The biology of cognitive decline and reduced survival in Parkinson disease

Prognostic factors in a population-based cohort

David Bäckström

Department of Pharmacology and Clinical Neuroscience
Umeå 2019
This work is protected by the Swedish Copyright Legislation (Act 1960:729)
Responsible publisher under Swedish law: the Dean of the Medical Faculty
Dissertation for PhD
ISBN: 978-91-7855-022-7
ISSN: 0346-6612
New Series Number 2006
Cover images (lower row): Diffusion Tensor Imaging, adapted from from paper V.
Drawings (top row and back cover) by Santiago Ramón y Cajal, from left to right:
1. Glial cells of the cerebral cortex, 1904, ink and pencil on paper.
2. Golgi stained pyramidal cells of the cerebral cortex (detail), ink and pencil on paper.
3. The pyramidal neuron of the cerebral cortex, 1904, ink and pencil on paper.
4. Astrocytes in the hippocampus of the human brain.
Back cover: Calyces of Held in the nucleus of the trapezoid body. The calyx of Held contain the largest synapses in the mammalian central nervous system.
All drawings by Santiago Ramón y Cajal are reprinted with the kind permission of the Cajal Institute, Spanish National Research Council (CSIC) Madrid, Spain.
Printed by: Umu print service, Umeå university
Umeå, Sweden 2019
To my family,

with a particular tribute to my grandfather.
# Table of Contents

Abstract  
Abbreviations  
List of original publications and manuscripts  
Kort sammanfattning på svenska  

**Background**  
*Introduction and a historical remark*
  - Parkinsonism
  - Parkinson disease
  - Atypical parkinsonism: The three ugly cousins
*The etiology of Parkinson disease*
  - Selective vulnerability of dopaminergic neurons in substantia nigra
  - Environmental risk factors
  - Alpha-synuclein and the prion-like spread of pathology
  - Genetic causes of Parkinson disease: impaired “cleaning” systems of the cell
  - Inflammation
*The variable prognosis of Parkinson disease*
  - Subtypes of Parkinson disease
  - Mortality
  - Cognitive impairment
*Biomarkers in parkinsonism*
  - Cerebrospinal fluid
*The causes of cognitive impairment in Parkinson disease*
  - Risk factors
  - The neurochemistry of cognitive impairment
  - Structural correlates of dementia (PDD)
*Rationale for research on the molecular diagnosis of neurodegenerative diseases*

**Aims of the investigation**

**Materials and Methods**
  - Ethics
  - Study populations
  - Investigations

**Results**
  - Neurodegenerative diseases diagnosed in the project
  - Mortality in a population-based cohort with parkinsonism (paper I)
  - Higher neurofilament in CSF in PSP than in Parkinson disease (paper II)
  - CSF patterns that preceede PDD in Parkinson disease (paper II)
  - Functional variability in dopamine-associated genotypes and cognitive decline (papers III and IV)
  - Neurofilament in CSF, disease severity and survival in Parkinson disease (paper V)

**Discussion**
  - "Benign" phenotypes and survival in idopathic parkinsonism
  - Immune reactivity and neurofilament levels in CSF in Parkinson disease
  - The different causes of cognitive decline in Parkinson disease: a hypothetical "wet-dry" model
  - Methodological considerations and limitations

**Summary and clinical perspectives**

**Acknowledgements**

**References**

**Appendix**
Abstract

Parkinson disease (PD) is a progressive neurodegenerative disease that affects about 1% of the population over 60 years. The cardinal symptoms are motor disabilities but cognitive decline is also common. About 50% of all persons with PD develop dementia within 10 years after disease onset. Dementia in PD account for high social costs and has large, negative effects on quality of life.

**Aims.** The aim of the study was to investigate clinical, neurobiological and genetic factors of importance for progression and for the prognosis in PD and parkinsonism. First, we aimed to describe mortality and risk factors for death, including possible associations with cognitive dysfunction, in patients with idiopathic parkinsonism. Second, we aimed to study if biomarkers in the cerebrospinal fluid (CSF) are useful for the diagnosis of different forms of idiopathic parkinsonism and prediction of cognitive decline in PD.

**Methods.** A population-based cohort consisting of patients with new-onset, idiopathic parkinsonism was studied prospectively. After screening in a catchment area of ~142 000 inhabitants in Sweden, 182 patients with parkinsonism were included. The patients were investigated comprehensively, including neuropsychological testing, multimodal neuroimaging and genetic and biosample analyses. During follow up, 143 patients were diagnosed with PD, 13 with multiple system atrophy (MSA), and 18 with progressive supranuclear palsy (PSP). A total of 109 patients died.

**Results.** Patients with MSA and PSP had the shortest life expectancy. PD patients who presented with normal cognitive function had a largely normal life expectancy. In contrast, the mortality was increased in PD patients with cognitive impairment, freezing of gait, hyposmia, and mildly elevated leukocytes in the CSF. Also of importance for the prognosis, patients with PD with an early CSF pattern of high Neurofilament light protein, low β-amyloid, and high heart fatty acid binding protein had an 11.8 times increased risk of developing PD dementia (95% CI 3.3-42.1, p <0.001), compared with PD patients with a more ”normal” CSF pattern. Variation in genes associated with dopamine function was also associated with some effects on cognitive functions in PD.

**Conclusions.** PD subtypes, for instance the subtype characterized by cognitive decline, have distinguishing clinical, neurochemical and neurobiological traits, which are of importance for the prognosis and the survival. An early CSF analysis is useful for predicting cognitive decline. The finding of a low-grade immune reaction in the CSF of patients with PD may have clinical implications. In clinical practice, CSF biomarkers could be useful for improving diagnosis and prognostication.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-synuclein</td>
<td>Alpha-synuclein</td>
</tr>
<tr>
<td>β-amyloid</td>
<td>Beta-amyloid</td>
</tr>
<tr>
<td>Aβ42</td>
<td>The 42-aminoacid form of Beta-amyloid</td>
</tr>
<tr>
<td>CamPaIGN</td>
<td>Cambridgeshire Parkinson’s Incidence from GP to Neurologist</td>
</tr>
<tr>
<td>CBS</td>
<td>Corticobasal syndrome</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase; an enzyme involved in dopamine degradation</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DAT imaging</td>
<td>Dopamine active transporter imaging</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRD2</td>
<td>Dopamine receptor D2</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy; a measurement in diffusion tensor imaging</td>
</tr>
<tr>
<td>FP-CIT</td>
<td>123I-(omega)-flouropropyl-2-ß-carbomethoxyl-3-ß-(4-iodophenyl)nortropane</td>
</tr>
<tr>
<td>HFABP</td>
<td>Heart fatty acid binding protein</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>MAPT</td>
<td>Microtubule-associated protein tau</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>MR / MRI</td>
<td>Magnetic resonance / magnetic resonance imaging</td>
</tr>
<tr>
<td>MSA</td>
<td>Multiple system atrophy</td>
</tr>
<tr>
<td>MSA-C</td>
<td>Multiple system atrophy, the Cerebellar subtype</td>
</tr>
<tr>
<td>MSA-P</td>
<td>Multiple system atrophy, the Parkinsonism subtype</td>
</tr>
<tr>
<td>NfL</td>
<td>Neurofilament light chain protein</td>
</tr>
<tr>
<td>NYPUM</td>
<td>New-onset Parkinsonism in Umeå</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson disease (also spelled Parkinson’s disease)</td>
</tr>
<tr>
<td>PD-MCI</td>
<td>Mild cognitive impairment in Parkinson disease</td>
</tr>
<tr>
<td>PDD</td>
<td>Parkinson disease with dementia</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PIGD</td>
<td>Postural instability and gait disturbance; a subtype of Parkinson disease</td>
</tr>
<tr>
<td>PPMI</td>
<td>The Parkinson Progression Markers Initiative</td>
</tr>
<tr>
<td>PSP</td>
<td>Progressive supranuclear palsy</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardized mortality ratio</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed Up and Go test</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
</tr>
</tbody>
</table>
List of original publications and manuscripts

(Presented in the order in which they are discussed).


Papers are reprinted in this thesis with the kind permission of the respective publisher.
Kort sammanfattning på svenska

Parkinson sjukdom (stavas också Parkinson’s sjukdom) är en kronisk och progressiv neurodegenerativ sjukdom som ger upphov till skakningar (tremor), långsamhet (bradykinesi) och rigiditet. Den är, efter Alzheimer sjukdom, den näst vanligaste neurodegenerativa sjukdomen, med omkring 10 miljoner drabbade i världen.

Utöver de klassiska, motoriska symtomen orsakar Parkinson sjukdom hos de flesta patienter även andra symtom, som nedsatt luktsinne, blodtrycksfall, livliga drömmar, REM-sömn störning, förstoppning och kognitiv (tankemässig) nedsättning. Demens har i tidigare studier visats utvecklas hos omkring 50% av alla med Parkinson sjukdom inom 10 år från debuten. Det är ett av de symtom som påverkar livskvaliteten mest hos de med Parkinson sjukdom.

I denna studie undersökte vi kliniska, biologiska och genetiska faktorer som kan vara av betydelse för prognosen, utvecklingen och dödligheten i Parkinson sjukdom och andra närbesläktade, neurodegenerativa sjukdomar. Vi undersökte 182 personer som nyligt hade utvecklat symtom. Sjukdomarna multipel systematrofi (MSA) och progressiv supranukleär paralys (PSP) var dödligare än Parkinson sjukdom, med en dödlighet som var över tre gånger högre än i den svenska normalbefolkningen. I Parkinson sjukdom hade överlevnaden att göra med om en patient hade en mild kognitiv störning (MCI, för engelskans "mild cognitive impairment") eller inte vid tiden för diagnos. De som inte hade MCI hade en i stort sett normal överlevnad, medan de som hade MCI hade en överlevnad som var 2.17 (95% konfidensintervall: 1.56 – 2.93, \( p <0.001 \)) gånger högre än i normalbefolkningen. Personer med Parkinson sjukdom som hade en mycket lätt pleocytos av mononukleära vita blodkroppar i cerebrospinalvätskan (vilket kan tolkas som en lätt inflammation i nervsystemet) hade också en markant ökad dödlighet.

Det är inte bevisat att en lätt inflammation i nervsystemet är kopplad till ökad dödlighet i Parkinson sjukdom men det är betydelsefullt att undersöka detta vidare eftersom det, i framtiden, eventuellt vore möjligt att behandla en sådan inflammation.

Demens utvecklades hos en betydande andel av de personer med Parkinson sjukdom som följdes och detta var kopplat till ett särskilt proteinmönster i cerebrospinalvätskan, i den tidiga sjukdomsfasen. De som tidigt utvecklade demens hade också en ökad dödlighet under uppföljningsperioden (som var cirka 8-14 år) men dödligheten var ännu större hos de som hade MCI vid studiens start.

Genom att göra några relativt enkla test, som test av luktsinnet, test av kognitiva funktioner, neurologisk undersökning och cerebrospinalvätskeprov får man mycket information om vilken typ av Parkinson sjukdom en person har.
Background

General introduction and a historical remark

Parkinson disease (PD) is a progressive and debilitating neurodegenerative disease that affects about 1% of the population over 60 years. It is the second most common neurodegenerative disease (after Alzheimer disease), with about 10 million affected worldwide. With increasing life expectancy in the general population, the number of people affected by PD is expected to increase and the relevance for the healthcare system will rise. The cardinal symptoms of PD are motor disabilities in the form of tremor, bradykinesia, and rigidity caused by lack of dopamine in key brain areas, mainly in the striatum, but cognitive decline is also common. Although PD is currently incurable, increasing knowledge about the pathology, as well as gene function in PD, creates opportunities and hopes for new disease-modifying therapies.

James Parkinson, from whom PD was named, missed the fact that cognitive decline is a feature of PD in his seminal "Essay of the shaking palsy", in which he otherwise succinctly describes the clinical features of PD (Parkinson 1817). Following Dr. Parkinson, PD was mainly regarded as a motor disease. Sixty years later, the influential French neurologist Jean-Martin Charcot observed in his "Lectures on diseases of the nervous system", that at a given point in PD (which he called "maladie de Parkinson") the "mind becomes clouded and the memory is lost" but these statements seemed to attract little attention. It was not until the 1970’s that dementia was recognized to be an important part of the clinical picture in PD (Marttila 1976).

During the last few decades, cognitive decline has again been widely recognized and studied, given that it is one of the major causes of severe disability in PD, but its exact causes remain largely unknown. Development of frank dementia in PD is estimated to occur in a high proportion of patients with PD, in about 50% of all cases at 10 years after disease onset (Williams-Gray 2013). PD dementia accounts for high social costs and has large, negative effects on quality of life and survival. However, a relatively large proportion of patients with PD lives for decades without apparent cognitive decline (Aarsland 2007). It is important to explain why some people with PD do not develop dementia. It is also important to explain the reasons for a shortened life expectancy in PD. This thesis presents data from a prospective, population-based study of parkinsonism (the NYPUM study) in order to investigate the prognosis.
**Parkinsonism**

Parkinsonism is an umbrella term for the symptoms and findings of bradykinesia (slowness of movements), rigidity, resting tremor, and postural instability and is called idiopathic when it occurs sporadically, without an obvious cause. By far, the largest occupant of this umbrella is idiopathic PD. However, several neurological diseases have features that overlap with idiopathic PD. The atypical parkinsonian diseases exhibit parkinsonism, hindering early differential diagnoses. Other diseases, such as essential tremor or normal pressure hydrocephalus, may have one or more symptoms in common with PD. Secondary parkinsonism, e.g. caused by neuroleptic drugs or multiple strokes (which is called cerebrovascular parkinsonism), also needs to be differentiated from PD.

**Parkinson disease**

The lifetime risk of idiopathic PD has been estimated to be about 3-4% in developed nations. According to a recent meta-analysis, the prevalence in all ages in the population is 315 PD cases per 100,000 (Pringsheim 2014), which makes PD the most common cause of parkinsonism. In PD, tremor, bradykinesia and rigidity are usually asymmetrical at initial presentation, is improved by dopaminergic therapy; and loss of nigrostriatal dopaminergic neurons is detectable by neuroimaging. Tremor is a presenting feature in only about half of all patients with PD, but 90 – 100% of patients with PD have tremor at some stage during their disease course (Jancovic 2008, Hughes 1993, Martin 1973, Rajput 1991). Current diagnostic criteria for PD require the gradual onset of bradykinesia, and at least one of tremor, rigidity or postural instability, and exclusion of other causes of parkinsonism (Gibb 1989). Postural instability is a typical symptom of PD but occurs later in the disease course compared to the other motor symptoms.

Although current diagnostic criteria mainly reflect the motor symptoms (Gibb 1989), it is well recognized that several non-motor symptoms are also typical for PD. Non-motor symptoms affects 98.6% of the patients with PD at one point or another (Stern 2012), and include, among others, a weakening sense of smell (hyposmia), constipation, orthostatic blood pressure dysregulation, sleep disorders - especially rapid eye movement (REM) sleep behaviour disorder, depression, apathy and cognitive decline. Many of these symptoms have a predominantly non-dopaminergic basis and resolve less-well to dopaminergic therapy than the classical motor symptoms. Although they often worsen with longer disease duration and give rise to a significant disease burden in advanced phases (van Uem), large cohort studies have shown that non-motor symptoms of PD are often already present in newly diagnosed patients. The early non-motor symptoms include constipation, urinary urgency, orthostatic symptoms, falls, forgetfulness, impaired concentration, hallucinations, sad feelings, excessive daytime sleepiness, and vivid dreams (Erro 2013, Khoo 2013). In fact, these symptoms often precede the onset of motor disease (Berg 2015). Prodromal symptoms such as hyposmia, constipation, and REM
sleep behavior disorder can be present up to 20 years before the characteristic motor onset of PD (Savica, 2018).

