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Prognostic subtypes in Parkinson's disease and related disorders. Clinical, genetical, and biochemical markers for long-term social, motor, and cognitive development.

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Abstract

Objectives: Neurodegenerative disorders such as Parkinson's disease (PD) progress with trajectories that may differ markedly between individual patients. This thesis investigates the effects of clinical, genetical, and biochemical factors on the long-term development of patients with neurodegenerative diseases with parkinsonism and cognitive decline.

Methods: **Paper I and II** were based on examinations and medical record search of a cohort of 142 PD patients. Serum neurofilament light chain (S-NfL), age, sex, disease duration and two different clinical classification systems: simplified clinical subtype (SCS) and motor-phenotype (MPS), were examined as explanatory factors for disease severity, mainly reflected by the time to reach five pre-defined milestones of disease progression.

Paper III and IV were based on examinations and genetic analyses of members of two extensive multi-incident families with parkinsonism and dementia. Genetic databases, cohorts of familial PD and controls as well as the medical literature were searched systematically for observations of the identified genetic variants.

Results: **In paper I and II** we found that SCS and S-NfL levels were associated with risks for developing four negative outcomes: walking-aid usage, nursing-home living, loss of independent locomotion and death. SCS groups also showed different risks for developing dementia. Some patients re-classified to a different SCS group at baseline compared to at re-examination after mean 8 years. Our findings suggest that combination of S-NfL with clinical parameters might improve classification of patients into groups with different disease progression. The MPS held no prognostic information, but we found associations with disease progression and measures of clinical and social affectio of PD when using only the postural instability and gait disorder component of MPS and not the tremor part. Age at onset and sex were confirmed to be important prognostic factors with large effect sizes.

In paper III we described the clinical picture associated with the identified MAPT p.(R406W) mutation in the studied family and 66 cases in the litterature as predominantly affecting memory. Behavioural and language dysfunction developped in most cases during the disease course, but parkinsonism was relatively rare. A particular pattern of atrophy was identified in the ventro-medial temporal lobe on imaging. This was confirmed on neuropathological examinations which showed progressive supranuclear palsy-like pathology with 4R tau isoforms in more abundance than 3R isoforms.

In paper IV the clinical and pathological disease was typical PD with marked cognitive detorioration in all affected family members. We presented a list of rare genetic variants that may be disease-causative in the family but had not previously been associated with familial PD. Variants in PGLYRP2 and the RUNDC3B genes were highlighted and were found associated to PD in the literature and in another patient with familial PD, respectively.

Conclusions: S-NfL, SCS, age at onset and sex are important factors associated with long-term disease outcomes in PD patients. These factors can be assessed easily through office-based clinical examination and serum analysis. Patients may benefit from improved prognostic information, and from fascilitated future research on pathogenetic and subtype-defining factors of PD is facilitated. The MAPT p.(R406W) results can improve clinical identification and radiological and pathological factors are suggested to further examine the differences between neurodegeneraitve diseases. New genetic candidates for causing familial PD with cognitive decline are proposed and theoretical models of environmental effects related to PD pathology and these genes are discussed.

Key words: Parkinson's disease, prognosis, neurofilament light chain, clinical subtype, motor-phenotype, MAPT R406W, frontotemporal dementia, enthorinal cortex, progressive supranuclear palsy, tau isoform, brain-gut hypothesis, proteoglycan recognition protein, PGLYRP, RUNDC3B

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To my family

now

then – late grandfather

and in the future – my daughter

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II.   Ygland Rödström E, Mattsson-Carlgren N, Janelidze S, Hansson O, Puschmann A. Serum neurofilament light chain as a marker of progression in Parkinson's disease – long-term follow-up and implications of clinical subtypes. *Manuscript submitted*

III.   Ygland E, van Westen D, Englund E, Rademakers R, Wszolek ZK, Nilsson K, Nilsson C, Landqvist Waldö M, Alafuzoff I, Hansson O, Gustafson L, Puschmann A. Slowly progressive dementia caused by MAPT R406W mutations: longitudinal report on a new kindred and systematic review. *Alzheimer's Research and Therapy.* 2018 Jan 9;10(1):2. doi: 10.1186/s13195-017-0330-2. PMID: 29370822

IV.   Ygland Rödström E*, Soto-Ortolaza A*, Englund E, Kafantari E, Dicksson D, Wszolek ZK, Ross OA§, Puschmann A§. Genomic analyses of a large Swedish multi-incident kindred with autosomal dominant Parkinson's disease with dementia. *Manuscript.* * and §: Equal contributions by the authors

Abbreviations

ACER: Addenbrookes cognitive assessment revised

AD: Alzheimer's disease

ADL: Activities of daily living

ApoE-ε4: Apolipoprotein E ε4 allele

APS: Atypical Parkinsonian syndrome

AUROC: Area under receiver operator characteristics curve

CBD: Corticobasal degeneration

CSF: Cerebrospinal fluid

DLB: Lewy-body dementia

DM: Diffuse malignant subtype

FTD: Frontotemporal dementia

HR: Hazard ratio

HY: Hoehn and Yahr stage

IM: Intermediate subtype

IQR: Inter-quartile range

LB: Lewy body

MAF: Mean allele frequency

MAPT: Microtubule associated protein tau

MCI: Mild cognitive impairment

MMP: Mild motor-predominant subtype

MPS: Motor-phenotype system

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MSA: Multiple system atrophy

NMS: Non-motor symptoms

NMSQ: Non-motor symptom questionnaire

NOD2: Nucleotide-binding oligomerization domain 2

PD: Parkinson's disease

PDC-Guam: Parkinsonism and dementia complex of Guam

PDD: Parkinson's disease dementia

PIGD: Postural instability and gait disorder

PGLYRP2: Peptidoglycan recognition protein 2

PPMI: Parkinson's progression markers initiative

PSP: Progressive supranuclear palsy

RBD: Rapid eye movement sleep behaviour disorder

ROC: Receiver operator characteristics

RUNDC3B: RUN domain-containing protein 3B

SCS: Simplified clinical subtype

SD: Standard deviation

S&E: Schwab and England activities of daily living scale

SNP: Single nucleotide polymorphism

TD: Tremor dominant

TDP-43: Transactive response DNA-binding protein of 43 KDa

UI: Undetermined/Intermediate

UPDRS: Unified Parkinson's disease rating scale

WES: Whole exome sequencing

WGS: Whole genome sequencing

Populärvetenskaplig sammanfattning (Swedish)

Parkinsons sjukdom (PD) medför dels rörelsesymtom (motoriska symtom) såsom stelhet, långsamhet och skakningar i vila, och dels icke-motoriska symtom såsom förstoppning, blodtrycksfall, kognitiva svårigheter och olika typer av sömnstörning. Sjukdomen finns i ärftliga former som har sin grund i genförändringar (mutationer) med mendelsk nedärvning, men majoriteten av patienterna räknas ha den vanliga icke-ärftliga (sporadiska) formen där orsaken är okänd och antas vara komplex. Sporadisk PD är mycket olikartad i sitt förlopp, både avseende vilka individer som utvecklar vilka symtom under sjukdomens gång samt avseende hur snabbt den framskrider. För vissa förlöper sjukdomsförloppet snabbt med allvarliga symtom såsom demensutveckling, för andra progredierar sjukdomen mycket långsamt med mildare symtom. Det saknas bra sätt att förutsäga hur sjukdomen kommer utvecklas hos en enskild individ med PD.

Vi ville undersöka det långsiktiga sjukdomsförloppet hos patienter med PD och om det kan förutses genom att använda väletablerade prognostiska faktorer samt (en anpassning av) ett nyligen föreslaget klassifikationssystem, som använder både motoriska och icke-motoriska symtom för att dela in patienterna i tre grupper: mild-motorpredominant, mellangrupp och diffus-malign subtyp (studie I). Detta subtyps-systemet jämfördes sedan med det mer etablerade motoriska motorfenotyp-systemet. Vi undersökte detta vidare i studie II där prognostisk nytta av halten av ett protein i serum, neurofilament light chain (S-NfL) var huvudfokus. Vidare så hade vi haft långvarig kontakt med två släkter med två olika varianter på sjukdom med parkinsonsymtom och tankepåverkan av demensgrad (studie III och IV). Vi ville beskriva deras kliniska bild och långsiktiga utveckling kopplat till den genmutation som orsakar sjukdomen, vilket i det ena fallet (studie IV) förväntades vara tidigare okänd.

Data insamlades genom forskningsbesök och strukturerad genomgång av journaler. En kohort med 142 tidigare undersökta individer var underlag för applicering av motorfenotyps- och subtyps-klassifikationssystem. Samtliga individer inbjöds till ett uppföljande forskningsbesök ca 8 år efter inkludering i studien, vilket genomfördes för 34 individer. S-NfL mättes vid både bas-besöket och uppföljningsbesöket. Familjemedlemmar i de två olika familjerna identifierades via intervjuer, släktforskningsdatabaser och kyrkböcker och en systematisk genomgång av tidigare rapporterade fall med den tidigare kända genetiska orsaken – en mutation i genen för proteinet tau: MAPT p.(R406W) – till demenssjukdomen hos den ena familjen genomfördes. Analys av alla gener (helexomsekvensering) och i ett fall av hela arvsmassan (helgenomsekvensering) utgjorde grunden i de genetiska undersökningarna.

Resultatet av studie I visade att det anpassade subtyps-systemet delade in patienterna i grupper med olika risker för samtliga fem indikatorer på

sjukdomsutveckling vi studerat: användande av rollator, flytt till vårdboende, osjälvständighet i förflyttning (Hoehn och Yahr stadium 5), demensutveckling och död. Påverkan av ålder vid insjuknande, sjukdomsduration och kön påvisades också vara stor. I motorfenotyp-systemet hade dock en stor del av individerna hamnat i gruppen med sämst prognos redan vid bas-besöket, vilket sannolikt medförde att systemet inte kunde användas framgångsrikt för risk-bedömning i kohorten. I båda klassifikationssystemen visades relativt stor omfördelning av grupptillhörighet vid uppföljningsbesöket. Resultaten kan tolkas som att subtyps-systemet ger bättre information om den förväntade framtida sjukdomsutvecklingen när PD patienter undersöks i mitten eller sent i sjukdomsförloppet. Anpassningen av systemet gjorde det lätt att applicera på ett vanligt läkarbesök.

Resultaten av studie II visade att S-NfL på gruppnivå var tydligt associerat till utveckling av ovanstående indikatorer och även till allvarlighetsgrad av andra aspekter av sjukdomen vid både bas-besök och uppföljningsbesök. Vi konstruerade ett explorativt diagnostiskt poäng-system genom att kombinera data från klinisk klassifikation, ålder vid debut och S-NfL. Det var främst de senare två delarna som bidrog till detta system. Resultaten talar för att S-NfL är ett värdefullt verktyg som återspeglar en stor bredd av sjukdomsförloppet och att särskilt dess kombination med debutålder är av stort intresse som prognostisk markör.

I den första studien om ärftlig PD, studie III, beskrev vi bilden av *MAPT* R406W-associerad sjukdom hos en svensk familj och i 66 fall i litteraturen som långsamt fortskridande och med flera symtom liknande Alzheimers sjukdom. Många individer utvecklade också beteendestörning och språkstörning, vilket oftare beskrivs vid frontallobsdemens, som *MAPT* R406W sjukdomen formellt tillhör. Hjärnavbildande undersökningar visade förtvining (atrofi) i mediala delarna av tinningloben och vi identifierade att detta sannolikt bör beskrivas tydligare av radiologer och föreslog ett radiologiskt tecken att använda för identifiering av sådan atrofi. Undersökning av hjärnvävnad av tre avlidna medlemmar visade ett oväntat fynd avseende fördelningen av former (isoformer) av tauproteinet, som till viss del går stick i stäv mot tidigare rapporter. Studien medför ökad förståelse och uppmärksamhet kring denna specifika genetiska sjukdomens symtombild inklusive dess likheter med Alzheimers sjukdom, vilket kan öka identifieringen av dessa individer och förbättra informationen till dem och deras anhöriga.

I den andra familjestudien (studie IV) visades en klinisk och neuropatologisk bild som vid sporadisk PD, med en mycket hög risk för demensutveckling. Det mest framstående resultatet bestod i en lista över 9 potentiella kandidater till ny genetisk orsak till PD. Två av dessa utmärkte sig bland annat genom att generna var associerade till PD, antingen genom tidigare studier vilket var fallet för *PGLYRP2* genen där vissa mutationer tidigare visats ge ökad risk för PD, eller genom att vi påvisade mutationen hos en annan person med PD och ärftlighet för PD, som var fallet för *RUNDC3B* genen. Om vidare studier bekräftar fynden som orsak till PD så kan det alltså betyda att vi identifierat en eller fler nya gener för PD. Vi

understryker dock att effekter av miljöfaktorer och nedsatt penetrans av sjukdomssymtom kan ha haft stor påverkan på slutsatserna i studien.

Summerat så visar avhandlingen på behovet att fortsätta undersöka olika varianter av PD, både ärftliga varianter och olika allvarlighetsgrad eller subtyper av sporadisk PD. Ökad förståelse av ärftliga former av PD kan förtydliga inverkan av kända eller föreslagna sjukdomsmekanismer för PD eller andra neurodegenerativa sjukdomar, såsom inverkan av miljöfaktorer eller olika påverkan på tau proteinet och dess isoformer, vilket diskuteras och föreslås i avhandlingen. Indelning av PD baserat på kliniska och biokemiska markörer bör fortsätta ske av liknande orsaker samt att individer med PD, dess anhöriga och vårdpersonal kan, efter bekräftelse av våra fynd i ytterligare studier, beredas prognostisk bedömning som kan medföra bättre beredskap för symtom i senare sjukdomsstadier. Det undersökta subtyps-systemet och S-NfL i kombination med insjuknandeålder påpekas särskilt som lämpliga alternativ för att på sikt åstadkomma detta.

Introduction

Overview

Neurodegenerative disorders are a group of medical conditions in which neurons slowly decay. Over time, neuronal loss impacts the affected brain and a progressive clinical disease manifests with symptoms such as typical motor symptoms in Parkinson's disease (PD) or marked cognitive or language dysfunction in the dementia-disorders. Such symptoms can gravely impact the lives of both the people suffering from the conditions and their close ones. The neurons atrophy in different patterns and speed, which causes continuously worsening symptoms that include cognitive and behavioural changes and often also involvement of motor functions. This thesis studies which role clinical, genetical, and biochemical factors play in the activity of neurodegenerative diseases with motor and non-motor affection. It also studies the long-term effects of neurodegeneration.

There are several pathological mechanisms and clinical features that overlap between the different neurodegenerative diseases and several factors affecting neurodegeneration are more thoroughly studied in other neurodegenerative diseases than PD. Although PD is a major focus for this thesis, it also encompasses other neurodegenerative diseases as the direct interest in paper III or as comparison to PD.

Clinical symptoms of neurodegenerative disorders

In general, cardinal features associated with Alzheimer's disease (AD), the most common neurodegenerative disorder, are symptoms affecting memory and spatial orientation. Patients with frontotemporal dementia (FTD) have changes of behaviour and language, and those with PD generally develop disturbances of motor function, gait, and balance, among other symptoms. However, the symptoms and their neuropathological background in these different neurodegenerative disorders are complex and can be overlapping. What is further complicating neurodegenerative conditions is that their causes are unknown and the concept of how a given neurodegenerative disease affects the brain may change due to new scientific understanding. For instance, PD, the second most common neurodegenerative disorder, was for a very long time reduced to encompass only

motor symptoms as its core clinical and neuropathological feature. More recently, it has been increasingly recognised that non-motor symptoms (NMS) of PD constitute a large part of the symptomatic spectrum and can help define different subtypes of PD (1). As we will see, this change has not yet been fully implicated in all aspects of knowledge of PD, as underlying mechanisms of the NMS are still not completely understood, and motor aspects still dominate clinical diagnostics (2). Also, and a key topic for a major part of this thesis, knowledge about the usefulness of the NMS to define disease subtypes of prognosis is still incomplete (3).

Disposition of this thesis

Directly after this introductory overview follows a detailed description of PD. Some information of other neurodegenerative diseases is also needed to understand certain disease features and parallels of pathological processes. The introduction thus includes a general part on neurodegenerative diseases before continuing with a list of some highlighted pathogenic mechanisms that can lead to PD and PD dementia (PDD). The rationale and aims for studying PD and families with genetic cause to parkinsonism and cognitive dysfunction then follows.

The methods, results and discussion sections of this thesis are divided into two different parts. Two of the studies (paper I and II) were based on a longitudinal cohort of patients with PD, and their methodology, results and discussion are described first. The two other studies (paper III and IV) were based on interviews and clinical examinations of individuals from two separate families with parkinsonism and cognitive decline and are described and discussed in a separate section thereafter.

Parkinson's disease

Paper I, II and IV in this thesis focus on PD. The first description of PD was made by James Parkinson in his essay on the shaking palsy in the year 1817. The disease was later named after him by another famous neurologist, Jean-Marie Charcot. The disease is defined primarily by motor hallmarks: bradykinesia (slowness of movements), postural instability, rigidity (stiffness) and resting tremor (4–6 Hz). A combination of bradykinesia together with any of the three latter mentioned symptoms has been considered the basis of parkinsonism, a symptomatic constellation which can be caused by many different conditions, both non-neurodegenerative and different neurodegenerative diseases (4). The typical parkinsonian disorders include PD, dementia with Lewy bodies (DLB), and three disorders categorized as atypical Parkinsonian syndromes (APSs): progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and multiple system atrophy (MSA). PD often include a characteristic progression of symptoms, such as unilateral perseverance early in disease and additions of other symptoms such as micrographia, hypomimia, dystonia, characteristic walking patterns with shuffling gait, start hesitation and other symptoms. The lack of some symptoms in clinical progression, such as NMS, or early involvement of some, such as cognitive decline, oculomotor paresis, profound postural instability, ataxia or others, are examples that suggest other causes to parkinsonism than PD. Revisions of diagnostic criteria have substantified these features into supportive criteria and red flags, which speak in favor and against PD to be the underlying condition, respectively (2). One can also note that these criteria disregard the postural instability as a part of parkinsonism, due to the risk of postural instability speaking for other causes than PD when present early in the disease.

PD is defined neuropathologically by the lack of dopaminergic neurons in the substantia nigra pars compacta along with accumulation of the protein α-synuclein into Lewy bodies and Lewy neurites (5). The substantia nigra pars compacta is a part of a group of midbrain neurons that belong to the basal ganglia, a group of subcortical nuclei involved in selecting desired motor and behavioral responses (4).

Epidemiology

PD is the second most common neurodegenerative disease and as with other neurodegenerative diseases, its prevalence increases with increasing age. The prevalence of PD rises in older individuals from being below 0.2% before 60 years of age to 1% in persons older than 70 years and 2% in persons older than 80 years of age (6). Like other neurodegenerative diseases, PD is predicted to increase rapidly in societies around the world as life expectancies increase. In fact, the number of patients with PD has already more than doubled over a 25-year period, to more than 6 million people affected today, and is predicted to increase to about 12–17 million people by 2040 (7). PD has one of the more-rapidly increasing incidences of neurodegenerative diseases and is associated with significant morbidity and 50–170% increase in mortality, which together with the rising number of patients highlights the importance of studying the disease (7).

