>

Abbreviations: HTRA2: Serine protease HTRA2, mitochondrial; PLA2G6: 85 kDa calcium-independent phospholipase A2; BST1: ADP-ribosyl cyclase 2; DNAJC6: Putative tyrosine-protein phosphatase auxilin; MAPT: Microtubule-associated protein tau; HLA-DRA: HLA class II histocompatibility antigen, DR alpha chain; SMPD1: sphingomyelin phosphodiesterase 1; GAK: cyclin G associated kinase; DGKQ: diacylglycerol kinase, theta 110kDa; PANK2: Pantothenate kinase; GBA: glucosidase, beta, acid HLA-DRB: acid lysosomal major histocompatibility complex, class II, DR beta 5; ACMSD: aminocarboxymuconate semialdehyde decarboxylase; Stk39: serine threonine kinase 39; LAMP3: lysosomal-associated membrane protein 3; SYT11: synaptotagmin XI; HIP1R: huntingtin interacting protein 1 related; STX1B: syntaxin 1B; FGF20: fibroblast growth factor 20; STBD1: starch binding domain 1; GPNMB: glycoprotein nmb. AD: autosomal dominant; AR: autosomal recessive n/a: not known.

Interestingly, carriers of mutations display different neuropathological phenotypes. At autopsy, patients with point mutations in SNCA display nigral neuronal loss as well as brainstem and cortical LBs. Triplications and duplications display similar pathology and, in addition, vacuoles in the temporal lobe. Additional α-synuclein copies lead to an earlier onset of the disease with dementia as a prominent feature (91). The various mutations that have been identified in LRRK2 lead to varying pathology. The most common mutation in LRRK2, i.e., G2019S, displays neuropathological ranges from non-specific nigral degeneration to a widespread distribution of Lewy bodies. Patients with parkin-associated PD reveal nigral neuronal loss at autopsy and varying α-synuclein pathology. By contrast, compound heterozygotes display LBs or tangle pathology. Pathological examination of GBA mutation-carriers revealed abundant α-synuclein pathology throughout the brain, specifically in the cerebral cortex and hippocampus. Patients with GBA-associated parkinsonism exhibit varying parkinsonian phenotypes but tend to have an earlier age of onset and show more frequently cognitive changes than patients with parkinsonism without GBA mutations (112). In table 3, mutations in genetic forms of PD and the corresponding phenotypes are summarized. Pathological substrates associated with other mutations have yet to be determined. The pathological phenotypes of identified mutations suggest that the different mutations observed in PD relate to separate pathogenetic mechanisms that define various forms of PD. Therefore, PD might not be a disease with a single cause but rather a syndrome with multiple causes and different pathological and clinical phenotypes.
<table>
<thead>
<tr>
<th>Locus/gene</th>
<th>Mutation</th>
<th>Neuropathology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1/4/SNCA</td>
<td>A53T, E46K, A30P</td>
<td>Nigral neuronal loss, cortical and brainstem Lewy bodies</td>
<td>(85, 92, 113, 114)</td>
</tr>
<tr>
<td></td>
<td>Triplication/duplicati</td>
<td>Cortical and brainstem Lewy bodies, temporal lobe vacuolation</td>
<td>(115-117)</td>
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<td>PARK2/PARKIN</td>
<td>Exon4Del/Exon4Del</td>
<td>Nigral neuronal loss, no Lewy bodies</td>
<td>(94, 118-120)</td>
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<td></td>
<td>K211 N/Exon4Del</td>
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<td></td>
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<td>O34fs/O34fs</td>
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<td></td>
<td>Exon3Del/Exon3Del</td>
<td>Nigral neuronal loss, no Lewy bodies, α-synuclein positive inclusions in the pedunculopontine nucleus</td>
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<td></td>
<td>R275W/Pro113fsX51</td>
<td>Cortical Lewy bodies, none in the brainstem but occasional Lewy neurites in the dorsal nucleus of the vagus</td>
<td>(122)</td>
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<td>Del1072T/Exon7Del</td>
<td>Lewy bodies in the locus coeruleus and substantia nigra</td>
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<td>PARK8/LRRK2</td>
<td>G2019S</td>
<td>Neuropathology ranges from non-specific nigral degeneration to widespread Lewy body disease</td>
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<td>R1441C</td>
<td>Varies from Lewy body disease to nigral degeneration with ubiquitin positive inclusions to severe tau pathology</td>
<td>(83)</td>
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<td></td>
<td>R1441G/I2020T</td>
<td>Non-specific nigral degeneration</td>
<td>(128, 129)</td>
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<td>Y1699C</td>
<td>Varies from Lewy body disease to nigral degeneration with ubiquitin positive inclusions or Alzheimer pathology</td>
<td>(83, 126)</td>
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<tr>
<td>PARK6/PINK1</td>
<td>Exon7Del/c.1488 g&gt;A</td>
<td>Nigral neuronal loss, Lewy bodies and aberrant neurites in the reticular nuclei of the brainstem, substantia nigra pars compacta and Meynert nucleus</td>
<td>(98)</td>
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<tr>
<td>PARK14/PLA2G6</td>
<td>R37X/c.1078–3C</td>
<td>Range from mild to severe Lewy body disease, with neurofibrillary tangles and axonal speroids</td>
<td>(82)</td>
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<td></td>
<td>T572I/T572I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK2</td>
<td>G521R/G521R</td>
<td>No Lewy bodies, diffuse tau pathology</td>
<td>(108)</td>
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<tr>
<td>GBA</td>
<td>N370S/L444P</td>
<td>Abundant Lewy body pathology, cortex and hippocampus</td>
<td>(112)</td>
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</table>
Molecular mechanisms involved in the pathogenesis of PD