**Atypical parkinsonism: The three ugly cousins**

The atypical parkinsonian diseases are multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS), which can be called the three ugly cousins of PD. They are neurodegenerative disorders with a more aggressive disease course and higher mortality than PD. A common feature of patients with atypical parkinsonism, as well as those with secondary parkinsonism, is that they respond less to dopaminergic therapy than patients with PD, or not at all. Lewy body dementia is sometimes also referred to as atypical parkinsonism.

MSA is characterized clinically by early autonomic failure, parkinsonism (predominantly in the MSA-Parkinsonism subtype), cerebellar ataxia (predominantly in the MSA-Cerebellar subtype), and pyramidal signs, in various combinations. MSA is an alpha (α)-synucleinopathy, with aggregated α-synuclein inclusions mainly affecting glial cells, while PSP and CBS are tauopathies, which are defined by intracellular inclusions of aggregated microtubule-associated protein tau (MAPT). The prevalence of MSA is estimated to be from about 2 to 5 per 100,000 in European and North American studies (Wenning, 2013, Faniculli, 2015), with a mean age of onset of 55 years.

PSP is characterized by supranuclear ophthalmoplegia (typically vertical gaze palsy), parkinsonism, recurrent falls and cognitive decline. The prevalence of PSP is approximately 6 to 7 per 100,000 (Schrag 1999), with a mean age of onset around 65-68 years. The median survival in both MSA and PSP is around 6 – 10 years after onset (Wenning, 2013, Faniculli, 2015, Schrag 1999). Pathological studies of patients diagnosed as having PD suggest that both MSA and PSP are underrecognized (Joutsa 2014). About 80% of the patients that are misdiagnosed as having idiopathic PD actually have MSA or PSP. CBS is more uncommon than MSA and PSP and is characterized by an asymmetrical, akinetic-rigid parkinsonian syndrome associated with atypical motor features like dystonia, ”alien limb” phenomena, and myoclonus and cortical findings such as apraxia, cortical sensory loss and variable degrees of progressive dysphasia.

**The etiology of Parkinson disease**

Although the exact causes for neurodegeneration in PD are still largely unknown, the underlying pathology of PD and many of the risk factors for the disease are characterized. In addition, during the last 20 years, the discovery of hereditary, monogenetic forms of PD has uncovered many important aspects of the cellular and molecular disease mechanisms in PD.
Selective vulnerability of dopaminergic neurons in the substantia nigra

The main pathological injury in PD is the death of dopaminergic neurons in the substantia nigra (SN) pars compacta, that project to the caudate nucleus and putamen (collectively termed the striatum). These neurons, together with their projections, are termed the nigrostriatal tract. At the time when motor symptoms emerge, it is estimated that about 50% of the nigral dopaminergic neurons and 80% of striatal dopamine are already lost (Fearnley 1991). Dopamine depletion, in particular in the dorsolateral putamen, is strongly linked to bradykinesia (Albin 1989). It has been hypothesized that the dopaminergic neurons of the SN are particularly sensitive to mitochondrial dysfunction and oxidative stress (Surmeier 2007). Furthermore, the dopaminergic SN neurons belong to a neuromodulatory control network, with diffuse, and highly branched axonal arbors that regulate other cell populations by tonic activity and release of neurotransmitters (Surmeier 2017). This is also true for other neurons that are affected by cell death in PD. The pathology in PD usually reaches other areas than the SN, which shows that other neurons are eventually susceptible. However, the exact mechanisms by which dopaminergic neurons and other neurons are selectively vulnerable in PD are currently unknown.

Environmental risk factors

The most consistent risk factor for PD is age. There are young-onset cases, but the risk of developing PD increases dramatically after 50 years. The mean age of onset is in the late ’60s, and in population-based studies around 70 years (Williams-Gray 2007, Moustafa 2013). PD affects about 1% of the general population over 60 years of age, and by the age of 85, it affects 4-5%. The peak incidence is around 80 years, which probably reflects underdiagnosis and diagnostic nihilism in higher ages. Although sparse data indicate a lower prevalence in Africa than in European or Asian populations, PD occurs worldwide and is slightly more common in males than in females.

Because age and sex are not modifiable, epidemiological studies have looked for environmental and behavioral risk factors. Risk factors that have been found to increase the risk of PD are exposure to pesticides, consumption of dairy products, a history of melanoma and traumatic brain injury. Smoking, caffeine consumption, higher serum urate concentrations, physical activity, and use of ibuprofen and other common medications have been associated with a lower risk in large cohort studies in several world populations (Ascherio 2016). When studied in animal models, many of these factors have neurotoxic or neuroprotective properties. The most consistent associations between pesticides and PD risk is for pesticides known to affect mitochondrial complex I (including rotenone) or to cause oxidative stress (including paraquat). This implicates that mitochondrial dysfunction and/or oxidative stress can contribute to PD (Tanner 2011). The mechanisms for the protective effects associated with smoking and coffee drinking
are not known in detail but are believed to be explained by exposure to nicotine and to the adenosine receptor blocking effect caused by caffeine, respectively (Ascherio 2016).

**Alpha-synuclein and the prion-like spread of pathology**

In 1912, Friederich Lewy described intracytoplasmic bodies in the dorsal motor nucleus of the vagus nerve from patients with PD, which were later termed Lewy bodies. Further research showed that the presence of the intraneuronal proteinaceous inclusions, termed Lewy bodies or Lewy neurites, in the substantia nigra in the brainstem is a defining characteristic of PD. The major content of Lewy bodies has been found to be filamentous forms of the synaptic protein α-synuclein and the small regulatory protein ubiquitin (Spillantini 1997). In PD, these inclusions also occur in other brainstem nuclei, and in diverse other locations, e.g. the olfactory bulb and the enteric nervous system.

In 1997, in a striking convergence of genetics and pathology, linkage analysis of the American-Italian Contursi kindred and in several Greek kindreds showed that mutations in the α-synuclein gene (SNCA), which change the α-synuclein protein, cause early-onset, autosomal dominant PD (Golbe 1996, Polymeropoulos 1997). Researchers initially identified a point mutation in α-synuclein and, following this discovery, duplications and triplication of the normal α-synuclein gene (SNCA duplications and triplications) were also shown to cause human PD (Chartier-Harlin 2004, Singleton 2003). This led to the conclusion that both elevated levels of normal, wild-type α-synuclein and mutated α-synuclein (which possibly is more resistant to degradation) could cause a toxic gain of function that led to the degeneration of dopaminergic neurons.

Several lines of research have established pathological deposition and aggregation of α-synuclein as a core cause of PD. In cellular and animal models using α-synuclein overexpression, aggregation and deposition of α-synuclein precede neuronal cell death, and strategies to inhibit the aggregation process reduce neurodegeneration and improve motor deficits in many species (Lashuel 2013). Furthermore, in animal models of PD, overexpression of α-synuclein is particularly harmful to dopaminergic neurons, which die selectively.

It is not entirely clear why α-synuclein is neurotoxic. However, studies of animal models as well as of human brains from patients with PD show that abundant α-synuclein polymerize abnormally into protofibrils and filaments, which eventually aggregate to form cytoplasmic Lewy bodies, which can impair the function of several types of neurons and glia. Upon reaching critical intracellular concentrations, α-synuclein can self-aggregate (Conway 2000). In some PD cases, α-synuclein aggregates can fill most of the cytoplasm of affected neurons, thereby potentially impairing normal cellular trafficking, and sensitizing the cells to death from other stresses.
The "prion-like" properties
In sporadic (idiopathic) PD, Lewy bodies appear in early phases in the lower regions of the brainstem, such as the dorsal motor nucleus of the glossopharyngeal and vagal nerves, the noradrenergic locus ceruleus, the olfactory bulb, and in the enteric nervous system (called Braak stages 1 and 2). As shown in the right column of Figure 1, the α-synuclein pathology then spreads in a seemingly specific pattern as the disease progress, first through the pons to the midbrain, including substantia nigra, and to basal forebrain structures including the nucleus basalis of Meynert (called Braak stages 3 and 4). In later disease stages, the pathology appears in the limbic system and diffusely in the neocortex (called Braak stages 5 and 6).

Figure 1. Distribution of β-amyloid, tau and α-synuclein inclusions in the human brain. Left: β-amyloid plaques develop first in basal temporal and orbitofrontal neocortex (Phase 1). They then appear throughout the neocortex, hippocampal formation, amygdala, diencephalon and basal ganglia (Phases 2 and 3). In severe cases of Alzheimer disease, the pathology spreads to the mesencephalon, lower brainstem and cerebellar cortex (Phases 4 and 5). Middle: Tau inclusions develop in the locus coeruleus and in transentorhinal and entorhinal regions (Stages I and II), followed by tau inclusions in the hippocampal formation and parts of the neocortex (Stages III and IV), followed by large parts of the neocortex (Stages V and VI). Right: The first α-synuclein inclusions appear in the olfactory bulb and the dorsal motor nucleus of the vagal and glossopharyngeal nerves of the medulla oblongata (Stages 1 and 2). From the brainstem, the pathology ascends through the pons to midbrain and basal forebrain (Stages 3 and 4), followed by the neocortex (Stages 5 and 6). The figure is based on the work of Braak, Del Tredici, and

Until recently, little was known about how the PD pathology, including α-synuclein containing Lewy bodies, progress in the CNS from one structure to other structures. It was hypothesized that the pathology occurred independently in different cells but, more recently, studies suggest that aggregates containing α-synuclein protein spread in the CNS through a prion-like transcellular propagation of "seeds".

Evidence that indicates that α-synuclein can spread from neuron to neuron is that Lewy bodies were found in embryonic midbrain dopaminergic neurons that were experimentally transplanted into the striata of PD patients more than 10 years after the procedure, likely demonstrating host-to-graft spreading (Kordower 2008). More recently, the demonstration that injected α-synuclein inclusions spread from the injection site to distant brain regions in animal models further supported the notion of a stereotypical spreading pattern in human PD (Desplats 2009, Hansen 2011). Aggregated, α-synuclein may spread trans-synaptically in structurally connected networks (also known as connectomes) in the PD affected brain. Filamentous α-synuclein, or other pathological proteins, released from cells may also be taken up by surrounding astrocytes and microglia, which could expand the pathology in the nervous system.

The spread of pathology in PD is called prion-like because of its similarity to the mechanism of propagation of brain pathology in another neurodegenerative disease; the rapidly progressive and fatal human prion disease Creutzfeldt-Jacob disease. Creutzfeldt-Jacob disease can be sporadic or (in about 7.5% of cases) familial and causes neurodegeneration through transcellular propagation of a misfolded prion protein, named PrPSc. The mechanism of spreading pathology in PD is also in agreement with recent hypothetical models of disease progression in common neurodegenerative diseases such as Alzheimer’s disease and tauopathies (including PSP and CBS), in which filamentous protein aggregates of insoluble Beta-amyloid and Tau, respectively, propagate in connectome-defined “spreading” patterns in the brain (Figure 1). However, in contrast to Creutzfeldt-Jacob disease, no examples of transmission of PD from human to human have been demonstrated (Beekes, 2014). Hence, it might be more reasonable to classify aggregated α-synuclein as an endogenous prion.

Genetic causes of Parkinson disease: impaired “cleaning” systems of the cell

During the last 20 years, several monogenetic forms of PD and, in addition, many genetic risk factors that increase the risk to develop sporadic PD have been identified. These investigations show that PD is genetically heterogeneous. The monogenetic forms of PD are caused by a single mutation in a dominantly or recessively inherited gene and are
relatively rare. They are estimated to account for about 5-10% of all cases of PD (Kalineri 2016). The remaining 90-95% of cases of PD are regarded as sporadic and idiopathic, although occurring more commonly in families than what is expected by occurrence by chance. In the population with familial PD, the known monogenetic mutations collectively account for about 30% of cases.

To date, at least 19 disease-causing genes for monogenetic PD have been identified (Deng 2018). Some highly penetrant forms of monogenetic PD are caused by autosomal dominant mutations in SNCA, LRRK2, and VPS35 and autosomal recessive mutations in PINK, DJ-1, and Parkin. These mutations can cause PD with a phenotype that is similar or identical to sporadic, idiopathic PD, but some of them are associated with atypical PD features, including early onset (before 40 years of age), dystonia and dyskinesia. The discovery that SNCA mutations (including multiplications of the gene) cause PD showed that abundant α-synuclein is a key feature in PD pathogenesis. PD caused by SNCA mutations is similar to sporadic PD, except for the tendency for more marked autonomic dysfunction, speech problems, behavioral changes, and cognitive decline. These cases often show widespread α-synuclein deposits, including in the cortex; both in neurons and in glia (Poulopoulos 2012). Mutations in the LRRK2 gene causes the most common variant of familial PD, which can be indistinguishable from sporadic PD. LRRK2 mutations are linked to defective endosome-to-lysosome trafficking, which may lead to dysfunction in vesicular transport in neurons.

Associated with recessive PD, Parkin gene mutations cause about 9% of young-onset (<50 years) and ~70% of the juvenile (<20 years) cases. These patients have an early onset PD (median 31 years), slow progression, levodopa responsiveness and commonly dystonia and motor fluctuations (Kasten 2018). Parkin is an E3 ubiquitin ligase, normally contributing to protein degradation, and mutations in this protein can, therefore, impair protein degradation of targeted proteins by the ubiquitin-proteasome system (Kalinderi 2016).

While sporadic, idiopathic PD was long thought to be a non-genetic disease, a large-scale meta-analysis of genome-wide association study (GWAS) data from 12 386 patients with PD and 21 026 controls recently showed that common genetic risk variants explained ~60% of the population-attributable risk for PD (Nalls 2011). Although the method may overestimate heritability, these analyses suggest that more than half of the PD in the population is explained by genetic makeup. In comparison to monogenetic forms of PD, genetic risk factor variants for sporadic PD are more common and less penetrant. Genes found to be associated with PD risk in GWAS include the SNCA and LRRK2 genes, that can also cause familial PD, and the MAPT and HLA-DRB5 loci. The most common genetic risk factor for PD known to date are glucocerebrosidase (GBA) gene mutations (Kalinderi 2016). These gene mutations lead to lysosome dysfunction and the failure of autophagosome-lysosome fusion (Schöndorf 2014) and are associated with PD with a
higher risk of cognitive decline than average in PD. Although there are also other possible routes of action, these mechanisms point to defective transport pathways to lysosomes as a common cellular pathology in sporadic as well as familial PD.

In summary, evidence from genetic PD indicates that factors that decrease the efficiency of protein clearance can cause PD. Experimental as well as genetic studies show that failure of the protein quality control systems, especially lysosomes, promotes the accumulation of misfolded α-synuclein and formation of inclusions in neurons (Desplats 2009). Although there are differences related to specific genes, PD gene mutations likely promote a toxic formation of α-synuclein aggregates in neurons, possibly in conjunction with environmental risk factors.