Diagnosis (and misdiagnosis) of PD

The current golden standard for a diagnosis of PD is neuropathological examination, but diagnosis ante-mortem is possible through clinical diagnostic criteria. Like in other neurodegenerative diseases, there are different levels of certainty in the clinically established PD diagnoses. In the relatively recently revised movement disorder sociaty criteria, a patient can be diagnosed with clinically probable PD, with both sensitivity and specificity aimed to be over 80%, and clinically established PD with higher sensitivity (2).

The revised diagnostic criteria further highlight differences to other causes of parkinsonism, including the APSs. This is done by weighing supportive factors for PD, such as rest tremor or an effect of levodopa treatment in the form of amelioration of symptoms or occurrence of dyskinesias, against red flags, such as progression to wheelchair or severe autonomic failure within 5 years of diagnosis (2).

Non-neurodegenerative parkinsonism

As parkinsonism can be a major or minor symptom of other neurodegenerative disorders, as in parkinsonistic forms of PSP or later stages of non-PD dementias, misdiagnosis early in disease is relatively common. Parkinsonism as a clinical symptom may also occur in conditions that are not considered a movement disorder nor a neurodegenerative disease. Examples for such conditions include complex neurological syndromes, medication-induced Parkinsonism, or isolated damage of the basal ganglia due to local infarctions or small vessel disease.

Atypical Parkinsonian disorders

A major contribution to diagnostic uncertainties is added by the APSs such as PSP or CBD. This group of disorders often affect motor functioning early in the disease course, with parkinsonism as a major symptom. PSP and CBD usually also involve rapid progression of motor and cognitive dysfunction and sometimes patients develop more unusual features such as limb apraxia or alien limb phenomenon in CBD and vertical gaze palsy in PSP (8-10). The APS MSA shows separate clinical subtypes that can include dysautonomia, cerebellar symptoms and/or parkinsonism (11). APS-patients often do not respond well to PD medications, which is an exclusion criterion in the newest diagnostic algorithm of PD when the lack of effect is clear (2). Radiological signs and biomarkers of APSs include atrophy in specific regions such as cerebellar peduncles or the midbrain, that can be measured or visualized, as for instance the hummingbird sign in PSP or the hot-cross bun sign in MSA (12, 13). Even though the APSs can be very similar to PD in the expressin of clinical symptoms, patients with CDB and DBS are displaying a different type of pathology in the brain at neuropathological examination compared to PD-patients, tau-related pathology instead of α-synuclein-related, which will be discussed in further detail below. There is a considerable overlap between pathological CBD clinical diagnoses, and patients found to have CDB post-mortem often have PSP but somtimes PD and different dementias as clinical diagnosis (10).

Diagnostic accuracy

Patients who are first considered to have PD need to be re-classified to another disease if signs or symptoms occur that indicate another cause for parkinsonism to be more likely. Even though the presence of some symptoms, associated with other reasons for parkinsonism than PD, have been marked as absolute exclusion criteria in the revised diagnostic criteria (2), and although physicians keep in mind that the initial diagnosis could be incorrect in PD patients, patients typically have correct clinical diagnosis in 65–90% of cases (14). Accuracy of clinical diagnosis in PD is increased during longitudinal follow-up, but can still convey a significant dilution of true PD patients in cohort studies, since clinical PD diagnosis will be somewhere around 90–99% correct at later stages (14). In one study, before the latest version of the diagnostic criteria, clinical diagnosis of PD was neuropathologically confirmed in a range of 26–88% of the patients diagnosed with *possible* PD at first visit, the lower level of certainty used, but higher leveled clinical certainty after 5 years of disease duration (15). The uncertainties of clinical diagnosis were also exemplified in the cohort studied in paper I and II of this thesis. Even though the patients were examined at baseline by an experienced neurologist (my main supervisor Andreas Puschmann) several patients were excluded at follow-up as they were found to have other causes of their parkinsonism than PD.

Heterogeneity of PD

PD is heterogeneous to such a degree that some doubt it is one single disease entity, and one could argue that several sub-diseases are combined in the term PD, suggesting it should rather be called Parkinson's syndrome (16, 17). The underlying causes of this heterogeneity are relatively unknown and this is one of the reasons for ongoing efforts to subtype PD (18).

Clinical heterogeneity

Patients with PD can have both varying types of NMS and motor symptoms and also different trajectories and progression speeds (18-20). For instance, some patients never develop tremor, some never develop PDD and some are still able to walk without assistance after 20 or 30 years of disease duration, while others become bed-bound, develop PDD or die within a much shorter time-span. Such heterogeneity is well-described in the literature and better means to measure it is considered a prioritized research focus (20, 21). The lack of an overall progression marker in PD to estimate the expected disease course brings a lot of uncertainty in both the work of the clinician and in the life of the patients with PD (22, 23). Numerous studies have assessed the topic of progression in PD and studied factors that can indicate if an individual will have an aggressive or mild disease course (24-27). New identification of replicable factors that are clearly correlated to certain disease traits or progression speeds have unfortunately been hard to identify but there are some factors known for some time that bring information on prognosis.

Pathological heterogeneity

The neuropathological hallmark of PD and the sister-disease DLB is the presence of a protein called α-synuclein, formed into complex deposits called Lewy bodies (LB). Even though neuropathology is considered the gold standard to diagnose PD, the neuropathological picture is not unanimous. Important areas proposed to be affected more consequently with LB pathology include the olfactory bulb, the dorsal motor nucleus of vagus in the brainstem, sympathetic ganglia and bowel mucosa (3, 28-31). Other areas of the brain are affected in later stages of disease, including the neocortex, where LB pathology is related to development of PDD (32, 33).

Perhaps as interesting as the suggested differences in spreading patterns of LB-related pathology is that accumulating evidence indicate that some genetic forms of PD (introduced in more detail in the next section), such as the LRRK2 p.(R1441G) mutation, does not harbour any Lewy-related pathology at all (34). Furthermore, while there are no full neuropathology reports yet on carriers of VPS35 p.(D620N) mutation, there is data from experimental models that suggest that instead of α-

synuclein pathology, depositions of tubilin associated unit – tau, is more likely in individuals with this genetic variant (35-37). This is a good example of the large heterogeneity of PD and in many regards poorly understood mechanisms behind the disease.

PD genetics

Sporadic and familial PD

As in most other neurodegenerative disorders, the pathogenic factors responsible for PD are not fully defined. The typical condition is thus labelled as idiopathic or sporadic disease which can not with full accuracy be diagnosed until death. In 10–15% of cases, though, there is a family history of PD, and there are known monogenetic forms of familial PD, caused by rare variants in a small number of genes and inherited in a Mendelian fashion, in about 5–10% of cases (4, 38). These specific forms of PD can be accurately diagnosed before death, and even if the clinical picture of the monogenetic disease-forms can differ somewhat from that of typical idiopathic PD, several mutations lead to clinical conditions and/or neuropathology indistinguishable from sporadic PD (39-41). Even though the sporadic disease is most likely multifactorial, studying the genetic causes can most likely improve our understanding of pathological mechanisms that are considered similar or identical to those in sporadic PD. Although the sporadic variants of neurodegenerative diseases are generally the most common forms, genetic disease could thus be considered a more solid ground for prospective clinical studies today since the cause is known before autopsy. Thorough examination of patients with familial monogenic disorders can increase knowledge on both potential underlying pathogenic mechanisms and varying clinical features. However, the low number of individuals with familial PD in a given geographical area can make statistical power in such studies hard to obtain (42, 43).

Historical overview of PD genetics

During the latest decades of the 20th century there has been an increasing recognition of PD accumulating in families. Although considerable research effort was made to establish linkage to chromosome areas, the first gene was not described until 1997, the gene for α-synuclein, *SNCA* (44). Superfluous expression of α-synuclein due to *SNCA* duplications and triplications was a few years later identified and then characterized as conferring a clinically severe form of PD with profound dysautonomia and cognitive decline (45, 46). During the following decade, the number of genes known to be directly causative of PD was expanded to 7 but since

then there has been but a few new contributions reliably associated with the disease (47). Nonetheless, PD-genetics as a research field has grown considerably, inspired by more efficient sequencing methodologies and the insights into pathology that the established mutations have contributed (48, 49).

The missing heritability in PD

The monogenic causes of PD are rare and account for about 0.5–5% of cases in most populations, which is lower than the proportion of patients with familial accumulation of disease compatible with mendelian inheritance pattern, reported to be aproximately 15–20% (38). The proportion of individuals with pathogenic mutations varies between different studies though, with 1.4%, 3.9%, 7.9% and 45.7% found in populations from the United Kingdom, Europe, China and North Africa, respectively (42, 50, 51). According to our previous findings when studying approximately 10% Sweden's PD patients, family history was reported in about 22% of cases included but we found a very low amount of known PD causative genetic variants: 0.54% of the individuals studied carried the LRRK2 p.(G2019S) mutation and 0.05% had any of the three genetic variants in *SNCA* studied (43).

Since the proportion of individuals with family history of PD is generally larger than the identified genetic causes, despite relatively many studies in PD genetics, there may be mono- or oligo-genetic causes to PD that are not yet discovered. Another explanation would be for several lower impact genetic factors that influence the risk, so called polygenetic factors, which could be found to add to disease development in both sporadic PD and the cases with family history without known cause (48, 52-55). Examples of genetic risk variants include variants in the *GBA* gene, encoding the lysosomal enzyme glucocerebrosidase, which are the most commonly identified genetic risk factors for PD known today, and LRRK2 p.(G2019S) that could be considered a monogenetic cause and also a risk-factor, given its commonness and relatively low grade of penetrance (56, 57). Given the complex nature of PD, *GBA* and other risk genes likely increase PD-risk in a background of other genetic variants or life-style factors.

Genotype-phenotype associations

Several studies have tried to establish connections between different genotypes and symptomatic phenotypes in PD (58). So far, the most common genetic modifier of sporadic PD phenotype identified is (again) *GBA,* which was shown, in two different studies, to constitute 1.4–4.4% of PD patients. Carriers of genetic variants in *GBA* showed higher mortality and risk for cognitive dysfunction compared to patients without (59, 60). Haplotypes in the gene for tau, *MAPT* (Microtubule associated protein Tau) has also been associated with age at onset and severeness of cortical pathology in sporadic PD (61, 62).

Apart from the copy number variants of *SNCA*, some genetic variants are associated with a specific clinical form of PD. Such is the case with VPS35 p.(D620N) associated PD, even though disease progression *per se* seem indifferent from idiopathic PD with this variant, the age at onset is reported to be much lower (63). *SNCA* variants are associated with a clinical picture with Parkinsonism, behavioral changes, cognitive decline and autonomic symptoms (40, 64). Similar phenotypes are also associated with recessively inherited mutations in the genes *Parkin*, *PINK1* and *DJ1* but the clinical trajectories of patients with these genetic variants are found to be slowly progressive and with very low frequency of dysautonomia and cognitive decline (65, 66).

Pathogenetic mechanisms of known mutations

Expanded research on the genes of established PD-causing mutations has continued and the cellular mechanisms conveyed by many of these are now known. Interestingly, most PD-causative mutations known to date are related to similar functions in the cells and the converging mechanisms thus suggest common grounds for several of the underlying pathological mechanisms in monogenetic PD. The mechanisms often involved include endocytosis, mitophagy or phagocytosis-lysosome pathways (67-69) and not on α-synuclein directly. If extrapolated to hypothesise similar mechanisms responsible in sporadic PD, potential treatment strategies aimed to remove α-synuclein might thus be unfruitful. Furthermore, *LRRK2*, one of the genes most commonly associated with monogenetic PD, is involved in a multitude of these cellular pathways (69). Since *LRRK2* variants have also been discovered to be risk-factors for sporadic PD, this infers support that converging mechanistic pathways in familial PD may be extrapolated to sporadic PD, which would be one major aim with research on high-impact genetic variants.

Non-motor symptoms of PD

The clinical picture of PD often involves NMS such as constipation, orthostatic hypotension, depression, cognitive impairment, daytime sleepiness, or sleep disturbances. This group of symptoms include many more examples and although the constellation of symptoms is very varying, most PD patients develop some NMS in the course of disease (70). Despite NMS in PD has been known since James Parkinson first described the disease, their importance in the course of disease has for a long time been underestimated (71). Today, however, the view of PD is continously expanding away from a motor-centred approach, as exemplified with larger contribution of several NMS to the resulting classifications when put together in data-driven clustering techniques, compared to motor parameters (1, 72). Implementing NMS into the way we look on PD is now regarded as a crucial effort

to divide this complex disease into more clinically accurate and biologically relevant subtypes (73). There is, however, not yet consensus on how to incorporate NMS with motor-symptoms in PD subtypes, given the large variation of NMS in PD patients. Also, a common presence of several of these symptoms in individuals without PD, obscure the usefulness of NMS in PD diagnostics and prognostics. NMS are, nontheless, used formally in clinic in diagnostics; as a supportive criteria for PD diagnosis (olfactory deficit or cardiac sympathetic denervation), or as a red flag when altogether not present after five years of disease (2).

NMS are associated with PD progression

The NMS are generally more heterogenous in nature than the motor symptoms of PD and can be harder to treat symptomatically (74). NMS are thought to be a part of pathological processes affecting more neuronal circuits than only the dopaminergic system, and noradrenergic, acetyl-cholinergic and serotonergic system affection in PD has been pointed out (4, 16, 75, 76). NMS have been studied more thoroughly in the latest years (77, 78), perhaps as a cause of the identification of the marked reduced quality of life inferred by increasing NMS burden which is often deemed more severe to the patients than the motor-symptoms (78-80). Several NMS have indeed been found to correlate with development of specific symptoms and pathology, such as affection of motor symptoms (79, 81, 82), development of motor complications (83) and cognitive decline (82, 84).

NMS in prodromal PD

The NMS seem particularly useful in establishing that an individual is in the end of the preclinical phase of PD and under definable risk to develop disease and could thus be defined as in a prodromal PD stage. It has been known for many years that hyposmia and constipation may precede PD development, sometimes by decades, and more NMS, such as anxiety, have also been shown before disease onset (85). Unfortunately, such symptoms and others, such as depression, that are typically easy to relate to PD in retrospect, are very common in the general population and these symptoms are thus not specific enough to establish individuals at risk for developing PD as long as other signs or symptoms are still lacking. Idiopathic rapid eye movement sleep behaviour disorder (RBD) on the other hand, has been very tightly linked to PD and around 80% of patients with confirmed idiopathic RBD could later develop PD or a neurodegenerative dementia (86). This feature has not only affected diagnostic criteria for prodromal PD, but also affected the view of development of manifest disease, as in subtype systems, the major interest of paper I in this thesis, and the *brain first versus body first* theory, explained in more detail in the discussion (3, 23, 87).

Cognitive symptoms in PD

Mild cognitive impairment or dementia

It has been established that a majority of PD patients develop some kind of negative affection on cognitive abilities, most often related to executive functioning. This can be thought of as relatively unsurprising, as the basal ganglia have important functions in cognitive decision processes as well as deciding which motor actions to perform. Although early established mild cognitive impairment (MCI) conveys risk for later dementia development in general, it has been noted that many patients also develop MCI that do not commonly develop to PDD, especially if frontal affection, corresponding to lack of executive functioning and other symptoms, is the most prominent neuropsychological feature (66). Even though the frontal MCI profile is more common, symptoms associated with impairment in posterior brain circuitry has instead been found to impose higher risks to develop PDD (84). Similar to PD motor symptoms, some cognitive affection by the disease thus seems inevitable but the type, timing and extent are highly variable (20, 84, 88). Constipation, a common prodromal symptom of PD and NMS in the disease, was associated with cognitive decline in one study on 621 patients (82). Other risk factors for PDD include genetic factors such as the apolipoprotein E ε4 allele (ApoE-ε4), explain in more detail below (32), and *GBA* genetic variants and *MAPT* haplotypes, mentioned above.

Hallucinations

Illusions and hallucinations are additional relatively common NMS in PD. The former is defined as a phenomenon with preserved insight that an experienced visual input leads to misinterpretation of an object that is present in reality, such as visualizing a tree as a person or a stone as a face. Hallucinations occur without an actual stimulus, can be more extensive and are not limited to visual nature, even though it is the most common modality. Hallucination can for instance be composed of different sounds or smells which was found to be a not uncommon phenomen while re-examining the patients included in work 1 and 2 in this thesis (data not shown). Hallucinations have been associated with cognitive decline in PD patients (89), but the exact relationship is not fully determined as hallucinations are common in PD and can also be induced by antiparkinsonian medication or deep brain stimulation surgery (90, 91).

How to measure PD heterogeneity

There are yet no established *authentic subtypes* of PD, a term henceforth used in this thesis as in a utopic classification with subtypes proven to directly reflect the additions of specific pathological mechanisms and clinical trajectories, when the

complex interactions of patient-internal or external factors are elucidated. Such subtypes could be based on which areas that are affected by specific spreading patterns of pathology and/or the types of pathogenic mechanisms involved, some of which are introduced in more detail below. There are, however, already some ways to assess the severity of disease on the level of individual patients, but if some of these constitute *authentic subtypes* of PD or are in fact surrogate measures of disease stage or the speed of the pathological process is not well-established. There are at least two relatively established means of classifying PD-patients into higher or lower risk for severe disease. These classifications have been known for several decades now, although this thesis will address some caveats when applying them and emphasize they do not identify *authentic subtypes* of PD.

Established classification methods

The two main factors that have been previously shown to harbour major prognostic information for PD patients are:

I)   Age at onset: Low onset age has been correlated with slower progression in PD (92). Early onset PD is often arbitrarily defined as before or until the age of 50 years as opposed to late-onset PD with an onset after 50 years, in spite of age should likely be considered a continuous influencer of disease where official cut-offs perhaps should be discouraged. Whether it really is onset age that is important rather than chronological age is debated, and this may differ between different aspects of PD progression. Chronological age could be more important for PDD development (93), but other studies have also found that high age at onset is associated with cognitive deterioration as well (89, 92).

II)   The motor-phenotype system (MPS): The notion that the severities of tremor relative to symptoms affecting posture, gait or balance can be used as a prognostic marker has likely been the most commonly used prognostic model of PD. This classification system has several variations (94), but a popular substantiation (95), can be determined based on scores in a very common clinical PD rating scale, the Unified PD rating scale (UPDRS) (96). This method is addressed as the MPS in paper I and II of this thesis and is further described below. This particular system has also been adapted to another version of the rating scale, the Movement disorder society-UPDRS (97). One potential problem with the motor-phenotype classification is that patients have been observed to re-classify during the course of the disease (98-100).

Subtypes based on cluster analyses

The above established classification methods were originally based on clinical observations which resulted in directed hypotheses. Even though their importance has since been well-documented, there can be benefits to avoid *a priori* hypotheses and explore other associations, since we do not have firm evidence on what is causing PD and should hence not be too strict in what we consider to define subsets of the disease. In the latest decade, more focus has thus been put on data-driven analyses, such as cluster analyses or artificial intelligence methods, attempting to use empiric observations rather than theoretical models to create PD subtypes. Using large datasets, several different symptoms from many domains can be included in such subtypes to predict PD progression. Replicability of these findings has unfortunately been low, which is to some extent a consequence of the methodology where the model is adapted depending on the data input. The sample at observation could thus influence the final models and reduce reproducibility (data would be over-fitted). Although similar conclusions were made from some studies, showing several general traits corresponding to subsets of disease trajectories, the lack of readily replicable clinical systems has impeded clinical introduction of such systems and called for future studies to focus on subtype-models that can be readily applied to different groups of PD patients (101, 102). An interesting variant called the clinical subtype system was constructed by Fereshtehnejad *et al* in 2017, who first made a cluster analysis and then constructed an algorithm to enable clinical application with similar results (23). This model was considered a base for the motor-nonmotor subtype in paper I and a constructed simplified version of it was further compared in relation to other PD-progression factors in paper II.

