Summary

Parkinson’s disease (PD) is a complex multisystem disorder causing progressive loss of dopaminergic and non-dopaminergic neurons in the central nervous system (CNS) and peripheral nervous system (PNS). Understanding disease progression and the multifaceted molecular mechanisms underlying the neuropathology in Parkinson’s disease (PD) is essential to develop novel neuroprotective or disease-modifying therapeutic strategies for this devastating disease. The theoretical framework on disease progression in the preclinical and clinical phase of PD presented by Braak and colleagues in 2003 (1;2) led to many discussions and studies on the clinical relevance of α-synuclein-immunoreactive Lewy bodies (LBs) and Lewy neurites (LNs) and selective vulnerability in early stage PD (3;4). Braak and colleagues showed that the distribution of LBs and LNs in the CNS is not random but predictable, which was later confirmed by others (5-8). The pathology most likely starts in predelicted sites, i.e. olfactory bulb (OB), dorsal motor nucleus of vagal nerve (DMV) or in the PNS (9;10). Importantly, the classification by Braak et al. (1), emphasizes the vulnerability of non-dopaminergic nuclei and systems, most likely contributing to non-motor symptoms in early stages of the disease.

Little is yet known about the molecular mechanisms contributing to neuronal cell death, selective vulnerability and LB pathology in the premotor stages of PD. Elderly with incidental Lewy body disease (iLBD) may serve as a model to study these mechanisms and discover novel therapeutic targets.

The overall aim of this thesis was to gain insight into molecular mechanisms leading to neurodegeneration and α-synuclein pathology in the premotor and motor stages of PD. To this end, we studied dopaminergic cell loss and LB pathology in the substantia nigra (SN) in pathologically-confirmed iLBD and PD donors with Braak α-synuclein stages ranging from 0 to 6 (Chapter 2). Furthermore, the transcriptome of postmortem brain tissue of iLBD and PD donors was studied in an attempt to identify known and novel molecular pathways contributing to neurodegeneration and α-synuclein pathology in selective vulnerable regions in early stage PD (Chapters 3-5).

In chapter 1, we reviewed the literature regarding non-motor and motor symptoms during disease progression of PD and the main neuropathological features of PD and PD-related disorders. The progression of the α-synuclein pathology throughout the brain is thought to correlate with the clinical progression of the disease and provides a framework for studying PD progression (1;3;11). Linkage, genome sequencing and GWAS studies have provided evidence for mitochondrial defects, oxidative stress, protein clearance and synaptic dysfunction in PD based on the identification of mutations or duplications in genes, such as DJ-1, Parkin and α-synuclein, implicated in juvenile and early-onset PD (12-14). Transcriptome studies using human post-mortem material have aided in our understanding of pathogenetic mechanisms involved in PD, pinpointing mechanisms such as inflammation, synaptic dysfunction, aberrant protein clearance and altered protein synthesis to play a key role in end-stage PD (15-17). Based on literature, we expect a role for autophagy and protein synthesis and synaptic dysfunction to play a role in the early stages of PD.

In chapter 2, we studied neuronal loss in the SN in donors with Braak α-synuclein stages ranging from 0 to 6. Donors with severe tangle, amyloid-β or other concomitant pathology were excluded, so degeneration based on Alzheimer’s disease pathology or other neurological or psychiatric disorders is highly unlikely. We demonstrated that nigral neuronal loss already occurs in elderly in which the α-synuclein pathology is limited to the OB, DMV and locus coeruleus (LC) (Braak α-synuclein stage 1-2). This may indicate that the presence of α-synuclein aggregates in these regions is associated
with nigral neuronal loss, suggesting that loss of trophic support or retrograde degeneration could contribute to nigral neuronal loss. However, there may be also as yet unidentified factors other than α-synuclein that play a role in the degeneration of nigral or other neurons in PD. Our findings are in accordance with the recently published data by Milber and colleagues (18), who demonstrated that dopaminergic cell loss may precede local α-synuclein pathology in the SN in iLBD. We showed that the progression from Braak α-synuclein stage 3 to stage 4 is associated with a significant decline in neuronal cell density (up to 46%) followed by a less pronounced loss between Braak stages 4 and 5, and 5 and 6. This suggests that the disease progression is not linear but rather negatively exponential, as previously suggested by others (19;20). Moreover, a negative correlation was observed between neuronal density and local α-synuclein burden in the SN of PD patients, which is also in line with the findings in other studies (19;21;22). In our study, no correlation between disease duration and nigral neuronal density was found, which is in contrast to the findings by others (23;24). One explanation would be that the variability in disease duration in our cohort was substantial and the number of PD patients in our study was relatively low.