Interestingly, genes and pathways that are involved in cellular trafficking have also been associated with other neurodegenerative disorders such as Alzheimer disease, frontotemporal dementia and amyotrophic lateral sclerosis (Abeliovich 2016). This overlap between PD and other neurodegenerative disorders points to common biological pathways in cells which should be fruitful to study further, and which may explain, for instance, why Alzheimer disease type pathology seems to be common in PD.

Other genetic influences
The human genome contains a vast number of variations, many of which have no effect on health. When occurring in a single nucleotide in the DNA, variants are called single nucleotide polymorphisms. When the variants increase the risk of complex diseases, or change the phenotype of a disease, they are described as “functional”. Several functional variants have been linked to PD and to cognitive function, including variants in the MAPT, COMT, APOE genes. Of particular interest in this thesis, the COMT Val\textsuperscript{158}Met and the C\textsuperscript{957}T polymorphisms in the COMT and Dopamine Receptor D2 (DRD2) genes, which occurs commonly in the human population, have been found to affect dopamine function in the brain. Carriers of two COMT Val\textsuperscript{158}Val-alleles (158Val homozygotes) show about 40% higher COMT enzyme activity compared to 158Met homozygotes (Chen 2004), and lower prefrontal dopamine activation as measured by PET (Wu 2012), which is associated with altered cognitive functions. The DRD2 \textsuperscript{957}C/C genotype correlates with higher number of D2 receptors in extrastriatal, thalamic and neocortical areas (Hirvonen 2009), which also seem to affect cognitive functioning (Li 2013). These genetic variations may have a larger effect in older ages and in disease states such as in PD, compared to healthy, younger individuals, because of declining levels of neurotransmitters and reduced redundancy of brain function.

Inflammation
Both neuroinflammation and systemic inflammation may play a role in PD. Dopaminergic neuron terminal loss in the nigrostriatal tract in early PD is associated with
activation of microglial immune cells in these areas (McGeer 1988). Furthermore, a feature of the immune system in PD is an increase of proinflammatory cytokines (such as tumor necrosis factor-α, IL-1β, and IL-6) in the striatum as well as in the CSF (Baufeld 2017). Recently, the use of $^{11}$C-PK11195 positron-emission tomography (PET) brain imaging of immune activity showed increased microglial activation in PD compared with controls (Gerhard 2006, Ouchi 2005). Although microglial activation is a non-specific reaction, one PET study showed increased microglia activation in the brainstem in patients with early-stage PD, which correlated with the degree of motor dysfunction and with dopaminergic denervation as measured by other imaging methods (Ouchi 2005).

A different type of support for a role for immunity in PD is that the risk of PD is influenced by variation in the HLA-DRB5 genome locus, which is central to the genetic regulation of the immune system (Nalls 2011). Furthermore, a Swedish epidemiological study found that 6 of 33 types of autoimmune disorders studied were associated with an increased risk of also having PD, including hyperthyroidism, hypothyroidism, amyotrophic lateral sclerosis, multiple sclerosis, pernicious anemia, and polymyalgia rheumatica (Li 2012). The correlation between multiple sclerosis and PD has been confirmed in other studies. A genetic study of genome-wide data suggested that several autoimmune disorders may have immune system defects in common with PD (Witoelar 2017). These data indirectly support a role for inflammation in PD pathogenesis. In addition, two large, observational studies have shown a lower risk of PD associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the general population (Chen 2003, Chen 2005). The effect of NSAIDs has, however, not been replicated.

Deposition of $\alpha$-synuclein has an important role in the initiation and maintenance of neuroinflammation in PD (Sanchez-Guajardo 2015), but it is debated whether this inflammation has a protective or a disease-causing role in PD. Innate immune cells of the nervous system have phagocytic properties and are capable of internalizing and degrading cell debris and protein aggregates (Rannikko 2015, Kim 2015). Their activation might, therefore, be a secondary response to overwhelmed protein clearance systems in affected neurons. If causing efficient degradation of cell debris and protein aggregates, through the lysosome pathway, the neuroinflammatory response could be protective, but if causing further release of $\alpha$-synuclein, the process could perpetuate prion-like spreading.

The variable prognosis of Parkinson disease

PD is characterized by variable patterns of loss of brain neurons; in both dopaminergic and nondopaminergic pathways. This gives rise to different phenotypes of PD, which are associated with different risks of important outcomes, e.g., cognitive decline. The differences in phenotype and prognosis are likely explained by different patterns of underlying neurodegeneration in PD.
Subtypes of Parkinson disease

A commonly used classification of the motor phenotype of PD is the division into a tremor-dominant subtype (TR-D) and an akinetic-rigid subtype with postural instability and gait difficulty (PIGD), and an intermediate subtype (showing mixed symptoms) (Jancovic 1990, Thenganatt 2014); see figure 2. The rate of clinical progression differs between these subtypes, with a faster progression of motor dysfunction in the gait predominant (or PIGD) compared to the tremor-dominant subtype (Jankovic 2001). The gait predominant (or PIGD) subtype also has a higher propensity for cognitive decline and anxiety than tremor-dominant PD (Alves 2006, Heeden 2016). Other PD classifications, based on genetics or cluster analysis, have been proposed but in clinical practice, a division into clearly distinguishable clinical phenotypes, like the PIGD and tremor predominant subtypes, may be most straightforward.

The motor subtypes of PD correspond to different patterns of underlying neuronal loss. High-resolution MRI scans from the Parkinson's Progression Markers Initiative (PPMI) study estimated brain grey matter atrophy in PD in correlation to subscores in UPDRS III for rigidity, axial symptoms, and tremor. The total UPDRS III score (which measures all motor symptoms of PD) correlated with reductions of grey matter bilaterally in the putamen and the caudate, while reductions in the anterior striatum were associated with more severe rigidity, and reductions in the left anterior striatum were associated with axial symptoms. In contrast, no significant structural brain measures correlated with the severity of tremor (Li 2018).

Figure 2. A simplified diagram showing characteristics of the PIGD- and Tremor-dominant subtypes of PD. The overlapping Mixed subtype is shown in the center. PD = Parkinson disease; PIGD = postural instability and gait difficulty; CTC = cerebellothalamocortical; DAT = dopamine
The pathological heterogeneity of PD is another important factor, which may create "overlap syndromes" between, for instance, Alzheimer disease and tauopathies and PD (Colom-Cadena 2017). Alzheimer disease brain pathology (β-Amyloid and tau aggregates) contributes to cognitive decline, gait impairment and a shorter period between motor onset and dementia in PD (Irvin 2018). Post-mortem studies show that most pathologies in the cortex, including neocortical Lewy bodies, Alzheimer disease pathology, and cerebral angiopathy is more prevalent in non-tremor-dominant (e.g. PIGD) phenotypes than in tremor-dominant PD.

**Mortality**

Despite advances in treatment, most studies report reduced life expectancy in PD, but survival differs considerably across patients (Macleod 2014). In 2016, a study showed that a patient with new-onset PD could expect an average remaining lifespan of 14.6 years (De Pablo-Fernandez 2017). The standardized mortality ratio (SMR) in most modern PD mortality studies has been in the range of 1.5 to 2.7, which means that patients have death rates that are 50% - 170% higher than in the general population (de Lau 2006). The reasons for the shorter lifespans in many patients with PD are currently unclear. However, the facts that mortality is higher in PD than in the general population independently from comorbidities of PD (Driver 2008) and that mortality correlates with the severity of PD symptoms, as measured by clinical scales (Marras 2005, Forsaa 2010) suggests that disease-specific features of PD (such as α-synuclein pathology) account for, at least partly, the increased mortality (Bäckström 2018). Pneumonia tends to be a slightly more common cause of death in PD than in the general population.

High severity of PIGD symptoms was shown to be an independent predictor of a shorter life expectancy (de Lau 2014). A few prospective studies have also found dementia in PD (PDD) to be an independent risk factor for higher mortality (Lewy 2002, Willis 2012). PDD has been estimated to antedate death by about four years (Kempster, 2010) and studies of populations of patients with dementia generally show higher mortality rates than in idiopathic PD. For instance, a Swedish study of memory clinic patients followed during ten years showed that the mortality was over three times higher in patients with Lewy body dementia (LBD) and Parkinson's disease dementia (PDD), compared with the general population (Larsson 2018). Taken together, these studies indicate that patients with PD that develops dementia have a high mortality rate.
Cognitive impairment

As a result of the relative success of dopaminergic treatments and advanced strategies like deep brain stimulation and dopamine-delivery pumps for the motor symptoms of PD, PD patients survive and remain mobile into advanced disease stages, where cognitive impairment, psychosis, and other non-motor symptoms are major causes of disability and morbidity. Cognitive impairment has an important impact on the quality of life of patients and their family members (Svenningsson 2012) and is associated with increased risk of nursing home placement and (for PDD) early death.

Dementia that occurs one year or more after the onset of motor symptoms in PD is classified as PDD, while dementia occurring before this time point in a patient with parkinsonism is usually classified as Dementia with Lewy Bodies (constituting an arbitrary "one-year rule"). Studies of the prevalence of cognitive decline in PD have shown variable results, owing to differences in case selection and diagnostic criteria that are used. Throughout the disease course, the incidence of dementia in PD is estimated to be up to six times higher than expected in healthy individuals of the same age (Aarsland 2001), with a point prevalence of PDD of 24 to 31%. Studies of higher quality, using prospective and/or population-based designs, tend to give higher estimates of the PDD prevalence, closer to 31%.

Within 10 years after disease onset, almost 50% of patients with PD might advance to PDD. In a prospective, population-based study of patients with new-onset PD in England, the CamPaIGN study, 46% had PDD after 10 years (Williams-Gray 2013). This proportion is in agreement with findings in other prospective studies (Hely 1999, Perez 2012). A high PDD prevalence of 78% was found in a population-based, prospective study in Norway, which included prevalent PDD cases (Aarsland 03). However, not all PD patients will develop dementia if they live long enough. Studies show that young onset PD and tremor dominant PD may confer very little or no cognitive impairment even after decades with the disease (Cilia 2015). This means that the long-term prognosis of cognitive functioning in PD is highly variable, and that cognitive phenotypes may function to delineate distinct subsets of PD.

Non-motor symptoms, such as cognitive decline, have a key role when evaluating disease progression in PD, and it has been suggested that they should be given a larger weight (Marinus 2018). Disease progression is also shown to be faster in PD patients with cognitive impairment. For instance, Burn and colleagues showed that the annual deterioration measured by the UPDRS III score in non-demented patients with PD was 2.6 points, but reached 4.9 points in patients with PD dementia (Burn, 2006). The slope of annual deterioration of cognitive function, measured by MMSE score, is also steeper in patients with PDD than in patients with PD without dementia (Aarsland, 2004).
Biomarkers in parkinsonism

The diagnosis of PD and other parkinsonian diseases currently relies on clinical evaluation. No universally accepted neurochemical biomarker is available to aid PD diagnosis in routine care. Therefore, biomarkers that aid diagnosis, predict the prognosis, and measure the activity of the neurodegenerative process in PD are urgently needed. Importantly, such markers could possibly also be used to measure treatment response in clinical trials.

Cerebrospinal fluid

Because the cerebrospinal fluid (CSF) is in direct contact with the fluid environment of the brain, it provides one of the best “windows” to study disease processes in living patients, by reflecting altered metabolic states and disease pathology. The CSF can be easily accessed by lumbar puncture, which is a routine procedure with few complications. While 80% of the proteins in the CSF derive from filtration of blood, 20% derive directly from cells in the central nervous system (Reiber, 2003). These proteins are an attractive source for biomarker discovery.

α-synuclein

On average, the concentration of α-synuclein is lower in CSF of PD patients compared to healthy controls (Eusebi 2017), but the finding is not reliably reproduced in individual patients. There is an extensive overlap of single values between the groups and many studies failed to show a difference (Magdalou 2015, Bäckström 2015). Standard ELISA measurement of α-synuclein in CSF is therefore not useful for establishing a PD diagnosis. The level of α-synuclein was found to be significantly lower in PD patients with non-tremor-dominant phenotype compared with tremor-dominant PD (Kang 2016). Furthermore, α-synuclein in CSF seems to be a general marker of neurodegeneration and neuronal loss, as shown by mildly increased levels in Alzheimer disease (Korff 2013) and extremely high levels in the rapidly progressive prion disorder Creutzfeldt-Jakob Disease (Llorens 2018).

More recent studies have used prion protein research technology in PD to induce α-synuclein aggregation in CSF from PD patients. The first, promising pilot studies using Protein Misfolding Cyclic Amplification (PMCA) and the Real-Time Quaking-Induced Conversion (RT-QuIC) showed a high sensitivity (about 90%) and a specificity of almost 100% in identifying PD (Fairfoul 2016, Shahnawaz 2017). Both assays are being validated independently within larger PD cohorts.

β-Amyloid (Aβ42) and cognitive impairment in PD

In Alzheimer disease, the CSF marker β-Amyloid 1-42 (Aβ42) is markedly decreased, reflecting the key pathological event of cortical deposition of β-Amyloid (Palmqvist
A few studies have found slightly lower concentrations of CSF Aβ42 in patients with PD, compared with healthy controls (Kang 2013, Alves 2010), and numerous studies have consistently shown decreased levels in PD associated with cognitive impairment and dementia (PDD). The finding of decreased levels of Aβ42 in CSF of patients with PDD compared to PD patients with normal cognition was confirmed in a meta-analysis, which showed that PDD was also associated with higher levels of total Tau and phosphorylated Tau proteins (Hu et al 2017).

These and other findings suggest an overlap between PDD and Alzheimer disease, which has led to the theory that brain pathology of the Alzheimer disease type contributes to cognitive decline in PD. Low concentrations of CSF Aβ42 in patients with newly diagnosed PD has been shown to correlate with a faster decline in the Mattis Dementia Rating Scale during 1 year of follow up (Siderof 2010). Interestingly, low CSF Aβ42 levels in early PD is not only associated with cognitive dysfunction but also with the development of L-dopa resistant impairments in gait function during follow up (Alves 2013). Aβ42 concentration is generally lower in the PD subtype of predominant postural instability and gait difficulty (PIGD) than in other PD subtypes (Kang 2013).

**Neurofilament**

Neuroaxonal damage and neuronal loss are general disease processes in many neurological disorders and cause permanent disability. Neurofilament proteins are promising markers of such processes, since they are exclusively expressed in neurons and their levels rise in the CSF upon neuroaxonal damage. Neurofilament light chain protein (NfL) is a species of neurofilament that leaks into the CSF, in particular upon damage to axons, and is elevated in amyotrophic lateral sclerosis, Huntington disease, frontotemporal dementia and inflammatory phases of active multiple sclerosis (Khalil 2018). NfL can predict important longitudinal outcomes in several neurodegenerative diseases. In familial Alzheimer disease, mild elevations of NfL in CSF can be seen ~10 years before the expected disease onset (Weston, 2017). In patients with frontotemporal dementia, NfL increases when symptoms begin to emerge, and high NfL correlates with brain atrophy and shorter life expectancy (Meeter 2016, Scherling 2014). High NfL correlates with clinical progression in primary progressive aphasias (Steinacker 2017). PD and the atypical parkinsonian diseases (MSA, PSP and CBS) have distinct neuropathologies, which suggests that there may be neurochemical differences detectable that may improve diagnosis. There is good evidence that NfL concentration in CSF is markedly higher in atypical parkinsonism, including progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal syndrome (CBS), compared to PD. This was first shown in 1998 (Holmberg 1998) and was later confirmed in two independent, large cohorts of patients with idiopathic parkinsonism (Hall 2012, Magdalinou 2015). High NfL in CSF can be used for distinguishing atypical parkinsonism from PD with a pooled sensitivity of about 82% and a specificity of about
85% (Ge 2018), but the accuracy for diagnosis in the early disease phase is less well known. Using highly sensitive assays, the atypical parkinsonian disorders PSP, MSA, and CBS can also be differentiated from PD by higher NfL analysed in blood samples, without extensive overlap, but the diagnostic accuracy is lower compared to CSF measurements (Hansson 2017).