Albeit identification of more robust subtypes in PD is a prioritized research effort, as expanded upon above, there have been only few longitudinal studies examining differences between proposed clinical subtypes (1). This was one of the rationales for paper I.

Neurofilament light chain

Neurofilament light chain (NfL) is one of a few biochemical markers that have been associated with PD. NfL is a protein situated in axoplasms of long myelinated neurons and has been described as *the troponin of the brain* in analogy to the extensively used myocardial markers that reflect tissue damage independent on cause (103). Indeed, axonal damage leads to increased NfL levels in CSF and blood in many different conditions where neurons degrade (104). NfL levels have been found to differ between some neurodegenerative disorders that are otherwise relatively similar (105), and NfL levels can help to differentiate between PD and APS (106-108). However, the role of NfL in PD diagnostics or prognostics has not been as robustly determined. NfL levels were not found to be different from healthy

controls in a systematic review that pooled data from other studies up to a total of just below 1000 individuals with PD (105). Another study found other clinical features such as severeness of RBD, psychiatric symptoms and radiological assessments to be significant prognostic factors but not CSF-NfL levels (24). NfL-levels were, however, correlated with worse PD prognosis in several other studies (109-112). Prognostic value of NfL levels in serum was the main factor of interest in paper II.

Combining markers from different modalities

Studies on several prognostic factors in PD have shown associations with individual outcomes, but each marker frequently conveys only a low–medium impact. There is thus a rationale for combining different prognostic markers (21). There have been combinations with genetic risk scores, for instance, that combine genetic variants that have very low odds-radios on their own, but together found to yield important risk-stratifications (113-116). Apart from prognostic purposes, studying combinations of different biomarkers can also improve our understanding of confounding risk-factors which will ultimately help further characterize the role of specific pathogenetic processes.

Other neurodegenerative diseases and pathogenic factors

PD will be the stepping-stone to understand neurodegeneration in this thesis, but elements of DLB and APSs can be similar to PD and these conditions will be frequently discussed in the thesis. Furthermore, the family in paper III developed an FTD-tau with an AD-like clinical phenotype. Thus, some details of these different diseases need to be addressed.

Similarities between neurodegenerative disorders

As of today, there is no cure for any neurodegenerative disorder, nor a drug that slows disease progression, and we still do not fully know the underlying reasons of any single disease or why the nervous systems of the patients are affected in different ways in the different conditions. Neurodegenerative diseases are generally described as complex, meaning that multifactorial causes are likely contributing to the pathological processes and the ensuing development of clinical symptoms. There are also several features described for PD above that are shared between neurodegenerative disorders in general, such as overlapping neuropathological features, clinical symptoms, internal and external risk factors, and mechanisms of disease propagation (33, 117-119).

Disease development

The different neurodegenerative diseases have preclinical stages where pathology is evident in brains of the affected, but no clinical symptoms have yet developed. These stages are considered to be relatively long and often identified several decades before the normal time for onset of marked clinical symptoms. Preclinical stages are of great importance as future treatments are more likely to be able to affect pathogenic factors at these stages (3). In some diseases, a prodromal stage is defined using distinct examination findings or combinations of other disease-associated features, which is increasingly studied in PD and AD (86, 120).

Neuropathological findings

Depositions of different proteins in living or dead neurons or glial cells, along with neuronal atrophy, characterize the different neurodegenerative diseases pathologically. AD is characterised by depositions of the protein tau and larger plaques of β-amyloid (surrounded by dystrophic neurites). Three disorders with parkinsonism as a common clinical symptom, PD, DLB and MSA, are characterised by accumulation of α-synuclein into LB. FTDs are more heterogenous but most often neuropathological findings include major deposits of tau, transactive response DNA-binding protein of 43 KDa (TDP-43), or a combination of proteins related to oncogenes including the fused in sarcoma protein (8). Even though most of the mentioned protein depositions were identified several decades ago, and the proteins have for some conditions been proposed causative, the relationship between pathological findings of the diseases and the clinical symptoms is incompletely understood.

Clinico-pathological overlap

Discrepancies between clinical and pathological diagnoses are yet a not too uncommon theme in neurodegenerative diseases. Patients with a certain pathological diagnosis may have varying clinical symptoms; for instance, clinic-pathological overlap is relatively common in PSP and CBD (8-10) and described in AD (121). Furthermore, in some types of dementias there are several overlapping clinical and neuropathological conditions. The clinical diagnostic group of FTD has a pathological base that is designated frontotemporal lobar degeneration (FTLD) and is sub-grouped by the mainly present protein deposition such as tau, TDP-43, or fused in sarcoma (8). There are three defined variants of clinical FTD: behavioural variant FTD, semantic dementia, and progressive non-fluent aphasia, the two latter sometimes grouped together as primary progressive aphasia. Also these clinical diagnoses do not always associate to the anatomical distribution of neuronal atrophy and/or proteinopathy (122). Until we know more about pathogenic and disease-influencing factors, the clinico-pathological overlap between different conditions highlights a need for improved disease-classifications.

An example of complexity and a change in nomenclature
An exemplification of the complexity in neurodegenerative diseases is available in findings from the patients in paper III in this thesis. The reader might note that the brief summary of the clinico-pathological overlap of this specific disease includes many aspects now introduced. The genetic cause of the disease in the family is grouped to be causing clinical FTD and pathological FTLD-tau. The clinical symptoms of several affected members in the family were, however, more reminiscent of AD, compared to other FTDs, but with increasing behavioural

symptoms with advancing disease and sometimes with parkinsonism. The pathological diagnosis was that of PSP.

Perhaps complicating the clinical and pathological classifications of this specific disease even further, the nomenclature of inherited FTDs was changed during the work with this thesis. When familial causes to inherited FTDs were initially identified this was made through a strong genetic linkage to chromosome 17. The patients were noted to express symptoms of both FTD and parkinsonism and when writing paper III the proper terminology to use was thus frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17). Shortly after publishing this paper, however, the name of the *MAPT*-associated disease was suggested to be changed to familial FTLD-tau (123), something we discussed in a published letter to the editor (124).

Factors influencing the diseases

As mentioned, the exact mechanisms responsible for neurodegenerative diseases are not known in any of the entities, although some, such as AD, are more well-explored. There are several known factors that have been shown to influence both the risk of developing some or several neurodegenerative diseases and/or the prognosis when a disease is diagnosed.

Genetic overlap

There are several genetic links between the different neurodegenerative diseases. For example, the well-established genetic risk factor for AD, ApoE-ε4, has been showed to also influence cognitive decline in PD (32). Similarly, haplotypes of the gene for tau, *MAPT,* modify age at onset of PD patients. The H2 *MAPT* haplotype is found to be protective in patients with both AD and PSP (61), while H1 haplotype is a risk factor for PD (125). Disease causing point mutations in *MAPT* instead give rise to clinical FTDs or PSP. Tau is hence tightly connected to several different neurodegenerative disorders and is described in more detail below.

Age as a common risk factor

Although several of the FTDs have a mean age at onset in the 50s and some patients with familial forms of PD can manifest as early as in their twenties, the incidence of most neurodegenerative disorders rises markedly when observing older populations. As stated above, PD-prevalence increases with age from 0.2–2 % in individuals of 60–80 years of age (6). For AD, the increasing prevalence with higher age is even more prominent and has been shown to be increased in the age groups 65-74, 75-84 and >85 years with 1%, 8% and 23% of individuals being afflicted by AD, respectively (126). Intriguingly, the fact that age is a marked risk-factor in most neurodegenerative diseases suggests large overlap in causative factors. For instance, age is a factor related to both increased mitochondrial dysfunction and reduced

capabilities of protein homeostasis, two factors discussed below as candidate mechanisms for causing PD (127, 128).

Protein deposits associated with neurodegenerative diseases

Are the proteins disease-causing?

The proteins associated with both AD and PD, tau and amyloid-β in AD and α-synuclein in PD and the other synucleinopathies, are composed of monomers that are improperly folded. These misfolded subunits aggregate into oligomers which then aggregate into fibrils that form amyloid depositions. The protein deposits accumulate in different cells and extracellular plaques in the brain. There is some support that these different proteins play a part in causing the diseases, especially as tau and α-synuclein oligomers hold toxic properties (129, 130). The notion that the proteins deposited in the brain contributes to the pathology also has support from the genetic forms of the diseases: *SNCA* duplications and triplications cause overexpression of α-synuclein in rare familial forms of PD, and several different mutations in *MAPT* lead to different neuropathological diagnoses (FTDs, PSP, CBD etc). Another important argument for the neurodegenerative diseases to be, in fact, proteinopathies is that injection of monomers and oligomers of α-synuclein protein into mice bowel causes protein aggregates. These aggregates form in previously healthy cells, and also healthy neuronal stem cells in human transplants have later been shown to have acquired α-synuclein pathology (29). These findings are in line with a cell–cell spread of pathology.

The role of proteinopathy in development of human disease is, however, not fully determined. The hypotheses that LB (131) or tau neurofibrillary tangles (NFTs) are driving pathogenic factors are for instance challenged by these findings:

- Phosphorylated tau oligomers are closely associated with cell-death in AD as compared to neurons with fully formed NFTs that were actually found to have better ability to survive (132).

- There are familial PD cases without α-synuclein pathology (34).

- Some familial PD variants are reported to have slower progression but increased rather than decreased amounts of α-synuclein aggregates in the brain (133).

- Spread of proteinopathy through cell-cell contact has been shown to be much slower than spontaneous formation in cells due to inflammation or other abnormal neuronal microenvironments (131).

Tau

Tau (tubulin associated unit) is often described in the context of one of the two major protein hallmarks in AD neurodegeneration, but different forms of tau aggregates are found in the brain in several other neurodegenerative diseases, known as primary tauopathies. This umbrella term includes a major part of the FTDs, known as FTD-tau, and the APSs CBD and PSP. As AD-related pathology is also a progression factor in PDD and DLB, and genetic haplotypes in the tau gene affect age at onset in PD, tau is involved in a majority of neurodegenerative diseases. Tau aggregates, including fully formed NFTs can, however, also be found in otherwise healthy elderly, often referred to as Pure Age Related Taupathy (PART) and whether such findings are associated with neurodegenerative diseases or not is debated (134-136).

Tau protein and the MAPT gene

The *MAPT* gene is located on chromosome 17q21–22 and the tau protein is expressed in both the brain and in peripheral tissues and have a function in stabilizing microtubules by binding to tubulin (137). This is proposed to enable formation and organization of axons and is achieved by microtubule-binding domains situated in the c-terminal half of the tau protein. The microtubule-binding domains are varying in numbers depending on alternative splicing of the 10th exon of the gene, so that transcripts without the 10th exon have three microtubule-binding domains and the other transcripts have 4. These are known as the 3-repeat (3R) and 4-reapeat (4R) tau isoforms. Because 0–2 exons at the N-terminal side of the protein (which does not bind microtubules) is also variably sliced, there are three 3R and three 4R isoforms of tau in total (138). Tau is also subject of several posttranslational modifications including, among others acetylation, oxidation and marked phosphorylation at multiple sites (139-141).

Tau in disease

When tau is associated with cellular damage, it is hyperphosphorylated on several of its phosphorylation prone regions and are aggregated into fibrillar forms and ultimately NFTs (140, 142, 143). Even though processes of tau phosphorylation have been vigorously studied, including the responsible kinases and phosphatases, the exact role of phosphorylation or other post-translational modifications of the protein, is not clear (132, 143). Tau in AD have both 4R and 3R tau included in NFTs and the filaments form a structure called paired helical filaments (139, 144, 145).

PD pathogenesis

To further substantiate some cellular factors that can be of importance in PD pathology and progression, a list of candidate pathogenic mechanisms for PD follows here. These are not directly studied in this thesis but give a theoretical background and are also mentioned in several of the papers because of the following reasons:

❖ Factors causing PD are currently not separated from factors leading to disease propagation and clinical progression. The aim of this thesis is to better explain clinical variability in PD, which could thus be a result of factors also initiating the disease or the markers identified could speed up already suggested pathological processes. Factors associated with developing the disease and PD-progression is thus in need of introduction.

❖ The work in this thesis highlights PD heterogeneity, which can be an effect of different pathogenic mechanisms at play in different individuals. A broadness of factors might thus be needed to explain individual differences.

❖ The complexity of PD and the identification of low-impact risk-factors indicate that several of the potential mechanisms in combination could be causing the disease. Markers that reflect different theoretical mechanisms of the neurodegeneration would likely be the best candidates for constructing advanced prognostic system, as markers associated to similar cellular processes are likely to confound one another if used together in a prognostic system.

Potential pathogenic factors in PD

A-synuclein

The finding that PD neuropathology includes inclusions of LBs has been known since the 1920ies, but it was not until in the late 1990ies that the content of the LBs was successfully determined as predominantly consisting of α-synuclein (146). There is yet no consensus on the physiological function of this protein, but it is proposed to be involved in vesicle recycling and endocytosis at the synapses, important for neuronal functions (147). It is located either as mono- or tetramers in the cytoplasm of cells, or in a membrane-associated form, which multimerizes under physiological conditions. Shortly before identifying α-synuclein in LBs, the first mutations in a gene found to be PD-causative was identified, *SNCA*, responsible for coding this protein (44), and the timing of these events might have contributed to the proposition that α-synuclein causes PD. As stated above, however, although α-synuclein is a hallmark of PD pathology, it is not unequivocally proven that the protein causes the disease (131).

Another important area to characterize further is the relationships between the sister-diseases with α-synuclein pathology: PD, DLB and MSA, where differences and similarities in the pathogenic factors of the diseases are not fully understood. Similarities between PD and DLB have been highlighted in the latest decades and speaking of these two diseases as a continuum has been more accepted (3), but MSA is defined as a separate disorder with α-synuclein pathology predominantly present in fibrillar forms in glial inclusions and not in LBs, suggesting a different type of pathology (148). However, additions of neuronal α-synuclein have been noted in a substantial proportion of MSA-patients, and neuronal death is ultimately also the cause of α-synuclein pathology in MSA (149). Future insights into α-synuclein function and dysfunction might help identify new ways to classify the synucleinopathies, which could include subtypes of PD more similar to DLB or with pathological addition of factors commonly seen in MSA.

Protein homeostasis

Misfolded proteins are normally sorted for degradation by ubiquitin-proteosome or autophagy-lysosomal pathways in the cell, and these pathways have in several instances been linked to α-synuclein pathology (150). One argument for dysfunctions in intracellular protein homeostasis to be a pathogenic factor in PD is that it is naturally decreasing with higher age and errors in protein sorting could thus be a key element for the associations with age in several neurodegenerative diseases. It is, however, not clear if such processes are initiating or propelling pathological spread or if they are a cofactor, as in gradual loss of protective cellular machineries.

For PD the balance between membrane bound, free and aggregated α-synuclein is of particular interest as disruption of these mechanisms could be responsible for disease and since it can be a promising therapeutic target (151). Although recent progress has been made in expanding our knowledge on protein homeostasis in synucleinopathies (152), it is still to be properly associated with clinical PD and is not further discussed in this thesis.

Neuroinflammation

Several studies have shown that there is a link between PD and peripheral inflammation markers (153) and an increase of inflammation in PD brains as the disease progresses has been shown, compared to healthy controls (154, 155). There is furthermore evidence that microglia are activated in PD patients (154) and specific symptoms have also been correlated with inflammation level of PD patients as shown by higher blood markers of inflammation in patients with cognitive deterioration (155). As with other proposed pathogenic mechanisms for PD, causation is not proven, however, and if neuroinflammation is upstream or downstream from the events leading to α-synuclein accumulation and cell-death in PD is not fully established.

Toxins and mitochondrial dysfunction

A basis for an environmental cause to PD was observed during an acute parkinsonism epidemic among narcotic users that started in California, USA, in the 1980s. These individuals were found to have developed a disease with symptomatology resembling that of PD. The cause was found to be intravenous administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound formed in the production of a synthetic opioid similar to pethidine/meperidine (156). MPTP exerted toxic effects especially on dopaminergic neurons in substantia nigra and the patients responded well to levodopa medication. MPTP was found to be taken up by dopaminergic neurons, after being converted to a more toxic form by monoamine oxidase B and excreted by glial cells. If not secluded by vesicular monoamine transporters in the neurons, the result was mitochondrial damage, a common pathway for several PD-causing toxins and genetic factors (157). MPTP was the first toxin that resulted in a parkinsonism resembling that of PD and led to associations of several environmental toxins to PD development. The fact that exposure to different pesticides leads to an increased risk of developing PD has since been relatively well-established and has been found in several studies from different continents since the MPTP discovery (158, 159). Worthy of special note is the quite extensively used insecticide rotenone, which has been found to replicate PD pathology well in rat models (160) and the herbicide paraquat, which has a similar structure as MPTP (161).

Another example of toxic exposure proposed to lead to an epidemic (or rather endemic form) of neurodegenerative disorders with parkinsonism, is local diseases on some islands in the Pacific Ocean, including the Parkinsonism and dementia complex of Guam (PDC-Guam). Both genetic and environmental factors have been suggested to cause PDC-Guam but the neurotoxic properties of parts of the cycad tree, used in traditional food on the island, or the accumulation of its toxins up the food-chain on the islands, has on several occasions been highlighted as a potential cause (162).

Even though PD is now generally considered to be caused by a combination of genetic and environmental factors, the studied environmental causes are not linked firmly enough to PD pathology to establish them as the main reason for PD. Studies on environmental factors are affected by a need for a large number of participants, since exposure varies considerably between individuals, and there are effects of protective factors as well, such as physical activity, smoking and coffee-drinking, whose combinations might yield more than an additive risk decrement (163).

Modes of progression of PD pathology

Apart from cognitive disturbance being associated with cortical LB pathology, there are few well-established associations of the distribution of pathology and specific

clinical symptoms in PD. There are general theories, though, of how the disease process may spread, that includes connections between pathogenic factors and development of clinical disease and/or some general symptom constellations.

The brain gut hypothesis

The brain-gut hypothesis is a theory stating that α-synuclein pathology in PD does not normally start in the brain, but pathological changes rather start in the neurons innervating the gut and then propagate to the brain. The hypothesis has its basis in the observed presence of α-synuclein pathology in colonic mucosa and submucosal plexus of the gastrointestinal tract (164, 165). A possibility of disease propagation between cells has provided methods for pathological spread in this manner, as misfolded α-synuclein oligomers have been found to be able to act as aggregate seeds and then convey the pathological confirmation in a prion-like manner (29, 150). Similar mechanisms are also proposed with tau in AD (117). Interestingly, the model of peripheral induction and subsequent propagation of pathology through the vagal nerve was substantiated by two Scandinavian studies finding that individuals that had underwent truncal vagotomy had decreased risk of developing PD (166, 167). As the major contact surface to the environment, the bowel mucosa is exposed to many toxic substances and microorganisms, and the nerves innervating the bowel, along with those involved in taste and olfaction, constitute the most direct link between our surroundings and the CNS. Toxins could more easily affect the gastrointestinal tract than cells in the CNS, because of the blood-brain-barrier regulating influx and actively secreting xenobiotics from the brain.