The genes associated with familial PD identified through association and linkage studies have provided some insight in the key molecular processes involved in PD, such as mitochondrial dysfunction, aberrant protein clearance and synaptic dysfunction (130-132). Also, inflammation and a prion-like propagation mechanism have been linked to the progression of PD (133, 134). Such molecular processes have been studied in several models of PD and will be discussed below. Little is known about the contribution of these key mechanisms in the early stages of PD or during the progression of the disease.

Mitochondrial dysfunction and oxidative stress in PD

Mitochondria are essential organelles that provide >90% of the energy in all eukaryotic cells through oxidative phosphorylation (135). Mitochondria are also the major source of cellular reactive oxygen species (ROS) (136). The role of mitochondria in the pathogenesis of PD was first suggested in 1983 after the discovery of the effect of the drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The intake of MPTP leads to a PD phenotype in illicit drug users in whom, upon autopsy, dopaminergic cell loss was observed in the SN (137). In 1989, mitochondrial complex I deficiency was first identified in the SN of sporadic PD patients (138). Recently, it has been shown that complex I deficiency is not confined to the SN, but is also observed in other brain structures, such as the prefrontal cortex (139).

Mitochondrial dysfunctions have been implicated in several missense mutations in genes, such as SNCA, LRRK2, PINK1, Parkin and DJ-1. Mutated forms of α-synuclein are localized to the mitochondria, resulting in decreased complex I activity in carriers compared to controls (140). Neurons of patients carrying LRRK2-G2019S showed a lower membrane potential and lower intracellular ATP levels, with alterations in mitochondria morphology and dynamics (141). PINK1 and parkin mutations that have been associated with certain forms of PD, are also involved in mitochondrial dysfunction. PINK1 is a cytoplasmic but mitochondrial associated protein kinase, which acts upstream of Parkin. Parkin is a E3 ubiquitin ligase that facilitates the degradation of damaged mitochondria (142, 143). The mutations in PINK1, Parkin and DJ-1 contribute to the mitochondrial dysfunction and oxidative stress. PINK1 and Parkin work in a common pathways to regulate mitophagy and DJ-1 is thought to be a redox sensor (93, 97, 144).