**The causes of cognitive decline in Parkinson disease**

**Risk factors**

The typical pattern of mild cognitive deficits in non-demented patients with PD, which is referred to as mild cognitive impairment (or PD-MCI), includes impairments in attention, working memory, complex planning, executive function and visuospatial abilities (Litvan 2012). These deficits may occur in one or several cognitive domains. When preexisting in PD, they are highly predictive of the future development of dementia. Other risk factors for dementia in PD are, ranked in the order from strongly predictive to less strongly predictive: hyposmia, hallucinations, high overall severity of motor symptoms, speech impairment, older age at onset of PD, axial impairment and gait difficulty (including a predominant PIGD motor phenotype), depression, and male sex (Baba 2012, Marinus 2018). Older age and low level of education are risk factors for dementia in the general population, as well as in PD, and are therefore not considered specific to PDD.

**The neurochemistry of cognitive impairment**

*Striatal and extrastriatal dopamine depletion*

The exact role of dopamine deficits in cognitive decline in PD is debated. The cortico-striato-thalamocortical loops connect the basal ganglia with the cerebral neocortex. Among these, the putamen is closely connected with the supplementary motor cortex and is believed to be involved mainly in motor functions. The caudate nucleus is connected with the dorsolateral prefrontal cortex and the lateral orbitofrontal cortex, and it has been shown that dysfunction in this system contributes to cognitive impairment in PD. PET studies showed that reduced 6-[18F] fluoro-L-dopa uptake in the striatum (Holtoff 1994, Ito 2002), and in particular in the caudate nucleus (Rinne 2000), is associated with cognitive impairment in patients with PD. These findings are supported by fMRI studies linking reduced activity in the caudate with cognitive impairment; especially in working memory. This was shown in the population-based NYPUM cohort of patients (Ekman 2012).

Loss of monoaminergic, including dopaminergic function, in extrastriatal regions is delayed in PD and seem to occur independently from nigrostriatal dysfunction (Pavese 2011). However, evidence shows that dopamine deficits in extrastriatal regions are
important for the impairment of memory and executive function in PD (Christopher 2014, Christopher 2015).

**Cholinergic dysfunction**
The cholinergic system of the brain is of major importance for attention, learning and conscious awareness, and has been found to be impaired in a number of dementia syndromes (Ballinger 2016). The major supply of acetylcholine to the cortex and the limbic system stems from the nucleus basalis of Meynert in the basal forebrain.

In patients with PD, accumulation of α-synuclein in basal forebrain cholinergic nuclei (primarily the nucleus basalis of Meynert) together with early atrophy of these structures, and a corresponding deficiency of cortical acetylcholine has long been recognized. The severity of degeneration of the nucleus basalis of Meynert is correlated with severity of cognitive impairments (Yarnall 2011), and hypofunction in the cholinergic system is thought to be a major contributor to the cognitive decline in PD.

In Alzheimer disease, deficits in memory and attention have a clear association with reductions of cholinergic function, such as measured by acetylcholinesterase (AChE) PET imaging (Ballinger 2016). However, in PDD, AChE PET imaging shows a greater cortical cholinergic deficit than in Alzheimer disease of equal global dementia severity (Bohnen 2003). In PD without dementia, the reduction in cholinergic function as measured by PET imaging is as severe or more severe than in Alzheimer’s disease (Yarnall 2011). Unsurprisingly, anticholinergic medications are associated with cognitive decline in PD (Ehrt 2010). In contrast, cognitive impairment improves by treatment with acetylcholinesterase inhibitors, at least at the group level. Since cholinergic deficits are consistently more severe in PDD than in PD in imaging studies, cholinergic dysfunction has been proposed to be responsible for the transition from PD to PDD.

**Structural correlates of dementia (PDD)**
The neurochemical and neuroanatomic substrates of dementia in PD (PDD) are incompletely understood, which hinders the development of new therapies. However, several mechanisms are implicated in PDD pathogenesis, showing a likely multifactorial origin. First, the results of many postmortem studies (Aarsland 2005, Braak 2005) point to the deposition of Lewy bodies in limbic and neocortical areas as a major cause of PDD. Autopsy series using a large number of cases show that α-synuclein pathology distributed in a neocortical pattern in PD, consistent with Braak stages 5 and 6, is a strong correlate of dementia during life (Irwin 2018). This pathology is associated with synapse disruption (Colom-Cadena 2017b).

Distinct strains or haplotypes of pathological α-synuclein may explain some of the variability in disease progression and cognitive phenotype (Guella 2016). Some species
of α-synuclein, or distinct "seed” strains, may have a propensity for wide-spread distribution in neocortical networks. This could explain why some PD patients are affected by early dementia.

Second, robust evidence shows that co-incident β-amyloid deposition in cortical and limbic areas affect cognitive function negatively in PD and is associated with PDD (Ballard 2006, Sabbagh 2009). Longitudinal studies show that patients with low Aβ42 in the CSF in the early phase of PD have a higher risk of cognitive decline (Siderowf 2010, Alves 2010, Bäckström 2015). These studies, together with pathoanatomical and PET imaging studies using Pittsburgh Compound B (11C-PiB), support the involvement of β-amyloid pathology in PDD and show a pathological overlap between PD and Alzheimer disease. Some studies have found the combination of cortical α-synuclein, β-amyloid and tau deposition to be the strongest correlate of PDD, especially in older patients, and that these pathologies may have synergistic effects (Colom-Cadena 2017, Jellinger 2012).

Third, as reviewed above, reduced acetylcholine function in the neocortex has been found to be a major contributor to cognitive decline. Atrophy of the nucleus basalis of Meynert found on MRI in early PD is a strong predictor of future PDD (Ray 2018).

The spread of α-synuclein and/or Alzheimer disease pathology gives rise to atrophy in brain networks, as shown by MR studies using voxel-based morphometry (Weintraub 2011). A meta-analysis of MR studies of brain atrophy in patients with PDD relative to healthy controls showed significant gray matter atrophy of the medial temporal lobe bilaterally, including the hippocampus, parahippocampus, and amygdala (Pan 2013). These are structures considered to be essential for memory and aspects of emotional and visual processing. Significant atrophy was also evident in the basal ganglia (mainly the striatum) in PDD. Furthermore, cognitive dysfunction in distinct domains may relate to atrophy in different anatomical structures. For instance, a decline in language correlates with atrophy in the temporal lobe in PD, while reduced performance in executive functions are associated with bilateral reductions in frontal and parietal gray matter (Duncan 2013).

Together with reduced levels of several neurotransmitters, all these structural brain pathologies are likely to be involved in the pathogenesis of PDD.

**Rationale for research on the molecular diagnosis of neurodegenerative diseases**

In clinical practice, the diagnosis of parkinsonism is often challenging. The finding of markedly increased NfL in CSF and blood of patients with MSA and PSP, compared to PD is, therefore, an important development. These studies were, however, with few exceptions, made on patients with already well established clinical diagnoses in
moderately advanced disease phases. Almost no studies have been carried out in the early phase. NfL as a marker for the atypical parkinsonian diseases (i.e., MSA, PSP and CBS) therefore needs to be investigated and validated in patients with early symptoms, when the diagnosis is in question.

Because subtypes of parkinsonian diseases may reflect differences in underlying pathobiology, identification of groups of patients with shared unique clinical features may improve research into subtype-specific biomarkers. Genetic diagnosis of parkinsonian and other neurodegenerative diseases is also likely to become important in the future, in order to enroll patients earlier in clinical trials, enable more effective treatment and, possibly, to predict prognosis.

All of the above reasons motivates efforts to differentiate PD and other parkinsonian diseases more clearly, based on underlying pathogenic mechanisms. These efforts will, likely, also lead to a better understanding of the causes of cognitive decline.
Aims of the investigation

Overall aims are to describe and characterize mechanisms and risk factors of importance for disease progression (motor and non-motor) in parkinsonism and PD, with special emphasis on cognitive aspects and mortality.

Specific questions addressed

**Study I**: Mortality is increased in the parkinsonian diseases PD, MSA and PSP. To what extent and what are the determinants for the increased mortality?

**Study II**: Are biomarkers in CSF useful a) for diagnosis of different forms of idiopathic parkinsonism, and b) for predicting future development of dementia in PD?

**Study III**: The role of dopamine deficits in cognitive decline in PD is unclear. Does the functional COMT Val<sup>158</sup>Met polymorphism in the COMT-gene and/or the Dopamine Receptor D2 (DRD2) gene polymorphism rs6277 affect cognitive functions and the risk to develop mild cognitive impairment or dementia in PD?

**Study IV**: A common polymorphism in the PITX3 gene (rs2281983) is of importance for the function of dopaminergic neurons. Is this polymorphism of importance for the development of dementia in PD?

**Study V**: Does the early neurofilament concentration in CSF reflect disease severity and neurodegeneration in early PD and can it be used to predict survival?
**Materials and Methods**

**Ethics**

All studies were performed in accordance with the Declaration of Helsinki. All participants provided informed consent.

The research project was approved by the Regional Medical Ethics Board in Umeå, Sweden (study I–V: DNR 03-387, DNR 05-077M, 2011-334-31M, and study V: DNR 2014-163-31M).

**Study Populations**

(1) *The NYPUM cohort*. A population-based, prospective study of new-onset parkinsonism in Sweden, that included patients diagnosed between January 1, 2004, and April 30, 2009, was conducted by a movement disorder team at a university hospital that represents the only neurology clinic in the region (the Department of Neurology at Umeå university hospital). Unselected cases of idiopathic parkinsonism from the geographic catchment area, which has ~142,000 inhabitants, were recruited to the study (a study denoted NYPUM; NY, which is Swedish for new, Parkinsonism in Umeå, Linder 2010). The studied area includes the southeast part of Västerbotten’s County in northern Sweden (Umeå, Nordmaling, Bjurholm, Vännäs, Vindeln, and Robertsfors).

To avoid selection bias and to make case identification as complete as possible, a careful population screening in the area was performed by many sources, including letters sent twice yearly to health practitioners asking for referral of all suspected cases with incident parkinsonism. Eldercare institutions were surveyed by visits (the largest institution) with an examination by neurologists or by an interview with healthcare providers (remaining institutions). After exclusion of patients with secondary parkinsonism (e.g., due to neuroleptic drugs or stroke) or dementia at baseline (e.g., patients with dementia with Lewy bodies), 182 patients with idiopathic parkinsonism were recruited to the study in the early motor (drug-naïve) phase and were followed prospectively. The patients were investigated with neurological, neuropsychological and genetic testing, biofluid collection, and multimodal neuroimaging, at baseline and at follow-ups (see Appendix for a table of the investigations), and these data were used in all studies of the present thesis. All laboratory analyses were performed blinded from clinical data.

A diagnosis of PD, multiple system atrophy (MSA), or progressive supranuclear palsy (PSP) required agreement among the examiners that the clinical criteria for the diagnosis
were fulfilled based on the UK Parkinson’s Disease Society Brain Bank criteria (Gibb 1989) or criteria for MSA or PSP (Gilman, 1999, Litvan 1996). In total, 143 patients were diagnosed with PD, 13 with MSA, 18 with PSP, 4 had unclassifiable parkinsonism, and 4 did not have idiopathic parkinsonism (figure 3) according to the diagnosis at the latest follow up. The diagnosis was confirmed by autopsy of the nervous system in 3 cases of PD and 2 cases of PSP.

(2) The Validation cohort. To validate findings in study V, a clinical cohort consisting of all patients with new-onset, idiopathic parkinsonism that was referred from primary care to the neurological department at Umeå university hospital from April 2009 through September 2018 was investigated. During this period, all patients that were diagnosed with PD were offered a lumbar puncture for analysis of the CSF around the time of diagnosis, and 193 patients with new-onset PD agreed to perform CSF collection. All these patients were included in the study and followed longitudinally (figure 3). In agreement with the exclusion criteria in the NYPUM stud, patients with secondary parkinsonism, dementia at baseline or atypical parkinsonism were excluded. Diagnoses of PD or atypical parkinsonian disorders were reached in the same way, using the same diagnostic criteria, as in the population-based NYPUM study.

(3) The healthy controls. An age-matched group of neurologically healthy controls (n = 31) agreed to participate in the population-based NYPUM study, performed the same investigations as the patients, and were followed prospectively with the same neuropsychological examinations as the patients.

Figure 3. Flowchart of patients included in (1) the NYPUM and (2) the validation cohorts. The diagnosis was established according to clinical diagnosis at the latest follow-up and confirmed by autopsy in 5 patients. MSA = multiple system atrophy; PSP = progressive supranuclear palsy. Mortality status was determined on (1) August 31, 2017 and (2) October 31, 2018, respectively.
Investigations

Clinical evaluation

All patients, in both patient cohorts, were assessed by neurological examination and motor assessments at baseline, and at least yearly thereafter. Baseline motor function was investigated by Modified Hoehn and Yahr Scale and the UPDRS in the early phase of PD, prior to the start of dopaminergic medication. The baseline UPDRS scores were divided into subscores for tremor (sum of items 20 and 21) and postural imbalance and gait disorder (PIGD; the sum of items 13-15, 29, and 30), in accordance with the DATATOP (Deprenyl and Tocopherol Antioxidative Therapy of Parkinson’s Disease) trial classification (Jancovic 1990). In the population-based study (the NYPUM cohort) olfactory function was investigated by the 12-item Brief Smell Identification Test (Doty 1996) global mobility by the Timed Up and Go (TUG) test, which is the time it takes to rise up from a chair, walk 3 meters, and sit down again (Podsiadlo 1991), and depression by the Montgomery-Åsberg Depression Rating Scale (Montgomery 1979). Non-motor symptoms were investigated by questionnaires, including the 39-item Parkinson’s Disease Questionnaire, and cognitive function by Mini-Mental State Examination (MMSE), in both cohorts, in addition to neuropsychological testing.

Neuropsychology

Extensive neuropsychological testing, which was used for PD-MCI and PDD diagnosis, in accordance with Movement Disorder Society (MDS) guidelines (Litvan 2012, Emre 2007), was performed at baseline and after 1, 3, 5, and 8 years in the NYPUM cohort (see Appendix). Since all cognitive domains (episodic memory, working memory and attention, verbal function, visuospatial function, and executive function) had been covered by the tests throughout the study period, PD-MCI diagnoses were applied according to the level 2 criteria of the MDS guidelines (Litvan 2012). Patients were classified as having MCI at baseline if (1) impaired in a minimum of 2 tests in one domain (single-domain MCI) or in a minimum of one test in 2 different domains (multiple-domain MCI), (2) impairments were at least 1.5 standard deviations below the mean of normative data of healthy controls, (3) self-perceived cognitive decline was reported in a questionnaire and/or directly by patient and/or family member, and (4) the patient had no functional impairment in basic activities of living (i.e., driving a car, social or personal care, medication management) due to cognitive impairment. The occurrence of such impairment due to cognitive decline is a characteristic of PDD. The neuropsychological test battery that was used is described in studies I – IV.