Brain-first and body first PD

The theoretical model of retrograde axonal transport along the vagal nerve is further nuanced by recent conclusions suggesting both a *body-first* PD and a *brain-first* PD variant, where the latter indicate that spreading of disease can also be directed from the brain through the brainstem and cortex and to the periphery (3, 29, 87). The identification of a possible way for propagation of pathology through protein-protein interaction thus convey a theoretical base for a peripheral–to–central spread of PD pathology and potentially *vice-versa*, which is an intriguing idea to explain some of the variability between PD-patients.

Rationales for this thesis

This thesis primarily aims to describe differences between patients with the same neurodegenerative disease, by investigating clinical, genetical, and biochemical factors that influence motor and non-motor function. The main focus is to provide long-term descriptions of clinical phenotype. Genetic causes or suspected genetic causes would then be associated with particular phenotypes and milder/more severe progression could be identified with prognostic biomarkers. Identifying associations between disease characteristics and clinical progression are prioritized research efforts for several general reasons:

- ❖ Information to the patients about their general prognosis can be improved and expanded to include if specific symptoms are to be expected in their form of disease.

- ❖ PD-patients of more appropriate degree of severity can be selected for future studies which would improve the possibility to finding disease-modifying treatments.

- ❖ Patients with different clinical phenotypes could increase our understanding of pathogenic factors in PD, via translational studies, leading to identification of improved subtypes caused by specific pathogenic factors.

- ❖ Examination of families that show Mendelian inheritance patterns, but without having a known mutation can lead to identification of previously unknown mutations associated with the disease.

Aims while investigating long-term PD heterogeneity

Paper I and II in this thesis aim to:

I) Compare a combined motor-nonmotor classification system with the more established MPS.

II) Investigate long-term prognostic value of S-NfL in PD and evaluate to what extent combinations of different prognostic parameters can add to prognostic accuracy.

Aims while studying families with cognitive and motor dysfunction

Paper III and IV in this thesis aim to:

III) Identify the genetic cause of the familial disease and describe disease-specific features.

IV) Propose a new candidate gene/genes causing PD.

Longitudinal cohort studies –
Methods and study designs

Paper I and II of this thesis were performed by following a cohort of patients with PD. The main outcomes were determined by medical record search and clinical re-examination. The explanatory variables in focus were clinical classifications by two different systems, measurements of S-NfL and clinical parameters such as age at onset and duration of disease, all assessed at baseline examination.

The PARLU cohort

Inclusion

Paper I and II in this thesis was performed by studying the Parkinson Lund (PARLU) cohort. The inclusion and baseline examinations of the individuals were performed by the main supervisor of this thesis, Andreas Puschmann, between 2007 and 2013. There were two different subgroups, included in different ways.

- ❖ The population subgroup consisted of individuals from three municipalities Sölvesborg, Olofström and Karlshamn, all in the Blekinge region of southern Sweden. Recruitment of the patients was made by announcing the study in local newspapers and through patient associations. Patients were also contacted directly after going through a registry of diagnoses in all hospitals of the neighboring regions, including medicine, neurology and rehabilitation clinics at the Blekinge hospital in Karlshamn, Skåne University Hospital in Lund, Kristianstad hospital, and primary health care centers in the three municipalities. All individuals residing in the three municipalities with ICD-10 diagnosis of PD or parkinsonism at the inclusion or 5 years prior were eligible and 76% of asked patients accepted inclusion in the study.

- ❖ The hereditary subgroup consisted of patients with family history of PD defined as having at least one first degree family member or more than one second degree family members with PD. The patients were

recruited internally on the neurology clinic at Skåne University Hospital (Lund and Malmö), Kristianstad and Ystad hospitals, or by the patients approaching the physicians responsible for the cohort at that time, associate professors Andreas Puschmann and Christer Nilsson. When the patients approached the physicians, they had typically obtained information on the study through patient associations or via contacts with relatives. The heredity group consisted to a large extent of probands although more than one individual from the same family was included for 6 families.

Exclusion

Patients with a follow-up time lower than two years were excluded since the time of observation was to short to enable reliable data with the methods used in paper I and II. All individuals that had a change of diagnosis to other than PD were excluded. This group included 8 individuals considered to be clinically affected by DLB or APS and five individuals with other causes of parkinsonism, such as mild parkinsonism with the main diagnosis of AD or ataxia, or parkinsonism after trauma or neuroleptics use. There were also 6 individuals with clinical symptoms of vascular parkinsonism and/or radiological evidence for infarction in the basal ganglia. The radiological investigations of all patients where infarction or white-matter lesions were noted in the basal ganglia or where symptoms that could fit with vascular parkinsonism were noted, were also re-evaluated by the main supervisor of this thesis, Andreas Puschmann. Clinical symptoms corresponding to vascular cause were defined as PD symptoms limited to the lower extremities for a prolonged period of time, at least three years after onset of motor symptoms, without developing full clinical picture of PD. Two individuals with known genetic cause were excluded, but three family members from the family studied in paper IV in this thesis were included, as they had no known genetic cause to PD and typical clinical picture of PD. After exclusion, there were in total five families where two individuals were included in the studies and one other family (the family studied in paper IV) where three individuals were included in the studies.

Some exclusion criteria were not identical between the two studies of the cohort, related to the amount of missing data that was imputed. In paper I, we excluded all individuals that had 20% or more of total subitems in UPDRS 2 or 3 missing. In paper II, individuals with 30% or more in the sum of UPDRS 2 and 3 (one individual) were excluded and individuals with 60% or more missing data points in the UPDRS 1–4 or postural instability and gait disorder (PIGD) sub-scores had the corresponding data considered missing but added to other analyses. Two individuals who were not included in the first paper due to the lower cut-off of missing values were included in paper II. Six individuals who were included in the first work did,

however, not have any blood-sampling, so 8 individuals in total were not the same for paper II as for paper I, two patients were added and 6 were excluded (see Figure).
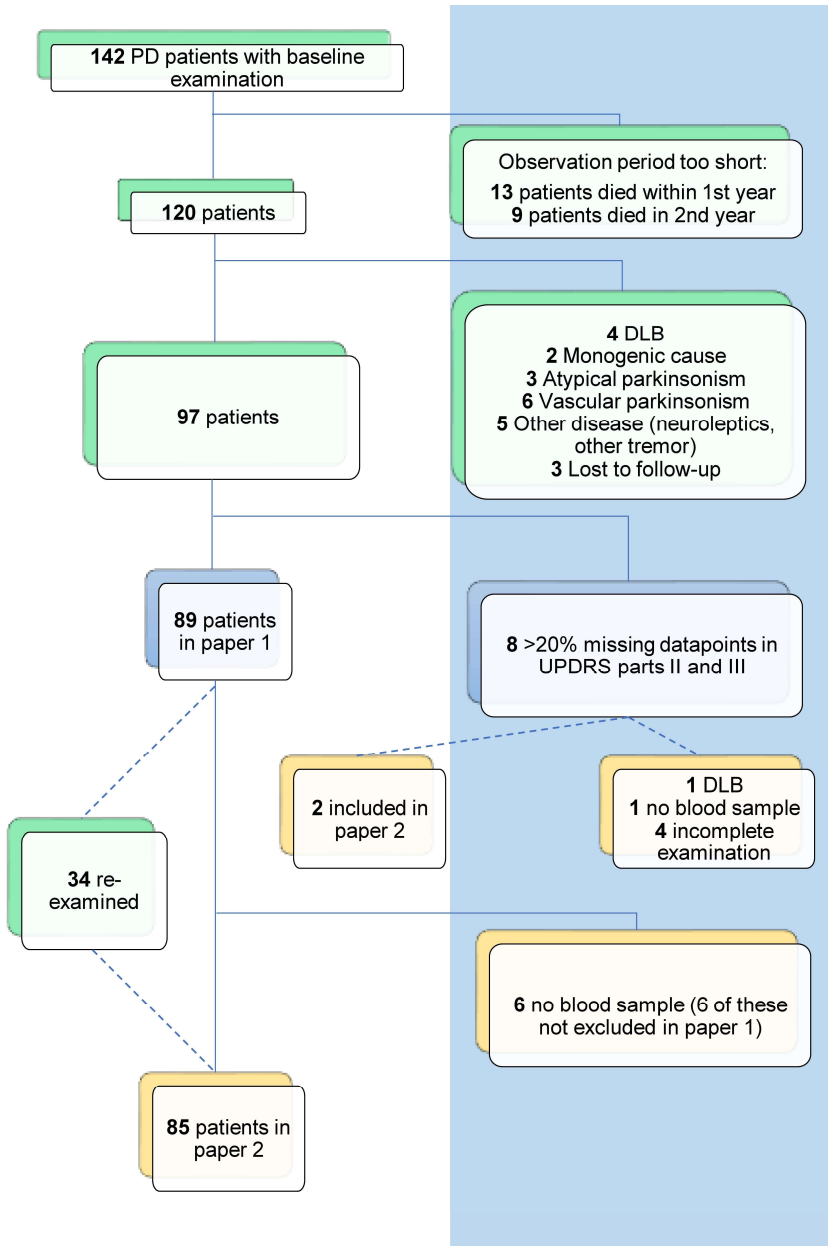
Figure showing flowchart for patient inclusion and exclusion in paper I and II
Patients included to the left, patients excluded and reasons for exclusion to the right (blue background). The green rectangles represent individuals who were the same in both paper I and II. Blue rectangles represent inclusion/exclusion for paper I and yellow rectangles represent inclusion/exclusion for paper II.

Diagnosis and other definitions

At the baseline examination, all individuals were confirmed to fulfil the following criteria for Parkinsonism: bradykinesia along with either rest tremor postural imbalance or rigidity. Continued evaluation that the patients were considered to have PD and no other diagnosis was performed at regular clinical follow-up but was checked for in systematic medical record search performed for all patients included patients, which encompassed evaluation of clinical and radiological signs of vascular parkinsonism. Age of onset was defined as the first symptom of PD noted by the patient. If there were ambiguities in the medical records and/or patient history, the examination where the disease was diagnosed or firstly suspected was prioritized. There were 6 cases where no reliable information about symptom onset was identified, and the date of diagnosis was instead used for these individuals.

Data collection

Examinations

The baseline examinantion of the cohort was performed in 2007–2013 by the main supervisor of this thesis, Andreas Puschmann, and included UPDRS (96) and NMS questionnaire (NMSQ) scales (79) as well as standardized neurological examination and medical history taking. To perform follow-up of clinical ratings and cognitive functioning we constructed a re-examination protocol for research patients visits, which was ethically approved by the ethical review board in Lund. All re-examinations were performed by the author of this thesis. Research nurse Christin Karremo coordinated the location and time of visits and took blood samples. Re-examinations were performed from November 2017 to July 2018 and included the same examination protocol and scales as the baseline examination with addition of the cognitive performance scale Addenbrookes cognitive examination revised (ACER).

Medical record search

Apart from the history and examination parameters collected during the baseline and re-examination visits, the author of this thesis established a method to perform a standardized and detailed search of the medical records of all individuals included in this cohort. The majority of the medical records were searched in parallel with the clinical re-examinations and the time-points of clinical re-examinations and search of digital records were generally not separated in time more than a maximum of a few months, with the exception of a few copies from primary health care and a memory clinic that were not acquired in paper form until some months later. As the

medical record search procedure could be performed for both living and diseased patients in the cohort, and the number of deaths during the observation period was relatively high, the medical records were considered the main source of information regarding patient outcome, and the results of the re-examination protocol were foremost used for reclassifications of clinical classification systems (paper I) and in separate analyses on subgroup level (paper II).

The medical records were foremost searched digitally, using standardized key words in the text concerning disease onset, all prespecified outcomes and other parameters (see Appendix for detailed definitions and Swedish search terms). The records were manually inspected in full-length if they were not available in the medical record system at Blekinge hospital or Skåne University Hospital. Manual search in full-length was therefore performed for the main parts of investigations, correspondence and records performed before 2002, available in a photo-archive of paper records. On several occasions, medical information was spanning back several decades, sometimes as far as the 1960s. Medical records were also acquired from memory clinics and if there were no neurologist notes on regular basis or if there were disagreements or ambiguities regarding symptoms, records were also acquired from primary health care and inspected in full length.

Definitions of milestones of disease progression

The primary outcome of the two studies on the cohort was the time from baseline examination to five pre-specified milestones of progression. These were chosen to reflect a broad spectrum of important events in the disease course and constructed to allow collection of information from medical records. The definitions used were the following:

I)   Use of walking-aids: When the individual was noted to have started using walker or other advanced walking-aids such as beta support or wheelchair. The walking-aid had to either be used indoors or for the majority of locomotion outdoors. Important exceptions thus included the use of canes and using a walking-aid only for long walks and not otherwise. Reversibility of the usage was not considered; if a patient fulfilled the criteria at any time the time-point was noted.

II)   Nursing-home residency: When the individual moved to a nursing-home permanently or started to have part-time care at a nursing facility for a total of 1 week per month or more. All types of accommodation that included healthcare personnel were considered a nursing-home in this regard, including assisted living facilities were the individuals still owned their own apartment and health care personnel was situated in a different building within the premises. Reversibility was not

considered; if a patient fulfilled the criteria at any time the time-point was noted.

III) Motor end-stage: To assess the time-point when an individual was considered to fulfil the Hoehn and Yahr stage 5 (HY-5) criteria (168), we used the first notion of confinement to wheelchair or bed, or the need to use a wheelchair without being able to walk without personal assistance. Reversibility of movement capabilities was considered; if an individual at a later time-point was being able to move without personal support the time-point of earlier fulfilment of the criteria was disregarded.

IV) Dementia: Individuals were considered to have developed dementia if they received any dementia diagnosis. As individuals with PD are not always routinely examined for, or asked about cognitive functioning, we concluded that only using this criterion would lead to many individuals considered not to have reached this milestone despite considerable impairment in cognitive skills that affected activities of daily living (ADL). We therefore considered clear descriptions of dementia state (also when examination of cognition was not opted for in individuals with only few years of expected lifetime and similar) or usage of acetylcholinesterase-inhibitor, as fulfilment of this criteria. Medical treatment with acetylcholinesterase-inhibitors was in four cases prescribed without formal dementia diagnosis. These cases had subsequent entries in their records supporting diagnosis of dementia though, such as subsequent cognitive deterioration or radiological findings coherent with a clinical picture of dementia. Reversibility of cognitive functioning was considered; if an individual had improved cognition or there were notions of confusion episodes or depression at the time of a medical record entry, the criteria was not considered fulfilled.

V) Death: Dates of death were collected through the Swedish tax agency. This governmental agency keeps register of all deaths in Sweden and all legal ways of being declared dead in Sweden is administered through this agency. Irreversibility was implicated.

Clinical classification systems

Motor-phenotypes and PIGD score

The motor-phenotypes are relatively well defined and we used the original description from 1990 where two sums of UPDRS items are used, namely a mean tremor score that then is divided by a mean PIGD score (95). The UPDRS items 16, 20 and 21 constitute the tremor scores and items 13, 14, 15, 29 and 30 constitute the PIGD scores. The quota between the means then determines the motor-phenotype classification group. Values ≥ 1.5 correspond to tremor dominant (TD) motor-phenotype (MPS-TD) and values ≤ 1.0 correspond to MPS-PIGD. The middle group with values between 1.0 and 1.5 is defined as an undetermined or intermediate motor-phenotype (MPS-UI). An important note is that the original MPS categories were primarily used in paper I. When starting to analyse the results of paper II, however, we noticed that the total sum of PIGD scores, instead of relating it to the tremor scores of the patient, had stronger associations with the different outcome measures than the different motor-phenotypes of the MPS. Results for PIGD score alone was then added ad hoc to paper I and PIGD score only, rather than the motor-phenotype groups were generally used in paper II after confirming that there were no significant differences in S-NfL levels between the original motor-phenotype classification groups.

Simplified clinical subtypes

The clinical subtype system classify patients into one of three groups, mild motor-predominant (MMP), intermediate (IM) and diffuse malignant (DM) and is based on a composite motor score and three different NMS (23). The non-motor parameters collected in the examinations of the PARLU cohort were different from that collected in the Parkinson's progression markers initiative (PPMI) cohort that was studied in the original work of clinical subtypes. Therefore, a direct reproduction of the subtypes constructed there was not possible. Since we had access to parameters that describes similar domains of symptoms in PD we opted for an adaptation of the system, with the aim of constructing a simplification for clinical use in a simplified clinical subtype system (SCS). See the appendix for a summarized figure of the subtyping algorithm. The scale of NMS encumbrance was changed from Scales for outcomes in PD-autonomic dysfunction to NMSQ. The scales and test-battery used to determine cognitive functioning and RBD in PPMI were replaced by dichotomic constructs of positive or negative details:

❖ RBD was simply assessed by asking the patients and their next of kin whether the patient was experiencing movement or verbal expressions that could be considered RBD. Such symptoms could be increased amount of

movement, as in the bedding always being in disarray in the morning or next of kin noticing highly increased or seemingly purposeful motions and/or abnormal amount of talking, singing, or shouting while sleeping.

❖ Regarding the cognitive functioning we used data on whether the patient had experienced hallucinations as a surrogate marker for cognitive decline. Data on hallucinations from examinations were, however, incompletely assessed. Furthermore, hallucinations can be a sensitive subject to speak about, especially given that the examiners were in most cases not the physician responsible for the patients' normal treatment. We thus opted to assess this feature more excessively than only through patient history and also checked the items on hallucinations in both NMS and UPDRS and also the patients' medical records for hallucinations prior to the investigations. Patients were considered to have had hallucinations if this was implied in any one of these assessments.

We used the same algorithm and the same cut-off at 75th percentile of the cohort values for the continuous parameters (composite motor score and NMS) as in the original work, but the simplifications of RBD and cognitive functioning was thus dichotomic. The composite motor score was derived in the same way as in the original work: by taking a mean of each individuals' z-value for UPDRS-2, UPDRS-3 and PIGD score (23). As the PARLU cohort has a relatively even spread of disease durations, we chose to mathematically reduce the complete expression, after inserting the SD and mean values from the cohort at baseline. The reduced formula (see Appendix) thus conferred a more straightforward way to calculate each individual's composite motor score. The 75th percentile cut-off of motor score were calculated using only values from individuals without imputation in UPDRS. The same formula was then used to calculate the motor score at the re-examination, but the cut-off (130.4) was adjusted to the 75th percentile of re-examination values (248.7).

Neurofilament light chain analyses

Collection of samples

Blood sampling was performed using standard procedures for venepuncture. The samples were the same day handed to standard chemical laboratories of the hospital where the sampling took place or transported to the chemical laboratory at Skåne University Hospital in Lund, after being stored a few hours in room temperature or maximum +4 degrees Celsius if stored longer. Laboratory personnel centrifugated the samples using standard settings and sera were aliquoted and then frozen to -80 degrees Celsius. Blood sampling techniques and laboratory techniques did not differ

for individuals who were examined at different hospitals and all samples were stored in the same biobank at the Lund University.