Several models have been used to study mitochondrial dysfunction in PD. First, over-expression/loss-of-function of the genes implicated in mitochondrial function have provided useful insights in the affected mechanisms and pathways linked to mitochondrial dysfunction (145). In addition, administration of specific mitochondrial complex I inhibitors, such as MPTP and rotenone allowed pharmacological testing in these models (for review: (145)). Environmental toxins leading to mitochondrial dysfunctions include Paraquat and Maneb. Paraquat is a potent redox cycler, which converts free radicals to ROS (146). Maneb inhibits mitochondrial complex III activity in rats (147), suggesting that this complex also plays a role. The construction of cybrids using mitochondrial DNA (mtDNA) of PD patients has demonstrated a defect in the encoding of complex I, and has identified deletions associated with complex IV (148). These findings confirm the role of mitochondrial dysfunction and related oxidative stress in PD, but little is still known about the contribution of mitochondria and ROS in the pathogenesis and progression of PD pathology.

Mechanisms related to dysfunction in misfolded protein clearance

The presence of LBs indicates that there might be an imbalance in production and clearance of misfolded, unwanted proteins in PD. Intracellular protein degradation is essential for cellular quality
control to eliminate damaged or altered proteins. Two major proteolytic systems exert this cleaning function: the ubiquitin-proteasome system (UPS) and the autophagic/lysosomal system. The UPS accounts for the selective degradation of most short-lived proteins, such that the faulty proteins are labeled for degradation by ubiquitin and, subsequently, degraded by the proteasome (149). Intracellular aggregates of α-synuclein are cleared through chaperone-mediated autophagy (CMA). In this process, first the cargo for autophagy is selected based on the binding properties with heat-shock protein 70 (HSPA70). Together with associated co-chaperones, α-synuclein is then delivered to the lysosomes, where it binds with lysosomal-membrane protein 2A (LAMP2A). Subsequently, the α-synuclein is translocated to the lumen of the lysosome, where it will be degraded by proteases (150) (summarized in figure 3). Extracellular aggregated forms of α-synuclein can be degraded in lysosomes after receptor-mediated endocytosis and their transport through the endosomal pathway (151). Mutant forms of α-synuclein prevent their degradation by the CMA pathway resulting in toxic aggregation in the cytoplasm as has been shown in cell cultures and post-mortem brain tissue (152). In addition, evidence for a role of dysfunctional autophagy/lysosomal clearance mechanisms has been found in post-mortem studies. Thus, immunoreactivity for lysosomal-membrane protein 1 (LAMP1), cathepsin D (CatD) and heat shock protein 73 (HSP73) has been shown to be significantly decreased within PD nigral neurons when compared to age-matched controls (153).

Some gene mutations have been linked to disturbed misfolded protein clearance, such as GBA, ATP13A2, and LRRK2. Studies using animal or cell models have demonstrated that GBA deficiency reduces lysosomal function and leads to the accumulation of α-synuclein (154). In addition, GBA can modulate ceramide metabolism and plays a role in α-synuclein processing (155). Mutations in ATP13A2, a lysosomal ATPase, also lead to impaired lysosomal degradation capacity (156). Furthermore, the LRRK2-G2019S mutation interferes with chaperone-mediated autophagy and enhances co-localization of α-synuclein with LAMP2 (157). Several models have been used to study the role of autophagy in PD. For example, in rotenone-based in vitro and in vivo PD models, decreases in autophagic functions have been found, while the number of autophagic vacuoles was increased (158). Targeted deletions of either Atg5 or Atg7 in mice resulted in the accumulation of poly-ubiquitinated proteins and sensitized neurons to degeneration. These findings support the hypothesis that autophagy is neuroprotective to neurons (159, 160). In addition, pharmaceutical-based modification of autophagy has been applied to interfere with the burden of misfolded α-synuclein. For example, inhibition of the mammalian target of rapamycin (mTOR) induces autophagy and rescues dopaminergic neurons (161).

To summarize, impaired protein clearance through dysfunctional autophagic and proteasomal mechanisms have been confirmed in the pathogenesis of PD. However, the upstream effectors leading to the altered protein clearance remain to be identified.

Synaptic dysfunction in PD

In PD, evidence is accumulating that synaptic dysfunction might be an early event in the degenerative process. An important observation in this context is that a loss of tyrosine-hydroxylase (TH) staining and of dopamine transporter (DAT) SPECT signal has been shown in the putamen, i.e., the terminal area of the efferents of dopaminergic neurons in the SN, in a phase of the disease in which neuronal loss in SN is still limited (44, 162). This suggests that synaptic dysfunction precedes neuronal loss in PD.