Genetic testing

In the NYPUM study, 134 of the patients with PD agreed to DNA analysis by peripheral
blood sampling. DNA was isolated from blood samples using standard procedures, in order to investigate genetic variations (polymorphisms) with previously shown functional effects. Polymerase chain reaction (PCR) is used to make many copies of a specific DNA segment. The variations of interest (the e2/e3/e4 polymorphisms in the APOE gene, the rs4680 and rs6277 polymorphisms in the COMT and DRD2 genes, and the rs2281983 polymorphism in the PITX3 gene) were genotyped using TaqMan Assays-by-Design, using the TaqMan 7900 HT Fast Real-Time PCR systems (Applied Biosystems, Foster City, CA).

CSF Analysis

Lumbar puncture was performed to collect CSF. At study entry (baseline) in the NYPUM cohort, CSF was obtained from 99 patients with PD, 11 patients with MSA, 12 patients with PSP, and 30 healthy controls (in total, 152 individuals) before initiation of any dopaminergic treatment. During treatment, collection of CSF was repeated after 1 year in 57 patients with PD, 6 patients with MSA, and 9 patients with PSP (in total, 72 patients). Collection of CSF was repeated once more in 35 patients with PD after 3 years. CSF levels of NfL were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) (NFLight; UmanDiagnostics AB), as shown in figure 4. The lower limit of quantification for the assay was 50 ng/L, and the coefficient of variation was 14.0%. CSF total tau concentration was determined using another sandwich ELISA which is constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described.18 CSF Aβ1-42 levels were determined using a sandwich ELISA constructed to measure Amyloid-β containing the first and 42nd amino acids. (19). Tau phosphorylated at threonine 181 was measured using sandwich ELISA. (20) CSF levels of α-synuclein were measured using a commercially available human α-synuclein ELISA. Lastly, levels of HFABP were measured using ELISA. CSF levels of NFL were measured in the Validation cohort with the same method as in the population-based (NYPUM) cohort. Board-certified laboratory technicians performed the CSF analyses, using procedures approved by the Swedish Board for Accreditation and Conformity Assessment. The cells in the CSF were counted by automated flow cytometry.

Figure 4. A sandwich ELISA. (1) A plate is coated with a capture antibody; (2) a sample, e.g., a protein-solution, is added and any antigen present binds to the capture antibody; (3) a detecting
antibody is added and binds to the antigen; (4) an enzyme-linked secondary antibody is added, and binds to the detecting antibody; (5) a substrate is then added, and is converted by an enzyme to a detectable form.

**Dopamine active transporter imaging**

Single-Photon Emission Computed Tomography (SPECT) uses a gamma-emitting radioisotope injected into the blood, and measures the binding of a specific radioisotope-attached ligand to a target in the brain. Binding to the dopamine active transporter (DAT) measures the amount of cell loss in the presynaptic dopaminergic system in patients with parkinsonian disorders. Of the 182 patients enrolled in the NYPUM cohort, 93.4% underwent DAT imaging by $^{123}$I-FP-CIT (DaTSCAN; GE Healthcare BV, Eindhoven, The Netherlands) SPECT. DAT imaging was done 3 hours after an IV bolus dose of 185MBq $^{123}$I-FP-CIT, prior to the start of medication at baseline. The imaging protocol was done within the framework of a nonprofit clinical trial (EU no. 2009-011748-20) and constituted a substudy within the population-based research project (Mo 2010, Jakobson Mo 2013). Semiquantitative analysis (based on regions of interest) and visual evaluation of the DAT SPECT. Normal reference values were derived from the age-matched group of healthy controls that participated in the NYPUM study. All patients that fulfilled diagnostic criteria for PD, MSA, and PSP and who participated in the DAT imaging (n = 163 in NYPUM) had a pathologic scan.

**Diffusion Tensor Imaging**

By measuring the random motion (or diffusion) of water molecules in brain tissue, especially in neuronal fibers, Diffusion Tensor Imaging (DTI) in MR scans can give an estimate of white matter integrity and connectivity in the brain, utilizing the fact that the directionality of diffusion is related to the structural integrity of the tissue. A crucial parameter measured by DTI is fractional anisotropy (FA), which represents an orientation distribution of the motion of water molecules, with a faster diffusion along the axons and fibers than that perpendicular to them. A decreased FA is a measurement of the severity of dysfunctional microstructural tissue in several neurological disorders. In the population-based study (the NYPUM cohort), 45 of the 99 patients from whom CSF was collected at baseline conducted a 3.0 Tesla MRI scan at baseline, and 20 and 14 patients with PD conducted both investigations at the 1 and 3 years follow up, respectively. DTI from these scans were acquired using single-shot spin echo (EPI) sequences.

**Mortality**

All-cause mortality was studied as the relevant outcome in study I and V. All surviving patients in the population-based cohort (NYPUM) were followed yearly for approximately 8.5 to 13.5 years, until August 31, 2017, while the patients in the validation
cohort were followed until October 31, 2018. The survival in both cohorts (figure 3) was analysed at these time points. For both cohorts, the survival data was complete, although a few older patients were followed by telephone rather than visits during the last few years. A death certificate, in which the cause of death was stated, was obtained for 98 (90%) of all the fatalities in the population-based (NYPUM) cohort.

**Statistical Analysis**

Baseline differences in clinical variables between different diagnostic groups (patients and healthy controls) were tested cross-sectionally by 1-way analysis of variance, and Kruskal-Wallis test, and correlation by Person r and Spearman ρ. In the studies where differences were tested with several tests, an adjustment was generally done for multiple comparisons, and significances were shown before and after this adjustment. In study I, age- and sex-specific standardized mortality ratio (SMR) was calculated by dividing the observed number of deaths in the longitudinally followed patients by the expected number of deaths. The expected numbers of deaths were calculated using the age- and sex-specific official Swedish National Statistics mortalities during 2004–2017 multiplied by the person-time from each patient group in the study. In all studies (I-V), prospective, longitudinal data and outcomes were investigated by Cox proportional hazard analysis, which was run with and without adjustment for potential confounders. Kaplan-Meier plots are used to show the effect of baseline factors that are of importance for the long-term outcomes.
Results

Neurodegenerative disorders diagnosed in the NYPUM project

Clinical characteristics at baseline for the patients with parkinsonian diseases that participated in the population-based study (the NYPUM cohort) are shown in table 1. In the NYPUM cohort, 143 patients were diagnosed with PD, 13 with MSA, 18 with PSP, and 4 had unclassifiable parkinsonism. The diagnosis was established according to the diagnosis at the latest follow up.

The mean age of the patients with PD was 71.2 years at study entry. The mean ages of patients with MSA and PSP at study entry were 73.6 and 75.0 years, respectively. MCI was more common in patients with PSP than in patients with PD or MSA. Orthostatic hypotension was more common in MSA than in PSP. Among the 143 patients with PD, 61 (43%) had MCI at baseline. During longitudinal follow up of the 143 patients with PD, the proportion of patients with PDD increased. At 3 years, 23 of the patients who were alive had PDD (26% had PDD or were dead). At 5 years, 29 of those alive had PDD (41% had PDD or were dead). At 8 years, 18 of those alive had PDD (64% had PDD or were dead); i.e., 36% were alive without dementia. Until August 31, 2017 (which was the last date of monitoring in study I), 54% of those with PD in the NYPUM study had died, compared to 89% of those with PSP and 92% of those with MSA. One of the two living patients with PSP died shortly (within a couple of weeks) after the last date of monitoring in study I.
Table 1. Characteristics of participants in the NYPUM study at inclusion.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PD (%)</th>
<th>MSA (%)</th>
<th>MCI (%)</th>
<th>PSP (%)</th>
<th>RBD (%)</th>
<th>Unified Parkinson’s Disease Rating Scale (UPDRS)</th>
<th>Bespoke Depression Score</th>
<th>No. of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>18</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>70.2 (19.2)</td>
<td>73.0 (19.3)</td>
<td>72.9 (18.9)</td>
<td>70.9 (23.2)</td>
<td>73.6 (19.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD / MSA</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSP</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBD</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unified Parkinson’s Disease Rating Scale (UPDRS)</td>
<td>2 (1-5.2)</td>
<td>3 (0.2-3.6)</td>
<td>2.3 (0.2-3.0)</td>
<td>2.9 (0.2-3.5)</td>
<td>2.5 (2.0-3.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bespoke Depression Score</td>
<td>8.1 (5.9)</td>
<td>8.1 (5.6)</td>
<td>8.0 (4.8)</td>
<td>8.1 (5.4)</td>
<td>8.0 (6.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>28.5 (17.7)</td>
<td>24.7 (24.2)</td>
<td>24.5 (24.3)</td>
<td>23.8 (23.0)</td>
<td>26.6 (24.0-32.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure difference after 3 minutes of standing</td>
<td>41.5 (27.7)</td>
<td>66 (42.7)</td>
<td>45.5 (37.4)</td>
<td>30.0 (30.0-33.0)</td>
<td>26.0 (19.0-35.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor impairment</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-motor impairment</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unified Parkinson’s Disease Rating Scale (UPDRS)</td>
<td>2.2 (0.6-3.1)</td>
<td>2.2 (0.7-3.0)</td>
<td>2.4 (0.5-3.1)</td>
<td>2.2 (0.6-3.0)</td>
<td>2.5 (1.6-3.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bespoke Depression Score</td>
<td>8.4 (5.8)</td>
<td>8.4 (5.8)</td>
<td>8.3 (5.6)</td>
<td>8.4 (5.8)</td>
<td>8.3 (5.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>28.5 (17.7)</td>
<td>24.7 (24.2)</td>
<td>24.5 (24.3)</td>
<td>23.8 (23.0)</td>
<td>26.6 (24.0-32.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure difference after 3 minutes of standing</td>
<td>41.5 (27.7)</td>
<td>66 (42.7)</td>
<td>45.5 (37.4)</td>
<td>30.0 (30.0-33.0)</td>
<td>26.0 (19.0-35.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor impairment</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-motor impairment</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mortality in a population-based cohort with parkinsonism (paper I)

The mortality in the NYPUM cohort, including all patients with idiopathic parkinsonism (n=182), was increased in comparison to the general Swedish population during the years 2004-2017, as shown in table 2 of paper I (Bäckström 2018). The age- and sex-standardized mortality ratio (SMR) was 1.84 (95% CI: 1.50-2.22, \( p <0.001 \)) for all patients with idiopathic parkinsonism, 1.58 (95% CI: 1.25-1.98, \( p <0.001 \)) for the patients with idiopathic PD and 3.32 (95% CI: 2.21-4.80, \( p <0.001 \)) for the patients with atypical parkinsonism (of whom 13 had MSA and 18 had PSP). Comparison between the patients with PD and the patients with atypical parkinsonism showed an age-adjusted hazard ratio for death of 2.76 in MSA and 1.42 in PSP, respectively. The survival was related to the age at first visit in PD, but there was no such relation for the patients with atypical parkinsonism (MSA or PSP). The most common cause of death for all patients was pneumonia.

In early PD, several factors correlated with shorter survival (figure 5 and 6). After adjustment for age, a diagnosis of mild cognitive impairment, a PIGD phenotype, high disease severity (as measured by total- and part III UPDRS scores), general slowness (as measured by the Timed-Up-and-Go test), freezing of gait and hyposmia at the first visit, and onset of dementia during the first three years, were clinical factors significantly related to a shorter survival. Tremor had no correlation to survival.
A relationship between cognitive function and lifespan in PD was found by several measures. Survival in PD was related to the MMSE score at inclusion in the study, with a 17% increase in the age-adjusted risk of death for each 1 point decrease in MMSE ($p = 0.011$). The onset of dementia during the first three years after study-inclusion conferred a 1.94 times higher hazard for death, while MCI at baseline conferred a 2.38 times higher hazard for death during the following years. The PD patients with MCI at the baseline visit had had a risk of death that was 2.17 times the risk in the general Swedish population (SMR: 2.17, 95% CI: 1.56-2.93, $p < 0.001$), while the mortality in PD patients (both male and female) with normal baseline cognition was not significantly different from the general population. Assuming that the average age at the start of the NYPUM study was about 71 for people with PD, the expected survival for people with no mild cognitive
impairment was 11.6 years, compared to 8.2 years for those with mild cognitive impairment.

Neurobiological factors at baseline that predicted a shorter survival in PD were reduced DAT uptake in the striatum, particularly within the most affected caudate nucleus (with a 1.48 times higher hazard for death for each standard deviation of lower uptake) and a subtle inflammatory reaction in the cerebrospinal fluid (as defined by a minor pleocytosis of mononuclear leukocytes of 2 to 10 cells per μL). Of all PD patients, 13.1% were found to have a subtle inflammatory reaction in the CSF and these patients had 6.31 times increased hazard for death during follow up (95% CI: 3.04-13.12, \( p < 0.001 \)) compared to the other PD patients. Given this finding, the CSF results were further analyzed and there was no evidence of CNS infection in any of the patients. Furthermore, the PD patients with a subtle inflammatory reaction in the CSF had higher progression of the UPDRS III scores at the one year follow up, compared to the other patients, despite treatment with equal dosages of dopaminergic medication.

Lower concentrations of Aβ42 in the CSF was moderately associated with higher mortality when the whole cohort of patients with idiopathic parkinsonism was investigated. This finding was mainly driven by a correlation between lower CSF Aβ42 and higher mortality in patients with MSA or PSP.
Figure 6. Predictors of mortality in parkinsonism. (A) PD compared to PSP and MSA. The effect of (B) a non-tremor dominant phenotype in PD; (C) hyposmia in PD, and; (D) a subtle neuroinflammatory reaction in CSF in PD. A subtle neuroinflammatory reaction was defined by 2-20 mononuclear leukocytes. Reprinted from Bäckström D, Granäsen G, Domellöf ME, Linder J, Jakobson Mo S, Riklund K, et al. Early predictors of mortality in parkinsonism and Parkinson disease: A population-based study. Neurology 2018b; 91(22): e2045-e56

Higher neurofilament in PSP than in Parkinson disease (paper II)

To neurochemically distinguish between early PD and atypical parkinsonism (MSA or PSP), a panel of six CSF proteins were analyzed in the patients with idiopathic parkinsonism (n = 128) and in the group of neurologically healthy control participants. The PD patients had a different CSF protein pattern compared to the healthy controls. At baseline, the patients with PSP had higher NFL and lower Aβ42 concentrations in CSF, compared to the patients with PD. The NFL concentration in CSF showed high accuracy
for discriminating PSP from PD, yielding a sensitivity of 75% and a specificity of 83% for PSP if a cut-off value exceeding 2020 ng/L was used. An NfL of 2020 ng/L was the cut off value with the highest Youden Index. At the one-year follow-up, the mean NFL concentration in CSF had increased by 27.1% in the PSP patients who contributed with 2 samples \((p = 0.005)\). Excellent discrimination of PSP from PD was obtained using NfL at one year (AUROC: 0.93; \(p <0.0001\)). At this time, CSF NfL exceeding 2916 ng/L had a sensitivity of 89% and a specificity of 93% for PSP against PD. In contrast, early MSA could not be clearly distinguished from PD by the CSF pattern.