Laboratory analyses

Serum NfL levels were determined by using Single Molecule array (SiMoA) platform and NF-light Advantage (SR-X) kit (Quanterix Inc., Lexington, MA, USA). All analyses were run in duplicates. Reanalyses were performed for ten samples; six where central variance had been >20% and four where there had been instrumental failure for one of the duplicates. Two samples were considered missing after analyses since central variances remained high. The analyses were performed in full by one of the co-authors of paper II, Ph.D. Shorena Janelidze, who was blinded to other data than sample numbers. All analyses were performed in September–October 2020.

Statistics

The milestones of disease progression were assessed with survival analysis, as in Kaplan-Meier survival curves and Cox regression models in both paper I and II, and the Cox regression results were generally considered the main finding of the studies. In paper II linear regression models were also constructed. Adjustment covariates were the same in all regression models in the two studies, namely age at onset, sex and disease duration. In paper I the SCS and MPS were assessed as categorical variables in which each category was contrasted against the group with the worst outcome in the respective systems, SCS-DM and MPS-PIGD. In paper II, the S-NfL levels were the main variable of interest and SCS group and PIGD score were entered as continuous variables separately to the adjusted models primarily to approximate combination potential by observing changes in the hazard ratio (HR) of S-NfL.

Linear regression models

In contrast to paper I, all variables were considered continuous in the analyses of paper II, but S-NfL levels were Ln-transformed in all analyses, since the distribution of base levels were positively skewed in the cohort. As commonly implemented with chemical analyses in medical sciences, Ln-transformation of S-NfL levels were applied, meaning that the calculated effect sizes or Hazard ratios corresponds to an amount of change in the correlated parameter/relative risk for each ~2.72-fold increase of S-NfL. In all linear models residuals were inspected and found not

heteroscedastic on scatter-plots and also found not to violate normal distribution assumption after inspection of histograms.

Survival statistics

In survival statistics the time under observation until either an individual is censored without reaching the outcome under consideration –developing a milestone of disease progression– or the time until the outcome is reached is used. Individuals were considered censored when they died or when the last contact with the patient was made; generally extracted from medical records as in an out-patient visit or telephone interview with the patient or someone living close to the patient. Log rank tests were performed to examine general differences between the three groups examined. Kaplan-Meier curves were constructed to enable a basic visual comparison and to construct survival tables and curves for presenting numbers of individuals at risk at different times. To enable the construction of the survival curves and tables, some continuous variables were grouped. Tertiles of PIGD score and individuals with S-NfL levels above and below the cohort median were used for this purpose in paper I and paper II, respectively. Assessment of the proportionality of the hazards assumption was performed for each Cox regression analysis using the cox.zph command in R v4.0.2 (survival package). R was also used for drawing and comparing receiver operator characteristics (ROC) curves in paper II (pROC package). For all other analyses SPSS v25.0 was used. Two tailed p-values ≤ 0.05 were considered statistically significant.

Combined models

We created explorative combined models using S-NfL and age at onset tertiles with or without either PIGD score tertile or SCS group. These factors were attributed 0–2 score points based on the tertile/rank of the classification group (0 for MMP, 1 for IM and 2 for DM) and summarized. A high score would thus correspond to high risk in all the parameters and *vice versa*. Individual scores were then assessed as diagnostic tests, as in constructing ROC-curves for reaching the different five progression milestones. The combined models were compared to the ROC curve of residuals of Ln-transformed NfL and age at baseline examination (when blood-samples were collected). The area under the curves were compared using Delong method (169).

Considerations and definitions of variables

Differences in reports between different patients or reporting health-care personnel could potentially affect survival statistics to a large degree. We used general and well-defined milestones with prespecified criteria to minimize inter-reporter

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differences. Any event happening when not observed constitutes an uncertainty to survival statistics, known as interval censoring. As the observation of reaching milestones of progression were based on clinical follow-up of the patients, there were differences in follow-up intervals between individuals, such as when different patients report new symptoms at different points in time or because of different caregivers having different routines for the frequency of follow-up visits, *etcetera*. To mitigate the effects of the differing intervals of follow-up in the medical records, the dates of reaching milestones and disease onset were extracted as calendar-year only. Using the exact dates would have inferred uncertainties to the statistical methods to be unequal between patients, since some patients were reported as fulfilling milestones close in time to the event actually taking place, and others considerably later, inducing a large effect of interval censoring for some individuals. Standardizing intervals to yearly report should mean that most individuals had time for their regular out-patient appointments, minimizing patient-delay of reporting. As deaths are generally reported without any long delay, date of death and date of birth was registered as the actual day. Furthermore, to minimize impact of severe comorbidity close to death of an individual, if reaching a previously unmet milestone of progression within 2 months prior to death, the milestone was counted as not fulfilled.

Longitudinal cohort studies – Results

Table showing the general demographics of the PARLU cohort in paper I and II
For specifics on the number of individuals included please see flow-chart in the methods section. S-NfL, serum neurofilament light chain; Yrs, mean ± SD years.

Baseline data	Paper I	Paper II	Re-examination
Number of patients (n)	89	85	34
Geographic inclusion	45 (50.1%)	41 (48.2%)	11 (32.4%)
Age at Onset (yrs)	59.7±9.2	60.1±9.2	55.3±8.2
Men (n)	54 (60.7%)	51 (60.0%)	18 (52.9%)
Duration at baseline (yrs)	7.9±5.3	7.9±5.1	6.9±5.2
Age at baseline (yrs)	67.6±9.1	68.0±9.1	62.2±7.7
S-NfL at baseline	21.9±14.3 (n=78)	23.1±16.8 (n=80)	17.1±9.9 (n=29)

Paper I

In this work we adapted a clinical subtype system and applied both this variant, the SCS, and the MPS in parallel on the patients in the PARLU cohort. This was performed on data from the baseline examination (n=89), when the patients had a mean ± SD disease duration of 7.9 ± 5.3 years, and on data from re-examination (n=34), 8.2 ± 2.0 years later. The cohort was observed in medical records during a period of 8.1 ± 2.7 years and we compared the capabilities of the classification systems to estimate risks for five important milestones of disease progression: walker-usage, nursing home living, developing HY5 or dementia or dying. The MPS-groups were ad-hoc replaced by individual PIGD scores.

Demographics and general outcome

Dementia
Of the 89 patients included, 27 (32.9%) were defined as having developed dementia at the end of the study. In the different Cox regression analyses performed for dementia development, disease duration, age at onset and male sex were significant risk factors. The relative risk increase inferred by a high age at onset for reaching the defined dementia-state was also indicated as the proportion of individuals with dementia was largely increased in patients with higher age at onset. Fifty-six percent

of patients with 70 years age at onset or more and 66.7% of patients with an onset age over 75 years developed dementia during the observation period of this cohort. HR of age at onset was, however, generally higher in the HY5, nursing-home and mortality models than the dementia models (see supplementary table 3 to paper I). Furthermore, male compared to female patients had relatively higher risks for developing dementia models with 7.6 (95% CI 2.1–27.8), 7.2 (95% CI 2.0–25.9) and 6.8 (95% CI 1.9–24.3) times the risk in the adjusted Cox regression models with SCS, motor-phenotype and PIGD score, respectively.

Table showing Cox regression results for dementia development for all covariates in categorical SCS model.
DM, Diffuse malignant subtype; MMP, Mild motor-predominant subtype; IM, Intermediate subtype; SCS, simplified clinical subtype system.

Dementia SCS	DM vs MMP	DM vs IM	Male sex	Age at onset	Duration at baseline
HR	4.21	2.32	7.62	1.15	1.18
95% CI	1.2–14.9	0.7–7.5	2.1–27.8	1.1–1.2	1.0–1.4
p-value	**0.026**	0.159	**0.002**	**0.000**	**0.014**

Table showing Cox regression results for dementia development for all covariates in categorical MPS model.
MPS, motor-phenotype system; PIGD, Postural stability and gait disorder motor-phenotype; TD, Tremor dominant motor-phenotype; UI, Undefined motor-phenotype.

Dementia MPS	TD vs PIGD	UI vs PIGD	Male sex	Age at onset	Duration at baseline
HR	3.32	2.13	7.19	1.14	1.22
95% CI	0.8-14.7	0.5-9.8	2.0-25.9	1.1-1.2	1.1-1.4
p-value	0.114	0.331	**0.003**	**0.000**	**0.002**

Table showing Cox regression results for dementia development for all covariates in the continenous PIGD score model.
PIGD score, postural stability and gait disorder score (sum of items 13, 14, 15, 29 and 30 in UPDRS)

Dementia PIGD score	PIGD score	Male sex	Age at onset	Duration at baseline
HR	1.07	6.84	1.14	1.20
95% CI	0.9-1.2	1.9-24.3	1.1-1.2	1.0-1.37
p-value	0.269	**0.003**	**0.000**	**0.009**

Mortality

Thirty-seven individuals (41.6%) died during the observation period. The mean duration at death was 15.1 (± 5.2) years and the mean age at death was 80.0 (± 5.9) years. Also in this regard, male sex was an important risk factor. Mean age at death was 79.2 (± 4.9) years for males and 83.7 (± 8.1) years for females and death HRs for men compared to women was 5.8 (95% CI 2.4–14.2), 6.5 (95% CI 2.6–16.2) and 5.3 (95% CI 2.2–12.7) in the three different adjusted models in paper I, assessing SCS, motor-phenotype and PIGD score, respectively.

Table showing Cox regression results for mortality for all covariates in categorical SCS model.
DM, Diffuse malignant subtype; MMP, Mild motor-predominant subtype; IM, Intermediate subtype; SCS, simplified clinical subtype system.

Mortality SCS	DM vs MMP	DM vs IM	Male sex	Age at onset	Duration at baseline
HR	2.67	2.00	5.85	1.17	1.18
95% CI	1.1-6.4	0.9-4.7	2.4-14.2	1.1-1.2	1.1-1.3
p-value	**0.029**	0.107	**0.000**	**0.000**	**0.001**

Table showing Cox regression results for mortality for all covariates in categorical MPS model.
MPS, motor-phenotype system; PIGD, Postural stability and gait disorder motor-phenotype; TD, Tremor dominant motor-phenotype; UI, Undefined motor-phenotype.

Mortality MPS	TD vs PIGD	UI vs PIGD	Male sex	Age at onset	Duration at baseline
HR	2.84	2.51	6.47	1.18	1.22
95% CI	0.9-9.4	0.9-7.3	2.6-16.2	1.1-1.2	1.1-1.3
p-value	0.088	0.091	**0.000**	**0.000**	**0.000**

Table showing Cox regression results for mortality for all covariates in the contineous PIGD score model.
PIGD score, postural stability and gait disorder score (sum of items 13, 14, 15, 29 and 30 in UPDRS).

Mortality PIGD score	PIGD score	Male sex	Age at onset	Duration at baseline
HR	1.11	5.34	1.18	1.19
95% CI	1.0-1.2	2.2-12.7	1.1-1.2	1.1-1.3
p-value	**0.038**	**0.000**	**0.000**	**0.000**

Milestone distribution in the classification systems

The proportion of individuals that developed the milestones of disease progression were ordered in both classification systems. Individuals categorized in the high-risk groups (SCS-DM and MPS-PIGD) had higher proportion of patients reaching all the different milestones than those of the middle-risk group (SCS-IM and MPS-U). The middle-risk groups had in turn higher proportion of individuals than the low-risk groups (SCS-MMP and MPS-UI). MPS-TD had the lowest proportion of individuals reaching all the outcomes and SCS-DM had the highest (see table 1 in paper I). The proportion of individuals within a classification group that developed the different milestones was relatively similar in SCS-IM compared to MPS-UI and slightly higher in SCS-MMP compared to MPS-TD. The groups with worse prognosis in the systems, SCS-DM compared to the MPS-PIGD, SCS-DM had 29.4–68.4% higher proportions of individuals that reached the different milestones of disease progression.

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a) SCS ▨ = MMP   ▨ = IM   ▨ = DM

Walker

Cumulative survival (%)

Log-rank p = 0.061

Follow-up time (years)

	0	2	4	6	8	10
n	35	34	29	19	18	9
at	30	25	23	14	11	3
risk	7	4	2	2	1	1

HY5

Cumulative survival (%)

Log-rank p < 0.001

Follow-up time (years)

	0	2	4	6	8	10
n	35	35	35	30	24	16
at	33	33	27	21	16	9
risk	18	13	9	6	2	2

Dementia

Cumulative survival (%)

Log-rank p = 0.016

Follow-up time (years)

	0	2	4	6	8	10
n	31	31	31	25	24	14
at	31	30	25	18	16	8
risk	13	12	7	5	3	3

b) MPS ▨ = TD   ▨ = UI   ▨ = PIGD

Walker

Cumulative survival (%)

Log-rank p = 0.413

Follow-up time (years)

	0	2	4	6	8	10
n	16	16	15	8	8	3
at	12	11	9	6	6	0
risk	44	36	30	21	16	10

HY5

Cumulative survival (%)

Log-rank p = 0.069

Follow-up time (years)

	0	2	4	6	8	10
n	16	16	16	13	11	7
at	14	14	14	12	9	4
risk	56	51	41	32	22	16

Dementia

Cumulative survival (%)

Log-rank p = 0.120

Follow-up time (years)

	0	2	4	6	8	10
n	15	15	15	12	11	6
at	11	11	11	10	10	5
risk	49	47	37	26	22	14

c) PIGD score tertiles ▨ = Tertile 1   ▨ = Tertile 2   ▨ = Tertile 3

Walker

Cumulative survival (%)

Log-rank p = 0.048

Follow-up time (years)

	0	2	4	6	8	10
n	31	30	29	19	15	7
at	28	24	16	10	10	3
risk	13	9	9	6	5	3

HY5

Cumulative survival (%)

Log-rank p < 0.001

Follow-up time (years)

	0	2	4	6	8	10
n	31	31	31	25	20	12
at	29	29	26	20	15	9
risk	26	21	14	12	7	6

Dementia

Cumulative survival (%)

Log-rank p = 0.065

Follow-up time (years)

	0	2	4	6	8	10
n	27	27	26	21	20	10
at	26	26	22	16	15	9
risk	22	20	15	11	8	6

Figure showing survival curves for reaching selected milestones
Survival curves of Kaplan-Meier estimates for individuals classified into the three groups of each classification system.
a) Simplified clinical subtype system, b) Motor-phenotype system. c) PIGD score tertiles. Individuals at risk at the beginning of each 2 year interval showed in table. The lowest-risk group showed in blue, the middle-risk groups in green and the high-risk groups in brown-red. Modified from paper I (170)

SCS showed long-term prognostic value

Kaplan-Meier survival curves

Development of the milestones was firstly assessed by survival curves of Kaplan-Meier estimates and Log rank tests over the three groups of each classification system (see Figure for selected survival curves). For the SCS, there were significant different risks between the groups for reaching all disease milestones but walker-use. Significantly different risks between the MPS were found only for mortality.

We also ad-hoc examined tertiles of the total PIGD score sum. Tertiles of the PIGD scores showed significant Log-rank tests for the walker-use, nursing-home, HY5 and death milestones of disease progression.

Cox regression results for SCS

The main result of this paper I was that the SCS could prognosticate the disease course of PD patients as assessed by the relative risks of when to develop five different milestones of disease progression (see Table). HRs of reaching all the different milestones in both unadjusted and adjusted analyses were significant for comparison between the SCS-MMP and SCS-DM groups. Differences in risk were moderate, with HRs between 2.8–4.2 when the whole cohort was analysed. When the HY5 Cox regression analysis was separated into two subgroups based on sex, in order to fulfil the statistical requisite for proportional hazards, there were indications of large differences in risk for reaching the motor end-stage HY5 between individuals classified as SCS-MMP and SCS-DM (HR of 9.9 analysing the men and 10.8 analysing the women of the cohort), although the low amounts of patients included in the analyses increased uncertainty with large 95% CIs (see table 2 in paper I).

Comparison between patients classified as SCS-DM and SCS-IM also showed capability of prognosticating PD, but to a lower degree. There were significant differences in risks in the walker, nursing-home and HY5 (female) adjusted models. However, the statistical uncertainty of the sex-sub-grouped HY5 models was especially prominent, with a 95% CI of 7.2–567.1 for women to reach HY5. The risk-assessment for mortality and HY5 (when sub-grouped for male only) showed significant HRs in unadjusted models but this did not hold for adjustments.

Table showing HRs for categorical classifications

Table from paper I, showing Cox regression results for the five different milestones of disease progression. Adjustments were made for age at onset, duration and sex. HY5 analysis was stratified based on sex since prerequisite for the statistical model (proportional hazards during the time of observation) was otherwise violated

SCS subtype	Unadjusted HR (95% CI)	p-value	Adjusted HR[a] (95% CI)	p-value
Diffuse malignant vs Mild motor-predominant				
Walker	2.89 (1.15-7.26)	0.024*	2.81 (1.10-7.15)	0.031*
Nursing home	5.24 (2.25-12.24)	0.000***	3.86 (1.57-9.52)	0.003**
HY5 (women)[b]	16.2 (3.63-72.34)	0.000***	10.79 (1.85-62.81)	0.008**
HY5 (men)[b]	13.01 (2.79-60.72)	0.001***	9.92 (1.99-49.46)	0.005**
Dementia	4.66 (1.49-14.58)	0.008***	4.21 (1.19-14.93)	0.026*
Mortality	4.74 (2.13-10.54)	0.000***	2.67 (1.11-6.43)	0.029*
Diffuse malignant vs Intermediate				
Walker	2.43 (0.96-6.17)	0.061	3.04 (1.14-8.11)	0.026*
Nursing home	3.54 (1.56-8.05)	0.003**	3.14 (1.31-7.48)	0.01**
HY5 (women)[b]	27.71 (4.43-173.45)	0.000***	63.66 (7.15-567.14)	0.000***
HY5 (men)[b]	2.9 (1.11-7.53)	0.029*	1.49 (0.54-4.07)	0.438
Dementia	2.74 (0.94-7.93)	0.064	2.32 (0.72-7.47)	0.159
Mortality	3.28 (1.51-7.1)	0.003**	2.00 (0.86-4.66)	0.107

Motor-phenotype	Unadjusted HR (95% CI)	p-value	Adjusted HR[a] (95% CI)	p-value
PIGD vs Tremor-dominant				
Walker	1.63 (0.74-3.56)	0.224	1.87 (0.85-4.13)	0.119
Nursing home	2.36 (0.82-6.8)	0.113	2.03 (0.69-5.95)	0.199
HY5 (women)[b]	2.34 (0.49-11.08)	0.283	2.10 (0.43-10.29)	0.361
HY5 (men)[b]	4.66 (0.62-35.08)	0.135	5.76 (0.74-45.04)	0.095
Dementia	3.41 (0.78-14.87)	0.102	3.32 (0.75-14.68)	0.114
Mortality	3.62 (1.1-11.88)	0.034*	2.84 (0.86-9.40)	0.088
PIGD vs Undetermined				
Walker	1.37 (0.6-3.13)	0.456	1.73 (0.75-3.99)	0.196
Nursing home	1.47 (0.56-3.85)	0.437	1.35 (0.50-3.61)	0.551
HY5 (women)[b]	0.85 (0.22-3.25)	0.817	0.83 (0.21-3.30)	0.788
HY5 (men)[b]	5.13 (0.68-38.63)	0.113	5.31 (0.70-40.50)	0.107
Dementia	2.56 (0.59-11.16)	0.211	2.13 (0.46-9.79)	0.331
Mortality	2.47 (0.87-7.01)	0.091	2.51 (0.86-7.27)	0.091

PIGD score	Unadjusted HR (95% CI)	p-value	Adjusted HR[a] (95% CI)	p-value
Walker	1.14 (0.98 – 1.33)	0.093	1.15 (0.97 – 1.36)	0.103
Nursing home	1.24 (1.10 – 1.40)	0.000***	1.19 (1.05 – 1.35)	0.007**
HY5	1.37 (1.22 – 1.55)	0.000***	1.34 (1.17 – 1.52)	0.000***
Dementia	1.17 (1.03 – 1.32)	0.017*	1.07 (0.95 – 1.21)	0.269
Mortality	1.19 (1.09 – 1.30)	0.000***	1.11 (1.01 – 1.22)	0.038*

PIGD score but not motor-phenotypes showed long-term prognostic value

Cox regression results for the MPS

The MPS-groups showed no significant adjusted differences in risk for any progression milestone. There was one significant unadjusted HR (mortality model) but this was not significant after adjustments.