As mutations in the presynaptic α-synuclein were the first to be identified, the initial focus of many PD studies after the discovery of this gene mutation in 1993 has been on synaptic dysfunction. α-Synuclein is required for maintaining complex function of Soluble NSF Attachment Protein Receptors (SNARE) at the presynaptic terminal. SNARE is involved in the docking and release of
synaptic vesicles (163). In PD, over-expression of α-synuclein results in alteration of vesicle fusion and SNARE complex assembly. Missense mutations and multiple copies of SNCA lead to dysfunction of vesicle release and docking (164, 165).

Other mutations in genes linked to synaptic dysfunction and familial PD have been identified more recently (105, 106). For example, DNAJC6 encodes the HSP40 Auxilin, a protein which is selectively expressed in neurons and has a specific role in the ATPase activity of its partner HSP70 in clathrin uncoating. In DNAJC6 null mice, it has previously been shown that the abnormally increased retention of assembled clathrin on vesicles and in empty cages leads to impaired synaptic vesicle recycling and perturbed clathrin mediated endocytosis (106). Also, the gain-of-function mutation LRRK2-G2019S has recently been implicated in the functioning of the synapse. This mutation leads to reduced EndophilinA regulatory activity, a protein that is involved in clathrin-mediated endocytosis of synaptic vesicles. Furthermore, a loss-of-function LRRK2 mutation also impairs vesicle trafficking at the level of the synapse (164).

UCH-L1 is a de-ubiquitinating enzyme that is selectively and abundantly expressed in the brain (166). Its activity is required for normal synaptic function. An in vitro study showed that suppression of UCH-L1 activity increased α-synuclein levels and resulted in a concomitant accumulation of presynaptic α-synuclein. In contrast, blocking UCH-L1 activity in α-synuclein over-expressing neurons decreased α-synuclein levels and enhanced its synaptic clearance (166, 167).

In addition, VPS35 and MAPT mutations are associated with the dysfunction of intracellular transport of endosomes. VSP35 is a component of a retromer system which mediates retrograde transport between endosomes and the trans-Golgi network (104, 168). MAPT plays an important role in promoting the assembly and maintenance of the structure of microtubules. Over-expression or
dysfunction of MAPT result in lesions consisting of fibrillar aggregates (169).

**Inflammatory response in PD**

Neuroinflammation occurs in a number of neurodegenerative diseases, including PD. The inflammatory processes entail the reactive state of astrocytes (astrogliosis) and microglia (microgliosis), and infiltration of other immune cells (133). Already in 1988, activated HLA-positive microglia cells have been identified in PD (170). Moreover, significant increases of cytokines such as interleukin-1α, 1β (IL-1β) and tumor necrosis factor-α (TNF-α) have been found in the brain and CSF of PD patients, in combination with high levels of nitric oxide in the activated microglia in the SN (171). Interestingly, genetic alterations in several immune function-related genes (e.g., DJ-1, LRRK2 and HLA-DR) can cause familial PD or increase the risk of developing PD. DJ-1 is thought to be a scaffold protein with anti-inflammatory function (172). This points to a key role for immune pathways in the pathogenetic mechanisms of PD which are possibly triggered by aging, oxidative stress, or abnormal forms of α-synuclein (reviewed in (173)).

Administration of neurotoxins in cell and animal models has provided insights into the role of inflammatory response in PD. For example, administration of the neurotoxic dopamine analog 6-hydroxydopamine (6-OHDA) revealed an important role for glial activity and inflammatory mediators in dopaminergic neurodegeneration (174). In addition, mice over-expressing wild-type α-synuclein display early microglia activation and a cascade of proinflammatory mediators such as TNF-α, IL-1β, interleukin 6 (IL-6) and Cytochrome c oxidase subunit II (COX2) have been observed in a dopaminergic cell line over expression α-synuclein (175). Also, in a rotenone model it has been found that through selective removal of microglia in cell culture, neuronal loss could be prevented (176).

Although extensive research has provided useful insights in the mechanism of inflammation in PD, it is not yet clear at what point of the cascade of the pathological progression the process of inflammation is involved.