**CSF patterns that precede PDD in Parkinson disease (paper II)**

Further analysis of the cerebrospinal fluid content of protein biomarkers in patients with early PD aimed to predict future development of dementia (PDD). Of the 99 of the patients with PD in the NYPUM cohort from whom CSF was available, 35 \((35.4\%)\) developed PDD during a follow-up period of 5 to 9 years. The levels of three CSF protein biomarkers were related to this decline. Higher NfL \((p <0.001)\), lower Aβ42 \((p = 0.005)\), and higher HFABP \((p = 0.004)\) in the CSF at baseline correlated with future PDD development. At cut-off levels above 1100 ng/L for NfL, below 626 ng/L for Aβ42 and above 500 ng/L for HFABP, the CSF biomarkers conferred hazard ratios for PDD of 2.6, 2.8 and 2.8, respectively, after adjustment for confounders. The incidence of PDD in PD patients with baseline NfL above/below 1100 ng/L, Aβ42 above/below 626 ng/L, and HFABP above/below 500 ng/L are shown in figure 7.

Prediction of PDD in PD patients was improved by combining the CSF biomarkers. A baseline triad of high NfL, low Aβ42 and high HFABP in the CSF conferred a high risk for future PDD development. Expressed as a ratio of NfL + HFABP to Aβ42, a value above 2.1 yielded 11.8 times increased adjusted hazard for PDD, compared to PD patients without this CSF pattern (see figure 7). In PD patients with baseline NfL + HFABP to Aβ42 values above 2.1, future PDD was predicted with a sensitivity of 90% (95% CI, 74%-98%) and a specificity of 71% (95% CI, 56%-83%). A simpler ratio of NfL to Aβ42 was also useful for PDD prediction, with values above 1.0 (higher NfL than Aβ42) conferring 6.7 times elevated PDD risk.

Adjusting the relationship between the CSF biomarker levels and PDD for age differences slightly lowered PDD hazard ratios. However, adjustment of the effect of age for the ratio of NfL to Aβ42 showed that the CSF measure was a stronger predictor of PDD than age, rendering age a nonsignificant covariate \((p = 0.229)\). This demonstrated that the ability of the CSF measure to predict PDD was more accurate than what could be achieved by using age as a baseline predictor. Essentially normal CSF profiles (defined by an NfL / Aβ42 ratio <1.0; which was similar to findings in healthy controls) were also found in some older PD patients. After adjusting for all covariates, high NfL was associated with the relatively rapid decline from PD with normal cognition to PDD.
A faster disease progression, as measured by an increase in the Hoehn and Yahr score at follow up, was associated with low baseline Aβ42. A high risk for disease progression in PD was found at baseline Aβ42 values <617 ng/L ($p < 0.001$).

**Figure 7.** The relation between CSF biomarker proteins and the future development of dementia in PD (PDD). The first three panels show the association of NfL, Aβ1-42 and HFABP with PDD, while the fourth panel show the predictive capacity of a combination of these three proteins. CSF = cerebrospinal fluid; NfL = neurofilament light chain protein; HFABP = heart fatty acid protein. Reprinted with the kind permission from the American Medical Association.
Functional variability in dopamine-associated genes and cognitive decline (papers III and IV)

Functional variants (single nucleotide polymorphisms) in two genes associated with dopamine metabolism and transmission, and one gene associated with survival of dopaminergic neurons, namely the COMT, DRD2 and PITX3 genes, were genotyped in 134 PD patients. These investigations included all the PD patients who participated in the NYPUM study and accepted DNA extraction. The genotypes had a relatively even distribution. During a follow up of 6-10 years, the PD patients with the a priori hypothesized risk genotypes COMT 158Val/Val (n = 26) and DRD2 957T/T (n = 37) were found to have a higher incidence of cognitive decline (including MCI or PDD), compared to the patients with other COMT and DRD2 genotypes. Kaplan Meier graphs for the incidence of cognitive decline in carriers of the COMT 158Val/Val and DRD2 957T/T genotypes versus other genotypes are shown in figure 8. The higher incidence of cognitive decline in PD patients carrying the COMT 158Val/Val genotype visible in figure 8 was driven by a higher incidence of MCI in comparison to patients with other genotypes. This was shown by a hazard ratio of 2.13 (95% CI: 1.11-4.08, \( p = 0.023 \)) for developing MCI among the COMT 158Val/Val carriers, while the PD patients with this genotype did not have a higher risk of developing PDD. PD patients carrying the DRD2 957T/T genotype had hazard ratios of 2.12 (95% CI: 1.14-3.94, \( p = 0.018 \)) for developing MCI and 3.22 (95% CI: 1.64-6.30, \( p < 0.001 \)) for developing PDD, compared with the patients with other DRD2 genotypes. The hazard ratios were adjusted for age, disease duration, sex, and baseline cognitive status (normal or MCI).

In the cognitive domains that were covered by the neuropsychological tests, there was no measurable effect related to the COMT genotype. The PD patients with the DRD2 957T/T genotype tended to perform worse in all tested cognitive domains, compared to the patients with other DRD2 genotypes. After correction for multiple comparisons, a significantly poorer performance remained in episodic memory (on average 0.25 standard deviations lower test scores, \( p = 0.007 \)) and in attention (on average 0.58 standard deviations lower test scores, \( p < 0.001 \)) in patients with the DRD2 957T/T genotype. The poorer performance in episodic memory was found in free recall conditions. Interestingly, in the group of healthy control participants, carriers of the DRD2 957T/T genotype performed poorer in tests of attention at an uncorrected statistical threshold, similar to the performance in patients with PD (although this difference was non-significant after correction for multiple comparisons). The PITX3 gene was also found to affect cognitive function during follow up.

Neurofilament in CSF, disease severity and survival in Parkinson disease (paper V)

In study V, neurofilament light chain protein (NfL) in CSF was analysed in patients with PD. The results show that a higher NfL concentration in the early phase of PD was related to more severe symptoms, as measured by clinical scales, hyposmia and shorter survival. The correlations were similar in the population-based (NYPUM) cohort, as well as independently, in the validation cohort. The correlations between symptom severity and NfL concentration were in the range of $r = 0.28–0.48$ ($p$ values 0.005 to < 0.001), and remained significant after adjustment for age. The NfL concentration also correlated with decreased fractional anisotropy in diffusion tensor imaging in several axonal tracts of the brain.
Discussion

In this thesis, the natural history of Parkinson’s disease, MSA and PSP was studied in a population-representative and prospectively followed cohort of patients; in most cases from shortly after the onset of first symptoms until the patient died. The main focus was on prognostic factors related to mortality and cognitive impairment in PD. The demonstrated relationships between long-term survival and early characteristics of PD, including the motor phenotype, CSF levels of NfL and possibly neuroinflammation (studies I, II and V) can provide clues for better understanding different disease progression patterns and differences in survival in PD. The dopamine-associated genotype variants and CSF protein pattern changes that correlated with the development of cognitive decline in patients with PD (studies III and IV) may provide insights to the causes of cognitive decline, in particular in relation to decreased levels of dopamine and aspects of neurodegeneration.

"Benign" phenotypes and survival in parkinsonism

In study I, patients with MSA and PSP were found to have a markedly worse prognosis than patients with PD, which is consistent with findings from several previous studies (Savica 2017, Nath 2003). The patients with incident, atypical parkinsonism (i.e., MSA or PSP) that were followed in the present population-based study were older than in most previous, hospital-based studies (Nath 2003, Wenning 2013, Low 2015), which could be a result of the population-based method of recruitment. Our results confirm the dire prognosis of these diseases, even when the age at onset is relatively high, as was the case in study I. It is worth noting that while age had a significant impact on survival in PD, age was not a significant predictor of survival in patients with MSA or PSP. This is likely explained by the strong effect on mortality caused by these disorders themselves (Bäckström, 2018).

The survival of patients with PD was reduced compared to the Swedish population during the years 2004 to 2017, with a mortality in the studied cohort that was 1.58 times higher than the general Swedish standardized mortality rate. Expressed differently, the standardized mortality ratio (SMR) was 1.58. Because the SMR in most modern PD mortality studies has been in the range of 1.5 to 2.7 (Macleod 2014, de Lau 2006), the mortality in the study was in the lower range. This may be a consequence of the population-based study protocol that was used (including patients with mild parkinsonism and explicitly diagnosing cases of atypical parkinsonism, which are diseases with a worse prognosis than PD) and the fact that all patients had access to comprehensive health care services throughout the disease course. In the 57% of patients with PD who had a normal
cognitive function at the beginning of the study, mortality was not different from the general population. These patients showed several traits suggesting a "benign" PD phenotype, including less bradykinesia and gait impairment, tremor predominance, and lower disease severity measured by the UPDRS, as described in a previous publication (Domellöf 2015). Patients who were cognitively stable, and did not develop PDD during follow up, also had a better olfactory function (Domellöf 2017).

"Benign" PD
Previous research on large cohorts with patients followed longitudinally has shown that a subpopulation of all patients with PD seems to have a "benign" disease, with a low incidence of dementia and a slow disease progression during follow up. In the CamPaIGN study, which studies a population-based cohort of prospectively followed patients with PD in England, a sub-population which represented 23% of all patients with PD had a good outcome at 10 years, being still alive without dementia or postural instability. This group of patients was younger at PD diagnosis, mostly had a tremor-dominant phenotype and had better scores of assessments of motor function, cognitive function and depression (Williams-Gray 2013). Similarly, cluster analysis of baseline data from 421 individuals from the PPMI cohort identified a “mild motor-predominant” form of PD (n = 223) with no or minimal cognitive involvement (representing 53% of the cohort), that had a significantly better prognosis, with less disease progression during follow up (Fereshtehnejad, 2017). Patients with these PD phenotypes may live for many years without cognitive decline, apparent disease progression or complications.

Patients with a "benign" phenotype have variably been described as having tremor predominant, young onset, mild motor, and cognitively normal (Bäckström 2018) PD. Study I shows an even larger proportion of patients with a "benign" phenotype (the 57% of PD patients who had a normal cognitive function at baseline) compared to the 23% found in the CamPaIGN cohort, who had a largely normal survival compared to the general population. These patients are likely to have a largely normal survival also in other settings; at least up to 13-14 years after onset, which was evaluated in study I. Some factors may actually contribute to an increased length of life in PD. There is evidence that PD is associated with lower risk of some diseases such as many cancers and a lower risk factor burden from tobacco-smoking and arterial hypertension (Vanacore 1999, Inzelberg 2007, Ritz 2007, Noyce 2012). In PD patients with normal cognition, who have a milder disease phenotype, such differences could counterbalance increases in mortality caused by neurodegeneration (Bäckström 2018).

However, the most important explanation for the normal life expectancy in some patients with PD is likely that they have a "mild" disease, which is more slowly progressive than average in PD. This "benign" PD phenotype may reflect a very slowly progressive pathology or even, in some cases, a "plateauing" pathology, conferring a low rate of clinical deterioration. The "benign" phenotype of PD, especially in the tremor-dominant
form, is also consistent with the sometimes encountered disorder of isolated resting tremor with nigrostriatal denervation (Ceravoldo 2008), which may represent a subtype of PD with very little clinical and pathological progression.

The findings in paper II and V indicate that a benign or "mild" form of PD may be identified by the early pattern of CSF proteins. In particular, PD patients with low NfL and/or a normal ratio of NfL to Aβ42 at the time of diagnosis (as shown by a higher concentration of Aβ42 than NfL; i.e. a ratio <1), are likely to have a "benign" phenotype at follow up; even in ages above 80 years. However, it is not well known if a "benign" and/or non-progressive disease course in younger PD patients could convert to a more typically progressive PD after many years with the disease.

**Rapidly progressive PD**

The subset of patients with "benign" PD is distinct from the PD patients with reduced survival compared to the general population. These patients can be said to have a more severe PD subtype. Cognitive dysfunction in PD was found to be a predictor of shortened life expectancy and the female PD patients also tended to live shorter than expected in study I. The reason for the shortened life expectancy in female patients with PD is unclear. It may be speculated that female PD patients are more affected by social isolation when they survive their spouses, which could contribute to mortality, or, alternatively, there may be neurobiological sex-differences in relation to disease progression.

In both male and female PD patients, the clinical phenotype that was related to a higher mortality was characterized by mild cognitive impairment, hyposmia, postural imbalance and gait impairment, freezing of gait, high disease severity as measured by the total and Part III UPDRS scores, and general slowness (as measured by the time required for the Up-and-Go test). This is consistent with previous findings that a non-tremor or PIGD phenotype of PD is associated with a worse prognosis and a higher load of several cortical pathologies (Jellinger 2012, Thenganatt 2014). Some previous studies also show a faster disease progression and higher standardized mortality in PD with disease onset in older ages (Jankovic 2001, Coelho 2012), which is associated with levodopa-unresponsive axial disability, such as gait unsteadiness, freezing, and dysarthria.

The PD phenotype that was found to be associated with higher mortality in study I is similar to the PD phenotype that has been consistently found to have a higher risk of cognitive decline and dementia in several longitudinal studies (Heeden 2016, Marinus 2018). Some of the traits of this PD phenotype, in particular, the high disease severity measured by the Part III UPDRS scores, the general slowness, and the bradykinesia, correlate with the severity of nigrostriatal dopamine denervation. This interpretation is consistent with the finding of a negative correlation between life expectancy and the severity of striatal DAT imaging deficits in study I.
**DAT deficits and non-dopaminergic dysfunction**

Surprisingly few studies have investigated the impact of DAT uptake deficits on survival in PD. A previous Finnish study by Järvelä and collaborators found no association (Järvelä 2014). Study I has a partially different design from this study, because the association between DAT uptake deficits and mortality was adjusted for age differences (in addition to possible differences in the SPECT equipment used, et.c.). Previous studies of nigrostriatal dopaminergic cell death have shown that the neuron loss that occurs as a consequence of normal aging is different from the pathological nigrostriatal degeneration that is caused by PD (Fearnley 1991). Interestingly, the association between DAT uptake deficits and survival in PD in study I was stronger after age-adjustment. The interaction with age indicates that it is the disease-specific DAT uptake deficits in PD (independent of age) that reduces survival, while the effect of normal aging (which causes a global decline of striatal DAT uptake) does not. This supports the interpretation that the severity of nigrostriatal degeneration gives rise to increased mortality in PD.

Reductions throughout the striatum (both in the caudate and putamen) were correlated to increases in mortality. However, DAT reductions in the caudate nucleus was a slightly stronger predictor of increased mortality in PD than reductions in the putamen. The difference may be due to a "floor effect" in putaminal DAT imaging in PD, rather than to fundamental differences in biological function between the nuclei. SPECT and PET studies consistently show more prominent degeneration of dopaminergic projections to the putamen compared to the caudate nucleus in early PD (Kaasinen 2017). The caudate nucleus is affected later, as a result of a more advanced disease progression (Brooks 2006). In consequence, early denervation in the caudate nucleus may be a more sensitive marker of a rapidly progressive and aggressive disease in PD, compared to putaminal denervation. Early caudate DAT deficits may give an indication of a more severe PD, predicting a faster pace of nigrostriatal (and, possibly, extrastriatal) degeneration.