In chapter 3, we confirmed that previously described molecular mechanisms such as the immune response, protein clearance and axonal function are deregulated in the SN of PD donors compared to age-matched controls (16;17;25). Interestingly, pathways such as mammalian target of rapamycin (mTOR) and eukaryotic initiation factor 2 (eIF2) signaling were already deregulated in the SN of donors with Braak stages 1 and 2. In addition, the mTOR and eIF2 signaling pathways remain altered in Braak stages 3 and 4 and Braak stages 5 and 6, which illustrates the importance of these pathways during disease progression of PD. The alterations in mTOR and eIF2 signaling pathways have previously been linked to PD by post-mortem and peripheral mononuclear blood cells transcriptome studies (26). Influencing these pathways may hold the key to alter disease progression in PD and as alterations in eIF2 are observed in the blood cells, deregulated elements of the eIF2 pathway may serve as biomarkers for PD.

Neuropathological studies have shown that OB is one of the first brain regions affected with α-synuclein pathology in PD (27). However, neuronal loss in the anterior olfactory nucleus or loss of dopaminergic neurons in the OB is not detectable in PD patients (28;29). Transcriptome analysis of post-mortem OB tissue of control, iLBD and PD donors (chapter 4) revealed deregulation of genes and pathways involved in protein transport and disposal systems, and in glutamate signaling in early stage PD. Deregulated processes included protein folding, ubiquitination and proteasomal degradation, Golgi membrane traffic, protein deglycosylation, protein import in mitochondria, cytoskeleton dynamics, apoptosis, transcription and pre-mRNA splicing in end-stage PD.

In chapter 5, we have used the transcriptome of the OB, medulla oblongata (MO), LC and SN tissue of iLBD, PD donors and age-matched controls, to gain insight in region-specific and common molecular mechanisms underlying α-synuclein pathology and disease progression in early stage PD. Comparative analysis of the transcriptome profiles showed that pathways related to the immune response, protein synthesis and autophagy are deregulated in all above-mentioned regions in iLBD donors compared to controls, suggesting that these mechanisms may play a role in the pathogenesis of PD. A significant down regulation of pathways involved in endocytosis was observed, as well as an up regulation in endosomal markers and proteins that are involved in the sequestering and autophagic degeneration of proteins, including heat shock protein 70 (HSP70), phosphatidylethanolamine (PE) and lysosomal-membrane protein 2 (LAMP2) (30). In addition, we observed a significant down regulation of synaptic vesicle genes and genes involved in synaptic transmission in the brainstem.
and OB of iLBD compared to controls including solute carrier family 10, member 4 (SLC10A4) and synaptic vesicle glycoprotein 2 c (SV2C) (31;32) and an up regulation of genes related to plasticity (33). All regions display an up regulation of inflammatory pathways and genes in PD and iLBD donors compared to controls (34), including CTLA4 signaling in cytotoxic T lymphocytes and B cell receptor signaling. In addition, major histocompatibility complex, class II, DR alpha (HLA-DRA) was deregulated which indicates that the immune system may already triggered in the early stages of the disease (35). Based on our findings, it is not possible to determine whether the inflammation and immune response serves as protective mechanisms or, alternatively, contributes to the progression of the disease. Molecular mechanisms involved in the formation of α-synuclein aggregates in all regions in iLBD included the ubiquitin proteasome system (UPS) and mitochondrial dysfunction. In the SN, prior to the presence of α-synuclein aggregates, few transcriptional changes linked to mitochondrial dysfunction were found. Significant deregulation of mitochondria was observed in brainstem tissue samples with α-synuclein aggregates. In line with our observations, it has recently been shown that an impaired autophagy, mitophagy and dysfunctional UPS results in accumulation of dysfunctional mitochondria and α-synuclein aggregation in a mouse model of neuropathic Gaucher disease, a lysosomal disorder (36).

We identified several novel molecular pathways associated with α-synuclein aggregation in iLBD. These pathways include down regulated isoleucine and valine degradation and up regulated polyamine regulation. Valine and leucine are branched-chain amino acids (BCAA) and are thought to interact with mTOR and EIF4. The latter are elements of the autophagy process (37). As shown in chapter 3, mTOR activity is decreased throughout disease progression in the SN. Therefore regulation of valine and leucine might directly or indirectly regulate mTOR activity. Furthermore, an increase in BCAAs may promote chronic inflammation and neurodegeneration in a primary microglia cell culture (38). Further studies exploring mechanisms affected by altered levels of BCAAs are needed to elucidate the role of these pathways in PD pathology.
References

20. Kordower JH, Olanow CW, Dodiya
37. Cota D, Matter EK, Woods SC, Seeley RJ. The role of hypothalamic mammalian target of rapamycin complex 1 signaling