**Prion-like mechanism in PD**

It has been postulated by Braak et al. that the progression of α-synuclein pathology might be caused by a yet unidentified pathogen that is capable of passing the mucosal barrier of the gastrointestinal tract and entering the central nervous system. By way of retrograde axonal and transneuronal transport, such a causative pathogen could reach selectively vulnerable subcortical nuclei and unimpededly gain access to the cerebral cortex (177).

In the past decades, evidence has accumulated supporting such a prion–like spread of α-synuclein pathology in PD (178). The post-mortem proof of α-synuclein containing Lewy bodies in embryonic dopamine cell transplants in PD patient suggests that the misfolded protein might be transmitted from the diseased host to donor neurons reminiscent of prion behavior (179, 180). Although being an intracellular protein, it has been shown that a small portion of α-synuclein is released from neuronal cells through exocytosis into the extracellular space (181) and that exocytosis is promoted under stress conditions (182). The mechanism works in a way that the extracellular, misfolded protein gains access to the cytosol of the recipient cell, and recruits and induces aggregation of the endogenous protein (183, 184). Experimental evidence supports the intercellular transfer of α-synuclein and the seeding of aggregation (185). In support of this, recent findings have shown that in cell culture, exogenous α-synuclein fibrils first aggregate in the neurites and then propagate retrogradely to the cell bodies, where in a later stage aggregates in the soma are observed (186). In a mouse model, α-synuclein aggregates and neuronal cell death are observed throughout the brain after inoculation of the misfolded protein in the forebrain (187).

To summarize, it has been hypothesized that a prion-like spreading mechanism is one of the key
players in the progression of PD pathology. However, the exact mechanism by which it operates in PD progression is not clear and currently investigated in mice and cell models.

**Identification of molecular mechanisms involved in sporadic PD using transcriptome analysis**

The genetic mutations and associations have provided insight in the molecular mechanisms underlying familial PD. The etiology of sporadic PD, however, is largely unknown, but associated with age (188). Also, sporadic PD is found to be more common in men (189) and linked to environmental factors, such that a higher incidence is reported in rural areas compared to urban areas (189, 190). An untargeted discovery approach to identify molecular mechanisms involved in PD is whole-genome transcriptome analysis. The transcriptome includes all mRNA transcripts in the cell. It can vary with external environmental conditions and reflects the genes that are being actively expressed at any given time. Transcriptome analysis has been used to provide insight in alterations in gene expression in sporadic PD donors compared to healthy control donors using specific regions of the brain. In human post-mortem tissue, the SN was investigated most frequently, either dissected out macroscopically (191-198) or using laser capture microscopy at the cellular level (199, 200). Other post-mortem studies focused on structures like the LC (201, 202), putamen (191, 197), frontal cortex (192, 197, 198) striatum (196) and cerebellum (193). These studies have revealed altered expression levels of genes involved in ubiquitination and proteasomal degradation of proteins, oxidative stress, vesicle trafficking, cytoskeletal stability, axonal guidance, dopamine neurotransmission and metabolism, neurotrophic signalling and programmed cell death. Recent studies have shown that these changes are specific for PD and do not occur in normal aging in the SN (203). Altered pathways found in PD compared to controls in multiple regions of the brain included Huntington disease signaling and BMP signaling pathway, which are both pathways linked to apoptosis. All canonical pathways identified in transcriptome studies in PD compared to controls are displayed in table 4. Canonical pathways identified include those related to pathways include pathways related to of the unfolded protein response (UPR), mitochondrial dysfunction and synaptic dysfunction in the pathogenesis of PD. In addition, new gene targets and interactions have been identified using transcriptome analysis, such as PGC-1α and FOXO1 (198, 204, 205). Although the transcriptome studies have revealed many possible pathogenetic pathways in sporadic PD, there is no consensus on how these pathways might be triggered and lead to cell death and α-synuclein aggregation. The causal factors are hard to discriminate since the pathways are interconnected and regulate each other. The role of processes such as mitochondrial dysfunction and synaptic dysfunction in the pathological cascade remain unclear, whereas cell death and protein aggregation are clearly a downstream effect. Therefore, transcriptome analysis of donors affected in the premotor and motor phase of PD might elucidate the mechanisms underlying the pathology in the early phases and the progression of PD pathology in later stages.
Table 4. Pathways deregulated in all investigated brain regions in PD compared to controls and previously identified in other transcriptome studies in brainstem, subcortical and cortical structures. The first column represents the Fisher p-value in our study, the columns on the right represent the lower Fisher p-value found in brainstem, subcortical and cortical brain structures.