Several of the symptoms that were linked to a reduced life expectancy in PD in study I, in particular, cognitive impairment (including attention and memory problems), postural imbalance and gait difficulties, and hyposmia, are also related to deficiency of non-dopamine neurotransmitters, such as acetylcholine. Cognitive impairment, postural imbalance, and gait difficulties are known to respond poorly to dopaminergic treatment, while some of these symptoms (e.g. cognitive impairment) may be partially treated with acetylcholinesterase inhibitors. The risk of both dementia and reduced survival in PD is likely to be affected by non-dopaminergic deficits, and, perhaps most markedly in older PD patients with comorbid brain pathology such as β-amyloid and cholinergic denervation. Several of the pathological processes that contribute to cognitive decline in PD are also likely to contribute to increased mortality. The exact causality of this correlation is not known in detail. However, a deficit which is common for both cognitive decline and gait disturbance in PD may be reduced attention, which correlates with cholinergic hypofunction (Ballinger 2016). Reduced attention impairs several cognitive
processes and has also been linked to gait disturbance in PD (Bohnen 2012, Müller 2015). Reduced attention may thus be one of the characteristics of a severe form of PD.

**Immune reactivity and neurofilament increases in CSF in Parkinson disease**

The collection of biosamples, including cerebrospinal fluid, in a population-based PD cohort such as NYPUM, allow investigation of biological factors contributing to disease progression and reduced survival.

**The neuroimmune system**

The neuroimmune system is important because it is a promising target for therapeutic disease modification not only in traditional neuroinflammatory disorders but also neurodegenerative diseases. Measures of systemic inflammation, such as a pro-inflammatory cytokine profile at diagnosis and small elevations of C-reactive protein (CRP) in blood, have been linked to a faster motor progression, faster cognitive decline, and reduced survival, respectively, in population-based and clinical cohorts of patients with PD (Williams-Gray, 2016, Sawada 2015).

In study I, the finding of a low-grade inflammatory reaction in the CSF of 13.1% of the patients with PD was strongly correlated to reduced survival (with 6.31 times increased hazard for death during follow up), (Bäckström 2018). The counts of mononuclear leukocytes in the CSF of PD patients with a subtle pleocytosis in the study ranged between 2 and 10 cells per microliter, which is not a large elevation, but the leukocyte counts could not be explained by infection in the nervous system (consistent with sterile inflammation) or blood contamination. The relation between minor increases of immune reactivity markers in the early phase and important future outcomes is consistent with recent findings. In the study by Sawada et al., a cohort of 313 PD patients were followed for a mean of approximately 4.8 years, and life-length was significantly reduced in patients with a baseline serum CRP >0.8mg/L compared to patients with a baseline serum CRP <0.8mg/L. In this study, higher CRP correlated with shorter life expectancy, after adjustment for multiple possible confounders. The elevation of serum CRP >0.8mg/L is small, and lower than what would be expected from an acute infection.

The possibility that the relevance of markers of systemic inflammation (e.g. CRP) in PD is confounded by the link to other diseases, like chronic inflammatory disease, coronary heart disease or widespread atherosclerosis (Danesh 2004, Captoge 2010) must be highlighted. However, the finding of a low-grade inflammatory reaction in the CSF, which reflects the milieu of the central nervous system, is less likely to be explained by such confounding. It is unclear if the inflammatory response in the nervous system is a primary event, causing or contributing to neurodegeneration, or simply is the consequence of aggressive and wide-spread neurodegeneration in PD. If it is a secondary
consequence, an inflammatory response might even be protective, in particular, by enhancing the clearance of abundant protein aggregates. Some observations, however, points towards a causal role of immune activation in the neurodegenerative process of PD. First (1), immune inhibition in animal models of PD can alter disease susceptibility and reduce the severity of the disease (Brochard 2009, Gao 2011), which implies a causal role of neuroinflammation. Second (2), genetic studies in humans, including genome-wide association studies, show a significant association between major histocompatibility complex genes (HLA-DR) and PD risk (Saiki 2010, Nalls 2011) which is not a secondary effect. Lastly (3), epidemiological studies of the normal population have shown that individuals who regularly take nonsteroidal anti-inflammatory drugs have a reduced risk of developing PD (Chen 2003, Chen 2005).

Inflammation, in particular activation of some types of microglia in the brain, has been linked to the pathological process in PD (Sanchez-Guajardo 2015). The neuropathology of PD is associated with activated microglia, which have been found close to dopaminergic neurons in the substantia nigra (SN) (McGeer 1988). Elevated levels of cytokines have been found in the striatum and SN (Garcia-Esparcia 2014, Mogi 1994). In addition, in vivo $^{11}$C-PK11195 PET studies show an increase of microglial activation in patients with PD in comparison with healthy control subjects (Gerhard 2006, Ouchi 2005). This neuroinflammation seems to be of importance for cognitive decline. In two PET studies, microglia activation in PDD was found to correlate with decreases of brain glucose metabolism and with worse functioning measured by MMSE (Edison 2013, Fan 2015). Furthermore, a larger increase in microglia activation can be visualized by PET in PDD compared with PD with normal cognitive function (Edison 2013). Some studies on CSF in PD also suggests an association between neuroinflammation and cognitive decline. One study showed increased IL-8 in CSF in PDD but not in PD without dementia, compared with healthy controls (Janelidze 2015). Another study found increases of cytokine IL-6 in PD with cognitive impairment, compared to PD with normal cognition (Yu 2014).

Investigating markers derived from CSF, a cross-sectional study of PD by Hall and colleagues, found that proinflammatory markers in the CSF correlated with both more severe motor dysfunction and with lower MMSE scores (Hall 2018). In CSF, relationships between CRP and the proinflammatory marker SSA, and non-motor features such as depression and fatigue was also found previously in PD (Lindqvist 2013, Hall 2018). Interestingly, in a PET study of Alzheimer disease, cortical levels of $^{11}$C-PK11195 correlated with dementia severity, measured by MMSE, while the cortical $\beta$-amyloid load did not (Edison 2008). This could indicate that, in Alzheimer disease, cortical microglial activation rather than the amyloid load drives the cognitive impairment.

Microglial activation and peripheral immune cells invading the brain may (together with a cortical pattern of neurodegeneration, and possibly acetylcholine deficiency) be one of
the links that connect cognitive decline with mortality in PD. An immune reaction in the CNS may enable or facilitate trans-synaptic spreading of misfolded protein aggregates in specific anatomical pathways in PD and therefore contribute to disease progression. An immune reaction may also contribute to disease progression in PD by enabling cytokine-mediated apoptosis of vulnerable populations of neurons (Hirsch 2009).

However, the distinction between primary and secondary pathologic events is important in this context. Further studies are needed to clarify whether immune changes contribute causally to the progression and pathology of PD or if they are secondary effects. The immune system is a promising target for neuroprotective therapy in PD, but only if immune reactions are primary pathogenic events that contribute to neurodegeneration.

**Neurofilament**

Identifying the amount of neuro-axonal damage in PD is a critical step in neurological care, as it may help estimate the prognosis of a particular patient. Neuro-axonal damage is the pathological substrate of permanent disability in many neurological diseases. NfL holds promise as a biomarker of such processes, as it is an abundant cytoskeletal protein that leaks into the CSF upon neuro-axonal damage, and reflects the severity of the damage (Khalil 2018).

In study V, a high NfL was shown to predict a more severe PD phenotype and shorter survival. In the Validation cohort of study V, which consisted of PD patients that were slightly younger and followed for shorter time periods than in the NYPUM cohort, high NfL concentrations outperformed the age at baseline as a longitudinal predictor of death. The increase in the hazard ratio for death during follow up, per unit increase of baseline NfL, was also higher in the Validation than in the NYPUM cohort. There were fewer fatalities in the Validation cohort than in the NYPUM cohort, reflecting the fact that the patients were followed for shorter periods. All these characteristics indicate that NfL is particularly sensitive to predict increased mortality in the early phase of PD (which was relatively more prevalent in the Validation cohort compared with the NYPUM cohort).

As discussed in study V, it is not clear from what specific part of the brain the elevated NfL emanates and what pathological process releases it. In the patients with PD, NfL associated lesions on diffusion tensor imaging (DTI) were found, among other locations, in corticospinal tracts unilaterally at the level of the thalamus at baseline, after 1 year more markedly, bilaterally in a similar location and, at 3 years, in right-sided corticospinal tracts in proximity to the cortex and in the pons. As stated in study V, the observed pattern could suggest a spreading PD pathology along axonal projecting systems of the brain, with a predominant caudo-rostral course (Bäckström et al. 2019). However, already at the early clinical PD stages (at baseline), several NfL associated DTI lesions were found in association fiber tracts of the cerebral hemispheres, such as the superior longitudinal fasciculus, which is consistent with previous findings in PD (Gattellaro 2009, Hattori
2012). Possibly, this pattern of lesions could represent the trans-synaptic spread of misfolded α-synuclein aggregates in connected brain networks. The lesions shown on DTI may also reflect other brain pathologies in PD, such as ischemic small vessel disease (Gattringer 2017) or co-morbid Alzheimer disease pathology (Olsson, 2016).

Even if not specific for PD, highly sensitive neurofilament measurements have the potential to fill a gap in the assessment of neuroaxonal damage in PD and other neurodegenerative disorders. The approach may allow a sensitive assessment of the consequences of brain tissue damage using a standard CSF sample measurement. As shown by the ability to predict motor severity and survival in two different PD cohorts (the population-based and validation cohorts) in study V, NfL concentration in CSF is likely to have clinical utility for prognostication. If validated in blood samples, NfL could constitute an important advance to aid research, for instance for measuring the efficacy of new neuroprotective strategies, and towards use in clinical practice.

The different causes of cognitive decline in Parkinson disease: a hypothetical ”wet-dry” model

Cognitive impairment, including dementia, is a common and highly problematic non-motor manifestation of PD. Accurate descriptions of the natural history of cognitive decline in PD are critical to the assessment of new therapies for this disorder. This pertains both to the clinical phenotype of cognitive impairment and to the brain imaging patterns and biochemical and pathological changes that lead up to cognitive decline in PD. Such descriptions are also important for correct information about the prognosis of individuals with PD.

Converging evidence points to a multifactorial basis of cognitive decline in PD. The results of studies III and IV highlights aspects of cognitive functioning in PD in relation to dopamine deficiency and availability and, possibly, in relation to the function of dopaminergic neurons, while study III highlights structural brain pathologies that are predictive of cognitive decline in PD.

The impact of depletion of dopamine and other neurotransmitters

Low levels of neurotransmitters are one of the earliest features of PD and may cause heterogenous symptoms. Patients with PD often experience memory problems characterized by an inability to recall information, which can partly relate to dopamine deficiency. Dopamine depletion in the caudate, as measured by F-DOPA uptake on PET, correlates with impairments in executive functions, such as planning and random number generation, and memory (Gratwicke 2015, Rinne 2000, van Beilen 2008). Furthermore, several lines of research indicate that dopamine transmission in the brain is of importance not only for so-called executive functions, but also for aspects of marking events in the external and internal world as ”salient” and for the encoding and retrieval of episodic
memories (van Beilen 2008).

The hippocampus is strongly interconnected with dopaminergic neurons in the mesencephalon (Lisman 2005), not only including the substantia nigra. More specifically, the dopamine receptor D2 (DRD2) subsystem of dopamine signaling is of importance for attention and memory (Floresco, 2006), and is linked to transient memory updating processes (Bilder 2004, Nyberg 2009). A reduction in D2 receptors has been shown in PD (Christopher 2015). In imaging studies, memory deficits in PD correlate with reduced extrastriatal D2 receptor binding in the medial temporal lobe, including the insula, in parts of the so-called "salience" network (Christopher 2014). The poorer performances in attention and episodic memory and the increased risk of dementia in DRD2 $^{957}T/T$ carriers with PD in study III could, possibly, relate to reduced function or increased vulnerability in D2-dependent "salience" networks of the brain (e.g., in medial, temporal structures). The memory and attention deficits in DRD2 $^{957}T/T$ carriers are in line with the proposed roles of D2 receptors in flexible memory updating, which may be influenced by "salience” signaling. Cognitive flexibility is also influenced by this system (Menon 2010).

The most pronounced impairment related to DRD2 $^{957}T/T$ genotype in study III occurred in attention and episodic memory. Furthermore, the patients with PD with a DRD2 $^{957}T/T$ genotype tended to perform worse in all domains. This was not statistically significant after correction for multiple comparisons, but the trend may imply that the DRD2 $^{957}T/T$ genotype impairs a wide range of functions and, possibly, that dysfunction in one network can disrupt other networks. A "global" dysfunction of this type is also consistent with the fact that dementia is, in essence, a multi-domain impairment.

In relation to COMT, previous studies show that functional COMT genotypes have an impact on prefrontal, cortical dopamine levels, while much less or no effect on striatal dopamine levels. The COMT $^{158}Val$ genotype seems to increase the risk of MCI mostly in the early phase of PD, with no effect on PDD risk, but this conclusion is also limited due to the small number of patients and the incomplete follow up in study III.

There are problems with confounding in studies of the effect of dopamine depletion on cognitive functions in PD (e.g. in imaging studies) because both dopamine depletion and cognitive functions share correlations with the underlying, neurodegenerative process of PD. However, even though they may become more evident in the context of the disease, differences in dopamine transmission caused by gene variants are mainly independent of the pathology of PD. Therefore, functional variants in the DRD2 and COMT genes, for instance, can show specific dopaminergic impairments in PD that are independent of the underlying, neurodegenerative pathology.

The genetic studies in this thesis show the importance of sufficiently large samples of
patients when studying possible influences of genetic variation on phenotype. The effect of DRD2 and COMT genotypes on cognitive functions merits further investigation in adequately powered cohorts. Clear proof of multidomain impairments and interactions between domains related to DRD2 genotype would only be detectable in a sufficiently large sample of patients; powered for investigation of small to medium size genetic effects over many domains. Despite the limitations, studies III and IV indicate that hypofunction in dopaminergic systems increases the risk of cognitive decline in PD and that the risk may be different depending on what part of the dopaminergic system that is impaired. The findings, as well as results in previous studies, suggest that both caudal and extrastriatal dopamine deficits (e.g. in the prefrontal or temporal cortex) are detrimental for cognitive functioning in PD. In general, degeneration of extrastriatal dopaminergic projections (in particular in the so-called mesocortical system) has been linked to the development of PDD (Hall 2014).

Because the underlying pathology that drives the dopamine deficits in PD is in itself progressive, the effect caused by dopamine depletion in brain networks is not likely to be stationary or non-progressive. Therefore, the mild and stationary effect on cognitive (executive) dysfunction caused by dopamine hypofunction in PD, that is hypothesized by the "dual syndrome hypothesis" (Kehagia 2013), may not be strictly correct. The effects of functional genetic variants are, often per definition, stationary, but this does not imply that the effect of declining levels of dopamine on cognition in PD is stationary. Hypofunction in caudal and extrastriatal dopamine systems may be progressive and can contribute to a decline in several different cognitive domains. The dopamine depletion that occurs early in the putamen is likely less detrimental in this regard. Further study, however, is needed to outline the exact contributions of different parts of the dopaminergic system during the evolution of cognitive decline in PD.

A hypothetical "wet-dry" interaction model

As stated above, cognitive decline in PD is multifactorial. Cognitive impairments in PD are caused both by deficits of neurotransmitters (which can be termed "wet" pathology) and structural brain pathology (which can be termed "dry" pathology) and their relative contribution to cognitive decline vary in relation to the disease phase. Therefore, the data presented in this thesis, as well as previous research, indicate more than two cognitive syndromes in PD.