Cox regression results for PIGD score only

Using the sum of PIGD scores, there were significant HRs for moving to nursing-home, developing HY5 and dying, which held for adjustments (see Table). PIGD score also showed significant unadjusted HR for dementia development, which did not hold for adjustments (see paper I).

Continuous PIGD scores were used which made direct comparison to the categorical classifications more abstract. If comparing two individuals with the same age at onset, sex, and disease durations, but with PIGD score that differed by 2 SD (6.0 points), the differences in risk would be a magnitude of 114, 204, and 66 %, for the developing nursing-home residency, HY5, and death, respectively. Thus, the differences in risk between the SCS-DM and SCS-MMP groups were larger than a change of 2 SD in PIGD score.

Adjustments and sensitivity analysis

HRs for adjustment covariates

Analyses were adjusted for sex, age at onset and duration of disease (result from dementia and mortality models are shown above and exact numbers for all analyses are available in supplement to paper I). The two latter covariates generally showed significant HRs in adjusted models of all milestones (except for when examining the time-point of HY5 development in women where age at onset HR was significant but disease duration HR was unsignificant). The duration of disease covariate had in general 0.01–0.10 higher HR in the different outcomes than the age at onset covariate (per one year increase). Thus, when comparing two individuals of the same sex and classification group, the difference in disease duration would have to be more than 10–100 times the difference in onset age (in years) for duration to be a more important risk factor. The mean ± SD of these covariates were 59.7 ± 9.2 and 7.9 ± 5.3 for age at onset and disease duration, respectively.

Sensitivity analysis

The adjusted Cox regression results for SCS were relatively unaltered for SCS-DM vs SCS-IM when analysing individuals with 100% complete UPDRS assessments (n=61) compared to the whole cohort (n=89). SCS-DM vs SCS-MMP showed

slightly decreased HR for nursing-home living and higher HR for dementia development, but lost significance level for the other analyses (p-values of walker use and HY5 development for males were increased to 0.058 and 0.053, respectively).

The MPS showed significant adjusted HR for walking-aid use (MPS-PIGD vs MPS-TD HR 4.5, 95% CI 1.3–15.6, p = 0.016) in sensitivity analysis. PIGD score showed unchanged Cox regression results for HY5 when removing UPDRS-imputed individuals, but lost significance level of nursing-home (p = 0.078) and death (p = 0.156) milestones of progression. Walking-aid use p-value was reduced, however, to just above significance threshold (p = 0.051).

Instability over time of classification systems

In both classification systems several patients changed classification group when reclassified at re-examination, compared to when classified using data from baseline visits 8.2 ± 2.0 years earlier (instability over time). The SCS showed higher number of patients that were reclassified, 22 compared to 10 for the MPS, but no patient was illogically re-classified using the SCS, as in reverting to a milder-risk group. In the MPS no group showed complete stability over time, as 14.3–100% of patients in each classification group changed group when reclassified (see Figure). Also, 25% of those classified as MPS-UI at baseline and 17% of those classified as MPS-PIGD, illogically changed group to MPS-TD after reclassification at follow-up.
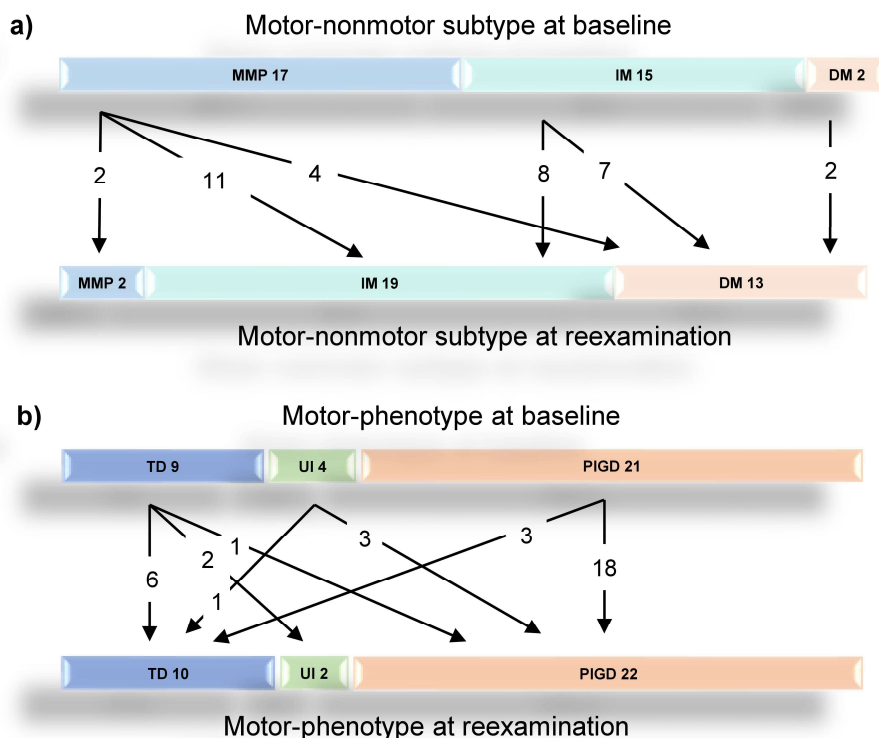
a) Motor-nonmotor subtype at baseline

MMP 17   IM 15   DM 2

2   11   4   8   7   2

MMP 2   IM 19   DM 13

Motor-nonmotor subtype at reexamination

b) Motor-phenotype at baseline

TD 9   UI 4   PIGD 21

2   1   3   3
6   1   18

TD 10   UI 2   PIGD 22

Motor-phenotype at reexamination

Figure showing classification change from baseline to re-examination
From paper I. Group belonging at classifications and individual changes (arrows) for the 34 patients available for both baseline and re-examination classification. The same patients classified with: a) Simplified clinical subtype system, b) motor-phenotype system. Modified from paper I (170)

Contribution of the NMS subparts in SCS

The NMS subparts of the SCS was applied using different scales but according to the same algorithm as in the original work establishing the subtype system (23). The ability of the SCS system to prognosticate the different milestones of disease progression, as assesed in log rank tests and Cox regression analyses, indicated that the adaptation from the original NMS measures maintained information relevant for risk assessment. When observing the frequencies of patients with and without the NMS parameters considered positive (NMSQ above 13.5, no symptoms indicative of RBD and no hallucinations reported in the NMSQ, UPDRS or medical records before examination, in for the different NMS) the NMSQ and hallucination subparts showed larger proportion of patients who died when considered positive compared to negative while the RBD did not.

Table showing patients who died with and without the different NMS parameters used in SCS classification at baseline visit
Number of patients (percentage of the whole cohort).

	NMSQ>13.5	RBD present	Hallucinations present
Deceased without	23 (36.5%)	18 (38.3%)	17 (27.9%)
Deceased with	11 (57.9%)	12 (36.4%)	20 (71.4%)

Paper II

Neurofilament light chain levels correlated to individual disease severity at baseline

At baseline examination there were significant, or p = 0.05, associations between S-NfL and the factors: age (unadjusted regression), UPDRS-2, PIGD score, HY-stage and Schwab and England activities of daily living scale (S&E) (see Table).

Table showing linear regression results at baseline examination
Age analysis is unadjusted. All other regressions were adjusted for sex, age at onset and disease duration. a, p-value of 0.050002; b, n=45

	Baseline S-NfL	p-value
Age	6.90 (3.63 – 10.18)	**0.000**
UPDRS-2	4.44 (2.20 – 6.67)	**0.000**
UPDRS-3	3.25 (-1.26 – 7.76)	0.156
UPDRS total	5.44 (-1.01 – 11.89)	0.097
PIGD score	1.29 (0.00 – 2.59)	**0.050[a]**
HY-stage	0.54 (0.19 – 0.89)	**0.003**
S&E[b]	-18.93 (-29.88 – -7.98)	**0.001**
ACER-score	n/a	n/a

Neurofilament light chain levels were associated to individual disease severity at re-examination

At re-examination, there were significant associations between S-NfL levels and age (unadjusted analysis), UPDRS2, PIGD score, HY-stage and S&E but not with UPDRS3 nor ACER scores. When instead examining the change of S-NfL between the baseline and re-examination, change in S-NfL levels were only significantly associated with change in PIGD score and absolute ACER score (see Table).

Neurofilament light chain levels can prognosticate PD outcome

Kaplan-Meier survival curves

There was a higher risk for individuals with S-NfL levels above the cohort median for reaching any of the milestones of disease progression examined (Log-rank p > 0.001 for all 5 outcomes, see paper II).

Cox regression results

The main finding of paper II was that S-NfL could stratify risks for four of the five different milestones of PD progression: using walking-aids, moving to nursing-home, developing HY5 and dying (see table). The HRs were significant and should be interpreted as an increase of risk of reaching the milestone measured when comparing two individuals with the same age at onset, duration and sex but ~2.72 times higher levels of S-NfL. The Cox regression model for dementia development showed unproportionally distributed hazards for the SCS, meaning that any result of Cox-regression cannot be reliably interpreted as hazards for dementia changed over time in this cohort. S-NfL were most clearly associated with risks for using walking-aids and dying, outcomes not specifically associated with PD but perhaps more to general burden of disease and disability.

Table showing results of linear regressions at re-examination

Re-examination S-NfL was transformed using the natural logarithm as base. Difference (Δ) in S-NfL as absolute numbers. Age analysis is unadjusted. All other regressions were adjusted for sex, age at onset and disease duration. a, n=31; b, n=15

	Re-examination S-NfL	p-value		ΔS-NfL[c]	p-value
Age[c]	6.78 (1.85 – 11.71)	**0.009**	Δ-aged	0.02 (-0.04 – 0.09)	**0.486**
UPDRS-2	5.88 (1.00 – 10.75)	**0.020**	Δ-UPDRS-2	0.11 (-0.67 – 0.28)	0.213
UPDRS-3	7.35 (-1.52 – 16.22)	0.101	Δ-UPDRS-3	0.18 (-0.23 – 0.58)	0.376
UPDRS total	16.82 (2.26 – 31.38)	**0.025**	Δ-UPDRS total	0.29 (-0.34 – 0.92)	0.351
PIGD score	3.73 (0.99 – 6.47)	**0.009**	Δ-PIGD score	0.13 (0.03 – 0.24)	**0.017**
HY-stage	0.79 (0.21 – 1.37)	**0.009**	Δ-HY-stage	0.02 (-0.00 – 0.05)	0.097
S&E[a]	-20.24 (-37.66 – -2.82)	**0.024**	Δ-S&E[b]	-0.12 (-1.34 – 1.11)	0.834
ACER-score	-12.26 (-26.23 – -1.71)	0.083	ACER-score	-0.82 (-1.38 – -0.26)	**0.006**

Table showing results of Cox regressions for reaching the different milestones of disease progression

The four right-most columns illustrate the classification systems added (as continous variables) to the S-NfL model (two left-most columns). All regressions were adjusted for sex, age at onset and disease duration. a, unproportional hazards indicated (see supplementary material for paper II).

	S-NfL HR	(S-NfL + SCS) S-NfL HR	(S-NfL + SCS) SCS HR	(S-NfL + PIGD score) S-NfL score HR	(S-NfL + PIGD score) PIGD score HR
Walking-aid	3.48 (1.43 – 8.50)	4.09 (1.58 – 10.59)	1.63 (1.0 – 2.66)	3.38 (1.36 – 8.35)	1.10 (0.90 – 1.34)
p-value	**0.006**	**0.004**	0.051	**0.008**	0.343
Nursing home	5.08 (2.07 – 12.46)	4.98 (1.87 – 13.28)	1.86 (1.13 – 3.07)	4.68 (1.79 – 12.22)	1.12 (0.97 – 1.28)
p-value	**0.000**	**0.001**	**0.014**	**0.002**	0.124
HY5	6.16 (2.13 – 17.79)	5.92 (1.79 – 19.66)	2.52 (1.44 – 4.41)	4.72 (1.48 – 15.02)	1.24 (1.06 – 1.44)
p-value	**0.001**	**0.004**	**0.001**	**0.009**	**0.006**
Dementia	2.77 (0.81 – 9.46)	2.01 (0.50 – 8.01)[a]	2.12 (1.12 – 3.98)[a]	1.71 (0.39 – 7.41)	1.07 (0.93 – 1.23)
p-value	0.105	0.322[a]	**0.020**[a]	0.474	0.33
Death	4.07 (1.72–9.66)	3.11 (1.16 – 8.31)	1.48 (0.92 – 2.38)	3.03 (1.20 – 9.18)	1.03 (0.92 – 1.16)
p-value	**0.001**	**0.024**	0.109	**0.021**	0.588

Potential of combining clinical and biochemical markers

S-NfL in the classification groups

There were differences in S-NfL level between the SCS-groups at baseline (p<0.001 as assessed with one-way analysis of variance, see Table for values), but not between MPS-groups (p=0.032 as assessed with one-way analysis of variance, see Table for values). There were similar results at re-examination (p=0.011 and 0.132 for SCS and MPS, respectively).

Table showing neurofilament light chain levels in patients as classified in paper I
S-NfL levels of the patients (pg/ml) as classified in paper I (mean ±SD).

	MPS-TD	MPS-UI	MPS-PIGD	SCS-MMP	SCS-IM	SCS-DM
S-NfL	17.5 ±10.0	23.1 ±9.5	22.8 ±16.0	17.9 ±9.6	18.8 ±8.4	32.8 ±21.2

Cox regression results

After establishing S-NfL's capability to prognosticate PD patients, we separately entered PIGD score and SCS group (as continuous variables) into the models. Although the S-NfL values were shown to be associated to both these parameters, their additions in Cox regression models showed no impact or very slight reduction of S-NfL's HRs, indicating that the clinical systems confer information additional to that of S-NfL levels. This was most clear for the HY5 outcome, where both PIGD score and SCS group showed significant HRs additional to S-NfL while S-NfL HR was relatively unchanged with maintained level of significance. In other words, this established that stratifying patients according to both S-NfL levels and either clinical system could be relevant for this PD motor end-stage. SCS group also showed significant HR for the nursing home outcome when combined with S-NfL, which could indicate a broader usability for SCS than PIGD score. For the walking-aid usage milestones, SCS did not significantly contribute to risk assessment (p=0.051) but S-NfL HR was increased when entering SCS group, which indicate that some part of SCS complements prognostic information of S-NfL.

ROC curves

After some degree of individual contribution of the factors were confirmed in Cox regression models, two combined models were created, combining S-NfL, age at onset and one of SCS and PIGD score, using data from the baseline examinations. A third combined model, with S-NfL and age at onset tertiles but without the clinical classification parameters was also constructed. ROC-curves for predicting individuals who were to develop the different milestones were constructed and the curves were compared visually and by evaluating differences in area under the ROC-curves (AUROCs) statistically between the individual models and age-adjusted S-Nfl levels (see Table and Figure). The three combined models showed

similar shapes of the ROC curves and similar AUROCs, that were all significantly different from that of age-adjusted S-NfL levels. The combined models that included clinical classifications showed slightly larger AUROCs than the S-NfL and age at onset combined model for prognosticating HY5-development, but slightly lower when examining mortality.

Table showing AUROCs for reaching the milestones of disease progression at any time
AUROCS for developing the five milestones of disease progression for age adjusted S-NfL levels and three combined models with either S-NfL and age at onset tertiles, S-NfL and age at onset tertiles and SCS-group-belonging or S-NfL, age at onset and PIGD score tertile. Delong method for comparing combined model AUROC vs age adjusted S-NfL AUROCs showed with p-values. Significant results highlighted in bold.

AUROC for milestone	Age-adjusted S-NfL	S-NfL + SCS + AaO	S-NfL + PIGD score + AaO	
Walking-aid	0.654	0.788 (0.128)	0.822 (**0.033**)	0.811 (**0.043**)
Nursing home	0.687	0.814 (**0.033**)	0.805 (**0.049**)	0.806 (0.050)
HY5	0.684	0.877 (**0.001**)	0.865 (**0.003**)	0.829 (**0.017**)
Dementia	0.590	0.765 (**0.021**)	0.775 (**0.005**)	0.772 (**0.016**)
Death	0.680	0.885 (**0.001**)	0.887 (**0.001**)	0.907 (**0.000**)

Sensitivity analysis

The sensitivity analysis performed in paper II showed that outliers in S-NfL levels might have been in part responsible for the association of S-NfL with HY-stage and the risks for dying and developing HY5. On the other hand, when removing the outliers, the walking-aid and nursing home results were instead strengthened, making it more likely that these parameters are not affected by extreme values of S-NfL. All the outliers at both baseline and re-examination had high S-NfL values. After removing the two outliers at re-examination there was improved significance levels of HY-stage, UPDRS2 and UPDRS-total.
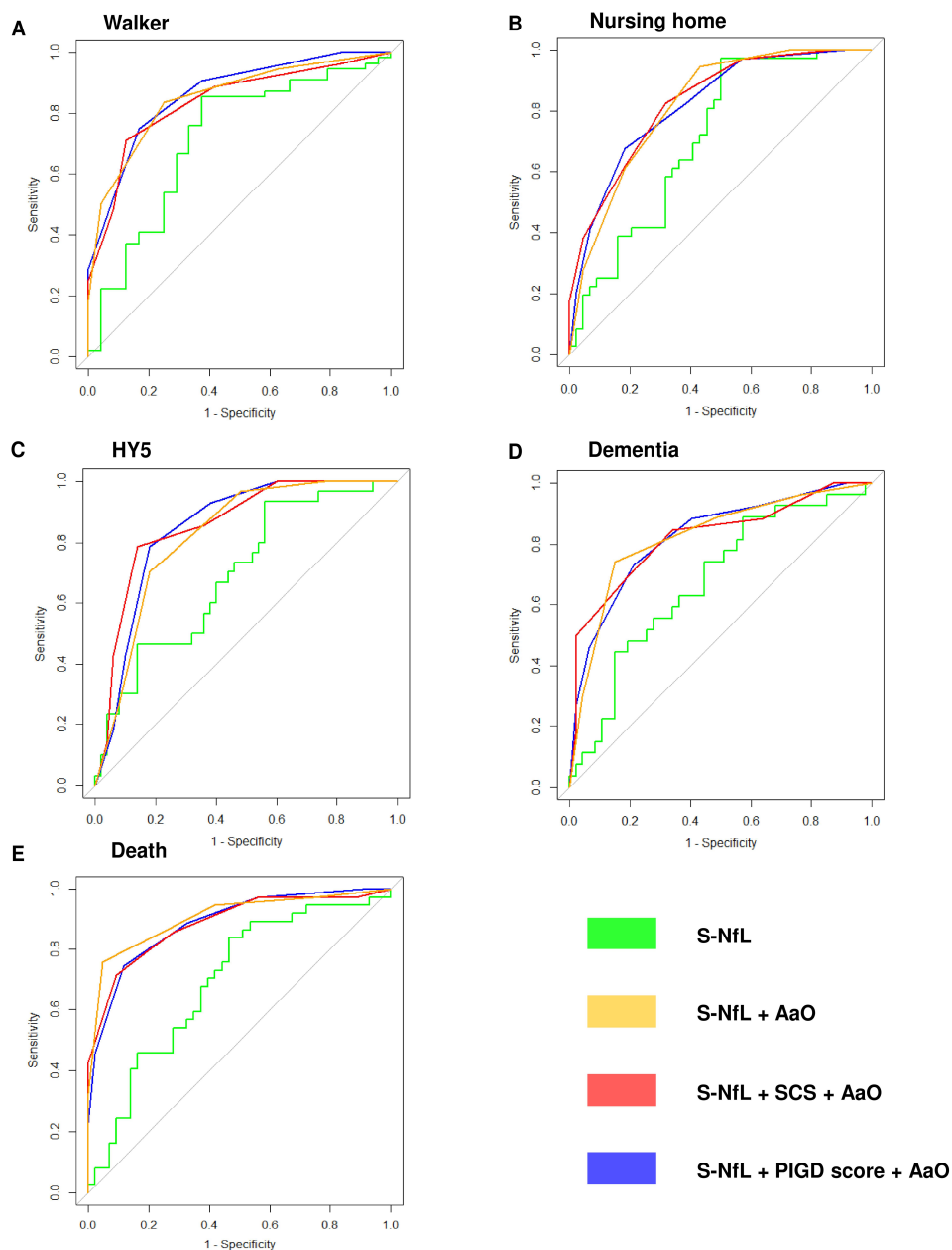
Figure showing ROC curves for S-NfL and combined models for developing the different milestones of disease progression
S-NfL, serum neurofilament light chain (as age adjusted and contineous when assessed alone and as unadjusted tertiles when assesed in combinations); AaO, age at onset (tertile); SCS, simplified clinical subtype (group); PIGD, postural instability and gait disorder score (tertile).