<table>
<thead>
<tr>
<th>Molecular pathway</th>
<th>Brainstem structures</th>
<th>Subcortical structures</th>
<th>Cortical structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Role of NFAT in Regulation of the Immune Response</td>
<td>0.017 193</td>
<td>0.00742 201</td>
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<td>B Cell Receptor Signaling</td>
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<td>0.029 201</td>
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<td>0.016 197</td>
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<td>Protein Kinase A Signaling</td>
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<td>α-Adrenergic Signaling</td>
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<td>0.0060 201</td>
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<td>Breast Cancer Regulation by Stathmin</td>
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<td>Androgen Signaling</td>
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<td>PI3K Signaling in B Lymphocytes</td>
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<td>0.0018 201</td>
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<td>IL-2 Signaling</td>
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<td>Huntington's Disease Signaling</td>
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Table 4 (continued)

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<tr>
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<th>Brainstem structures</th>
<th>Subcortical structures</th>
<th>Cortical structures</th>
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<td>0.043^202</td>
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<td>n/a</td>
</tr>
<tr>
<td>GM-CSF Signaling</td>
<td>n/a</td>
<td>0.012^201</td>
<td>0.025^197</td>
</tr>
<tr>
<td>Nitric Oxide Signaling in the Cardiovascular System</td>
<td>n/a</td>
<td>4.37*10^-4^201</td>
<td>n/a</td>
</tr>
<tr>
<td>ERK/MAPK Signaling</td>
<td>n/a</td>
<td>n/a</td>
<td>3.31*10^-4^197,206,207</td>
</tr>
<tr>
<td>Ga12/13 Signaling</td>
<td>0.026^196</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>NRF2-mediated Oxidative Stress Response</td>
<td>n/a</td>
<td>n/a</td>
<td>0.0014^206</td>
</tr>
<tr>
<td>Calcium-induced T Lymphocyte Apoptosis</td>
<td>n/a</td>
<td>0.007^201</td>
<td>0.049^198</td>
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Scope of the thesis

The overall aim of the studies described in this thesis was to unravel molecular mechanisms involved in the early stages of PD pathology and during progression of the disease using the transcriptome analysis of human post-mortem brain tissue. Our hypothesis was that the progression of PD pathology is not random, but predictable and regulated by specific genes and molecular pathways. The main objectives in our studies were:

1. Identifying the progression of nigral neuronal loss and local nigral burden of pathology throughout the Braak stages
2. To identify crucial pathways involved in the early stages of PD pathology
3. To identify region-specific and common molecular mechanisms during the progression of PD pathology and alterations upon presence of α-synuclein pathology

To answer these questions we have included the SN, MO, LC and OB, all brain regions that are affected by α-synuclein pathology in the early stages of pathological process, of control, iLBD and PD donors. We have included iLBD donors (Braak α-synuclein 1-3), PD patients (Braak α-synuclein 4-6) and controls (Braak α-synuclein 0), without concomitant neurological disorders and limited tangle (Braak tangle stage <3) and amyloid-related pathology (Braak <C). To control for age-related changes, the donors were age-matched.

In chapter 2, we report a study on the relationship between the local burden of α-synuclein pathology and neuronal loss in the SN in different stages of the pathological progression. To answer the second research question, i.e., which pathways are involved in the early stages of PD pathology, we investigated altered molecular pathways in the SN in controls, Braak α-synuclein 1-2, 3-4 and 5-6 (chapter 3). In chapter 4, we describe the molecular pathways identified in OB and in chapter 5, we compare the alterations in gene expression profiles in MO, LC and SN and OB, and focus on the alterations observed upon presence of α-synuclein aggregates and common altered pathways in the brainstem and OB during disease progression in PD. In chapter 6, a summary is given and the presented experimental work is discussed in the context of the current literature.
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