"Wet" pathology: PD is, especially in the early phase, a disease of neurons in neuromodulatory control networks. Neuromodulatory neurons; e.g. the dopaminergic neurons of the SN, the noradrenergic neurons of the locus ceruleus, and the cholinergic neurons of the nucleus basalis, have diffuse, highly branched axonal arbors and regulate other, diverse, cell populations by release of neurotransmitters (in particular dopamine, acetylcholine, and noradrenaline) and are selectively impaired in PD (Surmeier 2017). This affects neuronal activation in wide-spread networks (i.e., volume transmission). The
neurochemical network deficits that follow from this impairment (the "wet" pathology related to cognitive dysfunction in PD) causes a heterogenic cognitive dysfunction, with large inter-individual differences. This "wet" syndrome (deriving from impairment in, for instance, striatal and extrastriatal dopaminergic, cholinergic, and noradrenergic networks) is likely to vary from day to day in an individual patient, and causes impairments in attention, executive control, "salience" signaling, maintenance of working memories, and memory retrieval and encoding. These are typically functions of "cognitive control", which are subserved by the neuromodulatory control network neurons that are affected by PD. This type of impairment is different, for instance, from functions that are impaired by acute brain damage or stroke (which disturbs specific pathways). The "wet" dysfunctions occur early in the course of PD and may even be prodromal. Memory is likely affected (directly and/or indirectly) by these network deficits. Activated glial cells in the brain may be classified as part of a "dry" pathology, but the cytokines released by such cells is a wet pathology, that may contribute to cognitive dysfunction and other non-motor symptoms such as fatigue and depression. Evidence shows that fatigue and depression may contribute indirectly to cognitive dysfunction in PD (Lou 2015).

"Dry" pathology: The underlying, causative pathology of PD (Lewy body deposition) and co-morbid, structural pathology such as Aβ deposition in the cortex, is limited in the early phase of PD but progresses with the duration of the disease. When causing diffuse neocortical and limbic aggregation of proteins (α-synuclein, β-amyloid, and other pathological aggregates), ultimately with consequent synapse dysfunction and death of neurons, such pathology is strongly correlated with PDD (Braak 2005, Irwin 2018, Colom-Cadena 2017). This occurs most often in moderately advanced or advanced disease phases. The cognitive decline caused by this pathology is more stable from day to day, likely responds less-well to treatment such as acetylcholinesterase inhibitors, is related to structural disruption of neocortical networks (Gratwicke 2015), correlates with cortical brain atrophy in areas such as the medial temporal lobe (e.g. the hippocampus, amygdala and entorhinal cortex), the posterior visual cortex, as well as the prefrontal areas (Pan 2013, Duncan 2013), and is less reversible. The two structural, cortical pathologies of Lewy body and Aβ deposition and may interact to cause a severe, cognitively impaired PD phenotype (Compta 2011), which is possibly related to neuroinflammation (as reviewed above). However, the "dry" syndrome, characterized by cortical atrophy and pathological protein aggregation in the cortex, may co-exist with the "wet" (neurotransmitter deficit) syndrome, even in the advanced phase of PD.

PDD is multifactorial, and therefore different patients likely have different relative contributions of pathologies, explaining the cognitive decline. The severity of both the "wet" and "dry" disease processes can be investigated by different methods in early PD, and any one of the pathologies, or a combination, predict the future development of PDD (Figure 9). Early pathological findings that correlate with cognitive decline in PD in the long-term may be atrophy of basal cholinergic nuclei on MRI, a high NfL and a low Aβ42
in CSF, a risk genotype or MCI defined by neuropsychological testing, or a combination of these. These biomarkers may enhance the selection of patients in clinical trials aiming to prevent the development of PDD.

**Figure 9.** A wet-dry interaction model showing the evolution and characteristics of cognitive dysfunction in PD. PD = Parkinson disease, NBM = Nucleus basalis of Meynert, CSF = Cerebrospinal fluid.

**Interaction:** The "dry" pathology (i.e. α-synuclein) may actually spread via the dopaminergic and cholinergic projecting pathways to the brain and therefore predominantly affect the target areas of the brain that innervate and/or are innervated by these populations of neurons. In this case, the network pathology that occurs distributed in dopaminergically and cholinergically innervated areas might cause a pathophysiological effect that resembles the "wet" dysfunctions that are caused by neurotransmitter deficiency. However, if α-synuclein pathology spread from the vagus nucleus, other brainstem nuclei, substantia nigra basal forebrain via the connections (retrogradely or antegradely) to the limbic system and cortex, then α-synuclein pathology will seem to appear diffusely in many areas. There is also some evidence that the wet-dry interaction may work in the opposite direction, e.g., that neurotransmitter deficits could contribute to brain atrophy (Gennatas 2012, Markett 2017). This hypothetical "wet-dry" interaction model needs further support but could be used and tested empirically.

Studies II and V suggest that NfL is a biomarker of structural pathology in PD; possibly of the load of α-synuclein accumulation. This is evident by the correlations between NfL and severity of DAT imaging deficits in the striatum, disease severity, and hyposmia. In the younger subgroup of patients with PD with normal cognitive function at baseline in study II, high NfL predicted a rapid progression to PDD. A high NFL concentration may, therefore, be a sensitive marker of cognitive decline in younger PD patients (e.g. compared to Aβ42 in CSF- which is a traditional marker of Alzheimer disease pathology).
The sensitivity for (non-motor) progression in younger patients is also consistent with the findings in study V.

Elevated NfL in these patients probably reflects a more widespread and aggressive disease process, possibly α-synuclein spreading diffusely in axonal networks throughout the brain. This may be a more age-independent process in PD compared to cortical Aβ deposition, which is likely affected by other factors (Fearnley 1991). Patients with PDD who have significant Aβ co-pathology tend to be older (Irwin 2012, Jellinger 2002), and a low Aβ42 in CSF might therefore be associated with PDD with latency. In later disease stages of PD, other co-pathologies in the brain, such as cerebrovascular disease, may also confound the interpretation of the NfL concentration. In contrast, patients who have low NfL, as well as high Aβ42, in CSF in the early phase may have a low load of both α-synuclein and Aβ pathologies. This is consistent with the low risk of future PDD in study II. However, the exact relationships between CSF changes and the development of different brain pathologies in PD need further study, and should preferably be investigated by in-vivo as well as post-mortem methods.

**Methodological considerations and limitations**

In order to avoid selection bias in the population-based (NYPUM) study cohort, we included all, unselected patients with incident idiopathic parkinsonism in the studied area, rather than only PD, and we explicitly diagnosed atypical forms of parkinsonism. The population-based approach for recruiting participants favors generalizability and the results should, therefore, provide information that is generalizable to the “real-life” experience of the overall population with idiopathic parkinsonism, including PD. Likely because of the population-based design, the age at inclusion in the NYPUM study was relatively high (e.g. higher than in many previous clinical trials and hospital-based PD studies). The prevalence of MCI may, therefore, be higher than in some other studies. The definition of MCI which was used (<1.5 standard deviation of the age-appropriate normal values, and self-perceived cognitive decline) also affect the prevalence of MCI in the cohort.

The fact that the thesis is based on an observational study is also a limitation. Observational studies can contain uncontrolled confounding factors since participants are not randomized in relation to the studied variables. Participants that show differences in a studied variable (for instance cognitive function) may not be equal in other aspects (for instance age or level of education). The statistical analysis in all studies in this thesis tried to control for such differences, but there may, nonetheless, be confounding factors that are uncontrolled. For these and other reasons, there is a limited ability to infer causality from observational data.

In many cases, there was an uneven distribution of participation in investigations. In all
5 studies in the thesis, there was a bias for patients that were generally healthier and less severely affected by the disease to participate in all investigations (including lumbar puncture and neuroimaging), while the oldest patients participated to a lesser degree. This was seen for instance in the Diffusion Tensor Imaging of study V, especially at repeated investigations, which may give rise to an underestimation of the true pathology in the population with PD.

We have used ELISAs to measure NfL and other proteins in the cerebrospinal fluid. When measuring absolute values, this method may not always give the most exact precision which could be obtained by modern analytical methods. However, the ELISA methods are well validated and the analyses were performed at an established laboratory that uses procedures approved by the Swedish Board for Accreditation and Conformity Assessment.

Another limitation, similar to other studies of living patients with neurodegenerative diseases, is the limitation of clinical diagnoses. Studies have reported that neuropathologic confirmation of a clinical diagnosis of idiopathic PD at autopsy ranges from 65% to 93% (Adler 2014), although the accuracy of diagnoses is higher in expert centers (Hughes 2002) During the follow-up periods for the 5 studies in this thesis, neuropathologic diagnosis at a research autopsy of the nervous system was obtained in 5 of 109 deaths. The risk of incorrect diagnosis was nonetheless minimized by the long follow-up periods at a movement disorders unit, with evaluation by experienced neurologists, and the finding of pathologic uptake on DAT imaging in all of the examined patients (in both the NYPUM cohort and in the Validation cohort).
Summary and clinical perspectives

(1) The mortality is increased in the parkinsonian diseases PD, MSA, and PSP, compared to the general population. The standardized mortality ratio for all patients was 1.84. However, the survival is highly variable. Patients with atypical parkinsonism (MSA or PSP) had a mortality that was more than three times higher than the general population.

(2) In PD, mortality is related to cognitive function. Patients with PD presenting with normal cognitive function (no MCI) had a largely normal life expectancy.

(3) In PD, patients with a PIGD phenotype had shorter survival.

(4) Cognitive function as measured by MMSE, and olfactory function, were also related to survival in PD.

(5) CSF analysis revealed several patterns that were of importance for the prognosis in PD. Mildly elevated leukocytes in the CSF was associated with shorter survival. An early pattern predictive of the future development of dementia (PDD) was high Neurofilament (NfL), low Aβ42 and high HFABP (conferring and almost 12 times increased PDD risk). A high Neurofilament (NfL) at study entry was also predictive of reduced survival in PD.

(6) Functional variation in dopamine-associated genes (the COMT, DRD2 and PITX3 polymorphisms) were associated with effects on cognitive functions in PD in the studies in this thesis; in particular in attention (as measured by the Trail-Making-Test), episodic memory and visuospatial function. These particular polymorphisms may not be of clinical importance, but the fact that cognitive functioning in PD is affected by common genetic variation is an important concept.

In relation to diagnosis and prognostication in PD, four relatively simple tests and procedures were most useful:

- Clinical, neurologic evaluation, with a classification of the predominant phenotype.
- An olfactory function test (e.g., B-SIT), evaluating the degree of hyposmia.
- Testing of cognitive function (preferably neuropsychological evaluation of all cognitive domains but a shorter assessment such as the MoCA also has value).
- Lumbar puncture with measurement of Aβ42 and NfL concentrations.
Acknowledgement

The opportunity to complete a thesis has meant an enriching and developing time for myself and I am deeply grateful to all those who have made this possible. A large number of people have supported, inspired and helped me. I want to thank everyone but will just mention a few of you in particular.

My main supervisor, professor Lars Forsgren for your never-failing kindness and encouragement and for guiding me into a large, interdisciplinary research context. Thank you for giving me the opportunity.

Jan Linder, my co-supervisor, who did an invaluable job of collecting the data and the cerebrospinal fluid samples that made this thesis possible.

Gabriel Granåsen, for great support with statistics, who always made me feel that statistics are strangely uplifting.

Niklas Lenfeldt, who did an excellent work with the diffusion tensor imaging and Miles Trupp, the only man on earth from San Francisco living in Ö-vik, who helped me a lot with proofreading and who always had a brilliant perspective (on cells, as well as horses and many other things).

Magdalena Domellöf, my friend and amusing co-worker, who helped me to the right track of NYPUM. Jörgen Andersson and Mona Edström, who even more specifically made sure the foundation was always in place, and all the members who have contributed to the NYPUM project through the years. I still can’t believe the vastness of the data you collected! And of course the participants themselves.

Umeå University's medical faculty, who believed in me, and gave me the opportunities to conduct the research.

Johan Jacobsson, who at the end was my clinical supervisor, and the other boss, Hans Lindsten, who always encouraged us with a fantastic wall of laughs, which really was all that was needed to be said. Since Jonathan Salzer wrote his thesis in 2013, no paper has still been found that you have hesitated to sign!

Linda Eriksson and all the nurses in the Parkinson team (Åsa, Erika and Ulrika), who actually took care of the patients. Professors Patric Blomstedt and Marwan Hariz, Frida Nordin, the neurologist/synesthete who became a geneticist and all ST physicians; Jonathan, Måns, Elias, Sara, Simon, Thomas, Mattias, William, Thomas, Erica, Axel, Vivian - and everyone else - who makes the neurology clinic such a good place. Anders Svenningson for your catching will to bring the care of the neurologically ill forward and
Jan Malm for important tips along the way. Karin Forsberg, with the interesting career switch from neuropathology to neurology (thank you for choosing life!), who has recently become my roommate. Please, greet Heiko Braak from me and give him a copy of the book if he wants!

Richard Birgander, the eminent neuroradiologist, who always knew when it was time to invigorate with Norwegian death metal during the MR sessions. As it happened, I also learned something about MR imaging along the way.

Henrik Zetterberg and Lars Nyberg, because you give such inspiring examples of what science can be, and all the great "brains" at the UFBI lab, for inspiring lab meetings, even though my Meynert fMRI part did not make it into the thesis. Better luck next time.

Javier DeFelipe and Ricardo Martínez Murillo, at the Ramon y Cajal Institute in Madrid, who rescued me in spite of short notice, and gave me permission to use the World Legacy which consists of Ramon y Cajal's recorded works, as he saw the human brain in the microscope.

Maria, Hilma, and Tindur for all love and the life outside the box.

All friends and my dad, Torbjörn Bäckström, who in some inexplicable, yet tangible way inspired me to choose the work of science; always with a smile and a good story. His father, Carl Emanuel Bäckström, started the family's educational journey when, after his childhood under simple conditions, he moved to Lund and Uppsala to train himself. My mother and my siblings; Fredrik and Anna, for all the support.

Thanks!

The studies in this thesis were funded by grants from the Swedish Medical Research Council (K2013-62X-15224-10-4), Erling Persson Foundation, Umeå University, Västerbotten County Council, King Gustaf V, and Queen Victoria Freemason Foundation, Swedish Parkinson's Foundation, Kempe Foundation, and the Parkinson Foundation in Sweden.
References


Ballinger EC, Ananth M, Talmage DA, Role LW. Basal Forebrain Cholinergic Circuits and


Irwin DJ, Hurtig HI. The Contribution of Tau, Amyloid-Beta and Alpha-Synuclein Pathology to Dementia in Lewy Body Disorders. J Alzheimers Dis Parkinsonism 2018; 8(4).


Kalinderi K, Bostantjopoulou S, Fidani L. The genetic background of Parkinson's disease: current


<table>
<thead>
<tr>
<th>Time (years)</th>
<th>Baseline</th>
<th>1 year</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
<th>5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRI</strong></td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>SPECT</strong></td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><strong>SPG-36</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><strong>PDS</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><strong>SPG-39</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><strong>cT1</strong></td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td><strong>cT2</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>UPDRS</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Habits</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>SF-36</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>VF-12</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>SMP</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>SMP</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Appendix: Timeline for investigations**

All investigations were also performed at baseline. Familiar occurrence, motor function in daily activities, speech, gait, facial reflexes, eye movements, autonomic test (simple), SPECT IBZM, MDS-UPDRS, Hoehn Yahr, UPDRS, and SF-36 were also investigated (not shown in timeline).