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Longitudinal cohort studies – Discussion

General outcome of the individuals

The follow-up period of the PARLU cohort was at a relatively late disease stages, from 7.9 ± 5.3 years of disease duration and with follow-up for 8.1 ± 2.7 years. PD is more often studied closer to diagnosis (89, 171), even when considering studies on late events such as DBS (172) and mortality (173). Although there are several reports on cohorts with relatively long follow-up time (174-177), outcome after more than 15 years of PD has been relatively uncommonly reported (178-180). It is thus important to highlight some general findings in the cohort and set them into context of the previous work.

Prevalence and risk factors for dementia

A low prevalence of dementia

In paper I, 32.9% of the patients eventually developed dementia. There have been few studies made on cognitive outcome in PD patients with disease duration longer than 15 years (89). Hely *et al*. (181) studied a multicenter cohort in Sydney, Australia, where 136 patients were followed since onset. They reported that 83% of individuals with more than 20 years of PD duration to have developed dementia. In a multicenter, cross-sectional study, Hassan *et al*. (179) nuanced the picture with relatively high cognitive performance in all 187 individuals studied who had 20 years disease duration or more. Studies with shorter follow-up time reported 46–50% of patients with dementia (175-177), and a small study of 19 individuals followed during 20–30 years of PD duration, found 43% of patients to develop dementia (180). Compared to these studies, and especially the Sydney multicenter study (178), 32.9% is a low proportion, especially given that the durations of disease in paper I and II were relatively long. The low age at onset for the study by Hassan *et al*. (179) might have been a part of the good cognitive functioning observed there. Reasons for low proportion of patients who developed dementia in the PARLU cohort could include underreporting in medical records or selection biases. An important note, however, is that this low proportion of patients with dementia was

found despite the proportion of men in the cohort (60.7%) was relatively similar to what is generally reported (171). Male sex is a known risk factor for PDD. An odds ratio of 4.5 for PDD development in males compared to females was found in a study of over 400 patients, where men also were found to progress faster (182).

Clinical factors associated with dementia development

Apart from an overall low proportion of patients with dementia in the PARLU cohort, the effects of sex differences on cognitive development were very clear in the Cox regression results, where the risk of developing dementia was approximately 7 times larger for male relative to female patients if other characteristics were the same. The low percentage of patients with dementia was thus driven by a very low percentage of women who developed severe cognitive dysfunction. Age at onset was also a key determinant for developing dementia during the observation period, and was also important in the results on cognitive testing at re-examination in paper II, although the number of individuals available for this assessment was low (n=32). These finding were generally in line with results from previous cohort studies, although we found a larger effect of sex than previously reported (89, 182). Interestingly, one study followed a similar number of patients for 4.4 years after inclusion at a mean duration of 5.7 years, and found that male sex and age at onset were important determinators for dementia development, similar to the findings of paper I (183). If an individual also had RBD at the baseline of that study, a factor also compiled into the SCS, there was a prominent risk increase of dementia.

Age in general has been reported to have a stronger association with cognitive progression in PD than age at onset (93, 182). A recent systematic review, however, found larger effects of age at onset than age at examination (89). Chronological age was not directly entered into the models of paper I and II. Duration (years) and age of onset (years) was both included though and as exemplified in the results section, age at onset was the most important factor of the two. This was also supported in the sensitivity analysis of paper I, where the impact of duration of disease was rendered not significant in the dementia Cox-regression models. However, the impact of age *per se*, and the exact relationship between age at onset, duration and chronological age was not examined in this thesis.

S-NfL in relation to cognitive dysfunction

S-NfL levels did not impose increased risk of dementia development in Cox-regressions. Nonetheless, the change of S-NfL levels between examination, for a small subset of patients (n=27), correlated with cognitive performance at re-examination. Also, when S-NfL was used as a diagnostic test for developing dementia during the observation period, as adjusted for age or in combinations with age at onset or age at onset and classification parameters, the AUROC for developing dementia were similar to the AUROC for the other parameters assessed,

even though showing the lowest AUROC in all ROC variants with relatively modest AUROCs of 0.82–0.83. ROC-analyses disregard the timing of milestone development, and these analyses were not adjusted for sex, which was found to be an important risk-factor for dementia development in Cox regression models. A lower prognostic accuracy for dementia development is thus implied with S-NfL assessments in paper II. Albeit the association between ACER scores and change in S-NfL levels in the re-examined patients in paper II speaks in favor for S-NfL to hold some information on cognitive prognosis, that analysis was based on very few individuals and should not be able to rebutt the reported low ability of S-NfL as a prognostic factor for cognitive development.

An underperformance for S-NfL regarding cognitive aspects of PD was also suggested in at least two other studies: A large systematic review where no differences in NfL levels were detected between PD and PDD cases (105), and a longitudinal cohort study (109). The latter study found no difference of CSF-NfL levels in PD patients that were to develop cognitive dysfunction compared to those with withstanding cognitive functioning, but CSF-NfL levels were associated with HY-stage, UPDRS-III MOCA and other parameters in that study.

Motor functions

Thirty-two patients in the PARLU cohort (36%) developed the motor end-stage of HY5 (plus one patient that developed HY5 before the baseline examination). Although categorical comparisons of HY5 risks in paper I had to be divided by sex and were therefore based on relatively few individuals, the results were similar to that of the other milestones. There were no significant differences in risks found between the MPS groups while significantly different risks for developing the HY5 milestone were found between the SCS groups. There were, however, significant differences in risk also when using PIGD score only, but to a lower magnitude than when using the SCS groups.

When combined with S-NfL in paper II there was a slightly lower SCS HR for developing HY5 than what was found in the sex-stratified categorical analysis of paper I. The HRs for this outcome was, nonetheless the highest for the assessments using the SCS, PIGD score and S-NfL levels, in both unadjusted and adjusted analyses. Risk of developing HY5 could thus be relatively well reflected using the prognostic factors tested in this thesis.

Activities of daily living

ADL was reflected both in the UPDRS2 subpart and in a separate estimation using S&E scale. Both these measures at baseline and re-examination were associated with S-NfL levels at baseline and re-examination visits, with higher S-NfL levels

corresponding to less self-dependence in activities of daily living. The results thus suggest a role of S-NfL levels also for this important part of PD progression. Impact on ADL was, however, not directly examined in more ways in the studies of this thesis but the risk-stratifications shown for the HY5 and nursing-home (SCS and PIGD score) as well as the dementia milestones (SCS) can be interpreted as indirect assessments of ADL though, as these milestones of progression would imply a reduction of dependence.

Mortality

Male sex inferred a high risk for dying in the Cox regression models of paper I. The difference in mortality for men compared to women was higher than previously reported for PD in general; 5.3–6.5 in the mortality model in paper I compared to 1.5–2.7 in the literature (7). Nevertheless, the mean age at death was relatively similar to that of the general Swedish population, (84.3 for women and 80.6 for men 2020) and age at death was relatively sparsely reduced for males in the cohort, 1.4 years lower than that of the mean Swedish male (https://www.scb.se/hitta-statistik/sverige-i-siffror/manniskorna-i-sverige/medellivslangd-i-sverige/).
Increased S-NfL levels were associated with a higher risk of dying. An individual with 2.72 times higher S-NfL level than another has larger differences in risk than an individual classified as SCS-DM compared to one classified as SCS-MMP.

The classification systems in context

The simplified clinical subtypes SCS

Validations of the clinical subtype algorithm
In paper I and II in this thesis we adapted the NMS used in a previously established algorithm of combining motor and non-motor features in PD. This adaptation, the SCS system, was efficient in stratifying risks in the PARLU cohort and aside from the statistical results, the construction of the SCS in itself can be considered a type of confirmation of the original subtype classification system (23). The findings in paper I were a general confirmation of the algorithm of the original subtype system since the SCS showed prognostic usefulness for the development of the five different disease milestones assessed even though the NMS parameters were adapted and the disease durations in the PARLU cohort was highly varying. A usefulness of a similar approach to adapt the subtype system was also showed in a pathological patient series of 111 patients (184). Even though there was no evidence of pathological correlate to the SCS-groups in that study, there are now at least two studies that have adapted the original system, showing very similar results despite

different patient selections and different adaptations of the system. The study with neuropathologically confirmed cases also showed significant HRs of similar magnitude for using wheelchair, developing dementia, or dying. Nursing home residency was not assessed but a similar parameter, home care, showed HR (adjusted for severe depression and age at onset) of 3.33 (95% CI 1.35–8.20) in that study. Arguably, replication in different cohorts with variants of a general methodology constitute validation for this PD subtype system. Futhermore validation with pathologically confirmed cases and > 50% individuals recruited from a geographical area in the PARLU cohort gives some weight to the validations performed by de Pablo Fernandez *et al* (184) and paper I, respectively. The number of patients the variants of the system has been applied on is however still too low to directly enable large clinical use but establish that clinical prognostication in PD can be more successful when adding non-motor aspects of the disease. The simplified SCS variant also especially highlights a potential as a clinical tool for prognostication in neurological practices.

The longitudinal evaluation of SCS was a strength with paper I. Longitudinal follow-up for some patients included in the PPMI cohort was performed in another study and showed radiological and clinical parameters to be associated with clinical subtype (185).

SCS can predict cognitive development

Cognitive affection is a key concern for many PD patients and their relatives (186), and can be complicated to prognosticate in the clinical setting (187). SCS was relatively good at assessing risks of future dementia development at baseline examination. Furthermore, dementia-development and nursing-home living for the SCS showed the least changes of risk-gradients after sensitivity analysis in paper I.

SCS was a better prognostic marker than motor-phenotypes

In paper I, motor-phenotypes were generally not able to stratify risks for reaching the different disease milestones in the PARLU cohort. In paper II there were also no differences detected in S-NfL levels between the different motor-phenotypes. SCS groups, on the other hand, showed good usability for stratifying risks in general in the cohort with a contribution of risk-assassement that was significantly separated from S-NfL in more outcomes and to a higher degree than PIGD scores were.

Contribution of individual parameters in the SCS

As exemplified for this thesis, the distribution of patients that died during follow-up while considered to have the different NMS subparts of the SCS as positive, showed that NMSQ and hallucinations had relatively clear contribution to mortality, since there were high proportions of deceased individuals with these NMS domains. The proportions of deceased patients with and without RBD on the other hand were similar. RBD was to some extent assessed in a less detailed level than hallucinations

and NMSQ, which could be responsible for its relative non-contribution. Furthermore, RBD symptoms can be less prominent later in PD and since baseline examinations were performed after various disease durations, some patient might have forgotten details on symptomatology closer to onset, leading to a false low rate of patients with RBD designated as positive. The cohort that the clinical subtypes were conceptualized from had relatively low and homogenized disease durations, and RBD was in the original study assessed with a more detailed questionnaire (23). Both timing and method could thus have led to a more representative assessment of RBD in that study. The relative lack of prognostic information inferred by RBD in this phase of PD is in contrast to the relatively immense importance of RBD found at prodromal stages and around disease onset (3, 23).

In the PARLU cohort hallucinations were more informative than RBD which could indicate that a clinical subtype algorithm could perhaps be adapted to different aspects of PD as the disease progresses. In other words, RBD could be more important in the years close to diagnosis, speaking for an approach similar to that of the original clinical subtypes (23), while the impact of hallucinations later in PD might speak for using the SCS adaptation if prognosticating a patient with longer duration. Hallucinations has previously been associated with cognitive decline in PD (89), and might also have been indirectly associated with motor functioning in out studies as a potential side-effect of high medication dosage that indicates severe motor dysfunction. Both cognitive and motor decline have previously been associated with mortality in PD (173, 188), and hallucinations was recently included in a subtype system based on data from the same cohort as the study conceptualizing the clinical subtypes (189).

PIGD score and motor-phenotype

The original MPS, relating PIGD score mean to tremor score mean was not a successful prognostic factor in the PARLU cohort, even though it's a commonly used distinction between PD patients. Unexpectedly, patients with different motor-phenotypes showed no statistically significant different risks for using walking-aids or developing HY5, which would been expected by the system since these outcomes are highly dependent on motor functioning. Our findings were in contrast with some previous studies that found the MPS to have associations to amount of atrophy in PD brains (190, 191), and development of MCI (192). Several other studies have, however, discussed that this system could be considered a surrogate for disease stage rather than a disease trajectory, which would be in line with our results and is discussed in a separate section below (99, 193, 194).

PIGD score was more effective than motor-phenotype
Interestingly we found a clear use of the PIGD score of the MPS, as in removing the tremor scores aspect. When ad hoc examining only PIGD score in paper I, even

though risk of using walking aid could not be predicted, there were significant risk-stratifications of HY5, death and nursing-home living. Furthermore, PIGD score but not MPS was associated with S-NfL values in paper II. Some other studies have previously implied a longitudinal change in the prognostic usefulness of the MPS (99, 100), including studies finding longitudinal usefulness only of the PIGD score, similar to the findings of paper I (193). Theoretical explanations to why the tremor score would lose relative importance include:

a) The natural progression of disease; as tremor relative to other symptoms can declines with time, while postural instability and gait impairment continue to increase. This would lead to an ordered progression to MPS-PIGD for most individuals with PD, as observed in our and other studies (99). The gait part could be the driving factor of the system and presence of tremor might constitute a surrogate marker for the absence of PIGD symptoms, rather than have associations to disease progression on its own (3, 193).

b) For patients that instead have progressing tremor, there could be earlier ceiling effects in the tremor scores than in the PIGD scores of the UPDRS, as tremor can in some cases be severe. In other words, if a patient reaches a point where tremor scores hardly can increase further, relating the tremor part to PIGD aspects might diminish prognostic information rather than improving it, since the scores develop differently over time. There is also a specific form of benign tremulous PD (195). Heterogeneity among tremorous patients could perhaps dilute some of the information of tremor on cohort basis.

c) Factors concerning timing of diagnosis and misdiagnosis could also be a responsible factor. Presence of rest tremor might facilitate early clinical diagnosis, as both patients and physicians might be more prone to note this symptom early on. On the contrary, initiation of disease with mild rigidity would perhaps be more likely to be interpreted less objectively, as in considered to be normal aging by patients and their relatives. Also, MSA and PSP seldom present with tremor, leading to a confounder of tremor at onset to reduce the number of patients wrongly categorized as PD, or with more borderline pathology between PD and APSs. Similarly, if tremor patients are misdiagnosed, the alternative diagnoses are usually with beneficial prognosis (196). This theoretical factor should, however, rather be of lesser magnitude in paper I and II, given the relatively long observation period.

d) The relative success of PIGD score to the original MPS could be related to lack of statistical power. This was indicated by the uneven division of patients into the motor-phenotype groups, with most patients (66.3% in paper I) already being classified as MPS-PIGD at baseline. Uneven groups

increase the number of patients needed for analyses to be representative in survival statistics. PIGD score is instead a continuous parameter, making the statistical methods applied more prone to use the full information entailed.

e) Since we examined the patients with their normal medications, the change of tremor/PIGD relationship later in disease could theoretically be a surrogate marker for withstanding relative to deceasing effects of treatment. Since effects of medication on tremor can be variable (197), or less responsive to levodopa treatment in general (198), the values of the TD scores could be increased during the disease course relatively independent of medication type or dosage. PIGD scores could instead mark a medication dependent trait, at least in early–mid stages of disease (199), and the observed inadequacy of the MPS could thus be affected by confounding of medication effect, which would not affect tremor to as large an extent. Closer to diagnosis, treatment is often of lower dosage, and the confounding effects of medication on the MPS thus be lower. Other studies finding similar results with patients off medication speak against medication to have large effects, (94, 100), albeit one study found medication effects on tremor to affect changes in motor-phenotypes over time (200).

f) The original MPS could in theory be relevant only for individuals with specific spreading patterns of pathology or due to specific pathogenic causes (e.g. on or more *authentic subtypes*). Since 50% of the PARLU cohort was included from a geographic area, environmental exposure or genetic background might be somewhat more homogenous than in other studies, which could perhaps indicate a large proportion of a specific subtype of PD where tremor scores are not a valid prognostic feature.

Instability over time of classifications

Patients were reclassified at re-examination, resulting in several patients changing classification group as classified with either the MPS or the SCS. No patients changed to a group with lower risk when using the SCS, as four individuals did when using the MPS (11.8%). The processes underlying PD are today considered not to be of a naturally reversable or instable nature but due to relatively unchanging pathological mechanisms. The finding of illogically reclassified patients thus speaks against the MPS to be tightly correlated to pathogenic mechanisms. There were similar findings in two different studies classifying patients at mid disease (99) and in early disease (100). Several other studies have also confirmed a change of MPS-group over time (98, 200), including one study where three different variants of

motor symptom subtypes were examined (194), and one study using expansion of the MPS-UI as several subgroups (94).

However, the relative superiority of logical transitions of the SCS in paper I was likely affected by that we used the same formula for the composite motor score at both baseline examination and re-examination. This choice could have resulted in an avoidance of illogical progression as the SCS group might have differed if the composite motor score would have been calculated with higher grade of adaption to the cohort characteristics at re-examination, (as in the 27 of the 34 re-classified patients that had 0–2 NMS domains positive at re-examination and would have their classification fully dependent on motor score). Using the same formula was considered important, however, to simplify clinical use of the composite motor part of the system.

While writing this thesis we are still lacking accurate subtypes of PD as in *authentic subtypes* that can constitute a link between pathological processes and clinical phenotype. Such subtypes would arguably exhibit distinct features in both clinical symptoms, genetical background, cellular processes, and pathological end-result and would ideally not be subject to instability over time. On the other hand, change of classification group might be a part even of successful classifications, as different pathological processes could in theory drive the disease in different phases of the disease, instead of a single pathogenic mechanism determining progression in the whole disease span.

Impact of motor-phenotypes at re-examination

Besides the large proportion of patients who were classified as MPS-PIGD at the baseline examination, another observation clearly speaks for the MPS to be marker of disease stage rather than a prognostic subtype. Of the 34 patients available for re-examination in the PARLU cohort, all that reached any of the five major disease milestones where either classified as PIGD at baseline examination or at re-examination. A similar observation, that no relevant late-stage events in PD occurs before the patients have transitioned into MPS-PIGD, has been made by another group studying 171 PD patients from the same Norwegian community (99).

Are the clinical subtypes also a way of extrapolating disease stage?

Another study, replicating the subtype system SCS was based on, indicated that, similar to the MPS, the subtype groups would correspond to different stages of the disease course (201). If this would be considered true, the reason why prognostication from this system is possible would be attributed to extrapolation of the clinical state the patient is in at measurement: patients with few symptoms are more likely to remain that way than shift to a more aggressive form of disease, and

patients with an already more advanced symptomatic picture is probably more inclined to continue to progress. Whether clinical subtypes really are as stage-dependent as MPS remains to be determined by other studies. Even though our findings indicated that there was prognostic value in the SCS for many years after the prognostic accuracy of MPS was impaired, the large proportion of patients that re-classified in SCS could be in line with the earlier results showing clinical subtypes to be a stage-dependent measure.

Combining biomarkers

We found S-NfL levels to be associated with risk for both motor and social outcomes and also with two different ADL-scales (UPDRS2 and S&E). Our results thus highlighted the broadness of prognostic data in S-NfL levels. Furthermore, S-NfL showed wide risk-stratification capabilities in the PARLU cohort, as its values varied more than the PIGD score or the three SCS groups (SD 14.3 for S-NfL as compared to 3.0 for PIGD score in paper I, see Table and paper I).

As overviewed in the introduction, PD is suggested to be caused by a multitude of different pathogenic processes. An individual patient could thus have composition of factors that slow down some mechanisms but aggravates others (16), and combinations of different measures could be needed to make accurate prognostication in PD (202). When tertile of S-NfL levels were combined with tertile of age at onset as combined score models, with and without SCS groups/tertiles of PIGD score, the AUROCs for developing any milestones were improved, compared to that of age-adjusted S-NfL alone. Since S-NfL and age at onset constituted similar prognostic accuracy as the combined models with clinical classifications, S-NfL and age at onset could be a very effective way to evaluate risks on clinical appointments. However, the Cox regression models of paper II speaks in favor of also sex, SCS and PIGD score to have separate contributions to the risks of the patients, and more complex models might need to be constructed to put the information of these parameters to optimal use.

A theoretical ground for combining sex, age at onset, SCS and NfL could be that these parameters might measure different pathogenic aspects of PD. S-NfL constitute a marker of general degeneration of axons and thereby an indirect measurement of general neurodegeneration in PD, presumably without being especially connected to specific parts of the brain, as could be the case with motor-progression and the development of different NMS.

Implications of the findings and future perspectives

The importance of a simplistic system for clinical use

The MPS is commonly used in part because applying it in clinical conditions is relatively easy. The tremor and PIGD assessments used are directly compiled from the rating scale UPDRS, which is commonly used both in research and clinical follow-up. The clinical subtype system is a more complex system, combining NMS burden, cognition/hallucinations and RBD with the weighted contributions of three motor sub-scores: UPDRS II, III and PIGD scores. The SCS was an adaptation to this system that enabled quick risk assessment in a clinical out-patient setting by changing the NMS parameters to more simplistic ratings and adapting the formula for the composite motor part. Even though the results for the SCS generally showed high prognostic value of this simplification, the method likely reduce information, which could impede its use in research. The goal was, however, to build on the UPDRS assessments in clinic with as simple means as possible, enabling fast clinical application that might speed up the process of taking this system to neurological clinics around the world.

However, if the combinations of biomarkers suggested in paper II were to be validated as a more impactful prognostic factor, S-NfL in combination with clinical parameters could be considered the most convenient markers of general disease progression. Clinical examination and determining disease onset are harmless to the patients and venepuncture for S-NfL assessment is minimally invasive with low degree of side effects and can lead to quick and standardized laboratory analyses.

Importance for the patients

As stated in the introduction, reasons for the variability of clinical symptoms and progression rate in PD, are as of today insufficiently determined. Improved information gained from this thesis can have direct or indirect effects for the real-life situation and care of PD patients. The impact of the findings is in need of validation though, where number I–II below could be established in a near future and number III on the list corresponds to the ultimate goal of PD subtyping, which will require substantial more work to reach.

I) If validated, NfL and/or the SCS could inform PD patients whether they would progress rapidly with cognitive or motor dysfunction in a couple of years or if continued mild motor symptoms would be to expect during longer time.

   a. If a patient or his or her next of kin have an idea of whether the time frame is short or long until severe handicaps limit independence,

several things of personal importance could be planned before the symptoms become too much a limiting factor. In the case of high risk of future cognitive decline, wishes for living conditions and deciding who will aid in legal and economic matters might be of especial importance.

b. When reliable ways to identify individuals that are prone to progress fast are established, these individuals could be selected for clinical trials, which would shorten the timeframe and increase the possibility of detecting effects of therapeutic interventions.

c. With improved disease subtypes, ante-mortem diagnostics could be more accurate and in turn further potentiate studies on prognostics, further improved subtypes *etcetera*, since misdiagnosis could be minimized.

d. Detailed knowledge on how patients progress differently enables studies on why, which might in turn yield new knowledge on disease mechanisms, which, in a longer perspective would increase the chances of finding a cure.

II) NMS could be added to clinical diagnostics and classifications of PD, putting their known prognostic value to use. Apart from that establishing subtypes with NMS might lead to better markers of disease development, this might put the NMS in context, clarifying their role in PD prognosis and pathogenesis.

a. The patients might feel comforted by the NMS being a part of a particular type of PD, finding an explanation to why they are experiencing some NMS and others not, which could otherwise perhaps be frustrating.

b. With continued research, clinical and serum biomarkers might be found accurate enough that lumbar puncture or radiological investigations might be of less use in PD in the future. This could limit discomfort for the patients and reduce risks of side-effects (such as post dural punction headache or long-term effects of increased radiation exposure).

III) More detailed description of patients with different prognostic factors will facilitate research that can lead to identifying *authentic PD subtypes*, ultimately explaining the heterogeneity of PD.

a. Such subtypes would ideally include specific trajectories of progression, with different symptoms likely to develop before others and certain symptoms that would never develop in a specific subtypes. This would further improve optimization of the life with

the disease, as in planning for future cognitive or motor impairment, limited ability to perform household chores *etcetera* and would enable more optimally planned and timelier implemented actions to cope.

b. A concept of stable subtypes, corresponding to specific disease-causing mechanisms, are relatively likely to also be relevant in prodromal stages of PD. If such pan-disease subtypes would be established, the timeframe for accurate diagnostics could hence be improved to before onset of symptoms, which could be crucial for treating PD since the disease could be too widespread at onset of symptoms to cure. In other words, gaps between diagnosis of prodromal PD, clinical PD and neuropathologically confirmed PD can be diminished.

c. Treatments and ameliorating actions could be personalized to the specific PD subtype. As in physicians giving early recommendation of acetylcholinesterase inhibitor when cognitive decline is ensuing, or more strict recommendations of physical exercise if motor or specific non-motor symptoms were predicted to be a future part of the disease.

Future perspectives – Prognostic PD subtypes

Should we switch to PIGD only as motor rating?

The PIGD score parameter showed much better risk-stratifying ability than the original MPS, as indicated by the statistical analyses and the large proportion of patients that was categorized as MPS-PIGD and also the illogical instability of MPS-groups over time. These factors speak in favor of using PIGD score only instead of the original MPS. However, this change could be more relevant after some years of disease duration though, as our study was performed at a mean of 7 years of disease duration.

We need to examine re-classifications further

If both SCS and MPS are to some extent considered advanced markers of PD stage, this would mean that relevant progression markers should be studied in patients that change classification group. One previous longitudinal study did observe patients with stable MPS-TD after 5 years compared to patients that had converted to MPS-PIGD, though (203). The study showed that total NMS burden and severity of specific NMS such as cognition and sleep problems, were overrepresented in converters. Although sleep difficulty was the only factor that remained after adjusting for education, conversion could be predicted using years of education, MOCA results and NMSQ with an accuracy of 88,5%. This study was small but

confirms that NMS, including cognitive reserve, are important in PD progression. Another study on clinical subtypes also found that an increase of brain atrophy was associated with transitioning to a more severe subtype-group and *vice versa* (204). This concept is relatively unexplored and could contribute important prognostic information. However, the re-classified individuals in the present studies were too few to enable a proper examination of this subgroup.

Continue to combine different biomarkers

Several significant factors were identified in the adjusted cox regression models of paper I and II, including duration of disease, age at onset, sex and SCS or PIGD score. This substantiates that PD is a multifactorial disease and the notion to combine several, less powerful estimates should be valid. Indeed, the results of the Cox regression models and combined-score models in paper II in this thesis show that there is promising potential to combine biomarkers that are already established within current literature. Since the SCS, as being a mix of clinical factors, was showed potential as a prognostic marker in paper I and showed separate contribution when combined with S-NfL and age at onset in Cox regression models in paper II, continued research of how to best combine several markers of progression is a promising way forward for more advanced and personalized prognostication in PD.

As shown in the results of paper I and II, age at onset and duration at baseline examination had both relatively large impact on the risk for patients to develop the milestones of disease progression but age at onset was the most important risk factor of the two when comparing risks between most patients. In paper II we performed combined models using this parameter and S-NfL, showing promise as a simple clinical prognostication tool. As a major determinator of PD outcome, age at onset should be included in future research on prognostic models and other aspects of PD.

However, although the usability as a clinical diagnostic test was good in all the three combined models of paper II, the AUROCs were not above 0.90 despite the relative long observation period which most likely indicate that sensitivity and specificity is too low for general clinical usage as is. The concept of the combined models is not yet validated but could thus be in need of further improvement or selections, as in combining with sex or applying the prognostic systems on a subsets of PD patients, based on sex and length of disease duration for instance, to enable effective day to day use of these combinations without too large a number of inaccuracies. Although a relatively low number of patients was studied in this thesis, the results are a clear indication that combinations of different biomarkers are relevant to improve prognostication in PD.

Consider the number of subtypes

Although other studies on PD-classification have identified 3-5 grouped solutions (205-209), cluster analyses in the original work on the clinical subtypes suggested systems with two or three groups (23). We were unable to directly compare optimal

number of groups but there were KM curves with crossing longitudinal risk profiles between two of the groups, in the walker outcome. There might be incentives to consider a two grouped solution of PD to better reflect pathological processes. Usefulness of dividing PD into DM and non-DM patients was discussed in the study constructing the original clinical subtypes (23). A separate dichotomic variant of a clinical subtype system should perhaps be constructed and its combination potential with other neurodegenerative biomarkers assesed.

The SCS needs validation and cut-off determination

The study originally conceptualizing the clinical subtypes calculated the composite motor score as a mean of z-scores, based on the SD and mean of the cohort values. To be able to compare clinical subtypes between cohorts however, fixed coefficients to the motor-score components needs to be determined. We chose to apply the values of the three parameters in the composite motor score for the PARLU cohort at baseline examination. This enabled a mathematically transduction of the formula to a more simplistic one, enabling calculating of the SCS directly on the out-patient appointment as only the examination results of the patient would be the unknowns in the equation. We still used the 75th percentile of the cohort and the SCS is needed to be replicated to get indications whether this is a valid simplification, and to examine the proposed cut-offs. The PARLU cohort is relatively small but with variable ages at onset, geographical inclusion for half of the patients and have long and varying disease durations, motivating why we added the cohort mean and SD to the calculation. The simplified motor score calculation and the cut-offs might be used as a reference point for further examinations and might be shown to be representative for all PD patients but could perhaps be more likely to be valid only for those in a later phase of the disease. Cut-offs for the SCS closer to diagnosis and/or in prodromal stages are thus likely needed. Furthermore, the data on outcomes studied in paper I and II were retrospectively collected, although we had a predefined cohort and clinical examinations at inclusion. There has also been other validations of the clinical subtype algorithm with data retrospectively collected from pathologically confirmed cases of disease (184). Other studies have found radiological patterns of atrophy to be associated with the clinical subtypes (185, 204). Prospective use of the SCS and the original clinical subtypes should be studied to confirm their usability as clinical classification systems.

Future perspectives – General suggestions

Increase the number of studies with geographical inclusion

In paper I there were results that contrasted from those of previous studies, especially concerning the withstanding contribution of clinical subtypes as prognostic parameters and the lack thereof for the MPS. In paper II, there were furthermore unclear results concerning S-NfL's role for dementia development and

cognitive tests and what parameters that are best to incude in clinical combined models. Further studies, building on the findings of the clinical subtypes or establishing new ways to combine motor and non-motor aspects of PD, should be able to clarify ambiguities and should be able to improve prognostic subtyping solutions in PD further. It is of importance that the studies should be performed on and/or validated in cohorts with geographical inclusion, since this could be an explanation of some of the results of paper I and II that deviated from earlier studies. Also, motor-nonmotor subtype composition could differ between geographical regions, as NMS are found to vary between populations (78). Indeed, geographical inclusion has been highlighted to be crucially low in previous studies on PD subtypes (210).

Make more studies regarding the effects of age on PD prognosis

The matter of whether disease duration, age at onset or continuous age, is the more important factor for disease propagation is in the author's opinion under-researched. Our findings indicated that age itself has a larger impact on PD progression than has disease duration. Age-related mechanisms in the brain are perhaps an integrated part of the PD disease process or could be broken apart so that the disease processes are separated from age-related changes. If the effects of age were stated more clearly than today, it could be worth considerable research effort since identification of new mechanisms related to ageing could potentially have large roles in all neurodegenerative disorders. The interplay of age-related mechanisms with the underlying cellular malfunctions responsible for the different neurodegenerative diseases might even be key to finding successful treatments.

Strengths and limitations

Strenghts

The results of paper I and II were obtained from a cohort with 50% geographical inclusion, which improved validity of the results. Furthermore, we studied patients with diverse and relatively long durations of disease allowing us to draw conclusions for a large part of mid–late PD. The study was in general in a mid–late part of PD, which are stages that are relatively less studied, thus increasing the novelty of our findings. This is of special note with S-NfL levels, as it to the best of our knowledge was only previously studied for shorter time intervals. Furthermore, interrater difference is a potential problem with clinical examinations. We removed this bias in the cohort by having the same investigator for all baseline examinations and all re-examinations.

Limitations

There were likely relatively small selection biases in the population-subgroup, since inclusion was geographical. The researchers' special interest of hereditary PD was, however, known to colleagues which might have directly or indirectly imposed selection biases for the hereditary subgroup, where patients who were motivated and relatively healthy might have had higher probability of being directed to the study by a physician or being more inclined to ask a physician about ongoing studies.

For the vast majority of cases in the PARLU cohort no post-mortem verification was performed. Thus, a diluting effect of other conditions than sporadic or familial PD could arguably be affecting the results. The exclusion of patients with atypical findings and a long follow up time should, however, minimize the contribution of APS patients and cases with vascular or iatrogenic causes to Parkinsonism, since these diseases often progress with different patterns and speed than that of PD. The patients studied had regular neurologist-visits to detect such avoidances from the typical disease course, but these were not standardized but in large parts reliable on the individual skills of many different neurologists. There was a low proportion of patients with PDD and cognitive testing was only performed in a subgroup, which might have impeded analyses of risk for cognitive decline in the cohort.

Conclusions – Longitudinal cohort studies

The SCS was a successful simplification of clinical motor-nonmotor subtypes that can readily be applied at out-patient appointments. This system showed promising results regarding risk-stratifications for all the milestones studied.

The MPS was unsuccessful as a prognostic marker in mid–late PD. Removing the tremor part of the system and only using PIGD scores resulted in a relatively good long-term risk-stratification.

We found a low incidence of cognitive decline to dementia level. The risk of dementia could be predicted by the SCS but S-NfL levels might include less prognostic information regarding cognitive outcome.

We verified the importance of sex, age at onset and motor severity for PD progression, including increased risk for dementia and mortality. These effects were previously known but we noted a destinctly high magnitude of risk-increase of male sex compared to what has been previously reported (7). We concluded that higher age at onset and male sex are the most important factors for progression in PD.

We confirmed that S-NfL levels are associated with PD severity at mid- and late-stages and its longitudinal change was associated with change in clinical parameters. S-NfL levels also hold general prognostic information regarding risks of the milestones studied.

We showed that the concept of combining S-NfL levels with age at onset and/or clinical classifications could prognosticate PD-outcomes with medium–high prognostic accuracy as diagnostic tests, but more research to evaluate and refine such models are needed.

Family studies – Methods and study designs

Contact with the families

Two different families with symptoms of cognitive decline and parkinsonism as well as a family history indicating dominant autosomal inheritance of some or all disease traits, constitute the study populations of paper III and IV in this thesis. Both families were selected for detailed examination and longitudinal observation since there were no known causative mutations found in relevant genes when assessed in clinical visits (family in paper III) or through previous screening in the PARLU research setting (family in paper IV) (211).

Initiation of the studies

The family studied in paper III was followed for several decades by the memory research unit in Lund. The disease was well-known to most family members as a large number of individuals developed the disease, and family members expressed their wishes for a search after a genetic cause. The phenotype was described as AD and genetic analyses of the then known disease-causing mutations in *PSEN1*, *PSEN2* and *MAPT* did not show any pathologic variants. However, this clinical genetic testing was performed in the one family member who had dementia of another cause (individual III-2 in pedigree, denoted with arrow, see Figure).

The family in paper IV also had a clear accumulation of PD and/or cognitive decline in some family branches. Even though the family was large and held four cousins with a phenotype very similar to normal PD who were all four initially included in the PARLU cohort, genetic screening in 2007 for all up to then known genetic causes of PD did not find the cause (211). One of the more severely affected individuals was considered proband (individual III-2 in pedigree, denoted with arrow, see Figure).

Expansion of pedigrees

Affected individuals were interviewed regarding family connections and pedigrees were constructed. The pedigrees were further expanded by contact with other family members through either:

- ❖ Further interview with unaffected family members during their clinical examinations

- ❖ Letters and/or phone-calls after family members providing contact information

- ❖ According to genealogical information of the civil registries from the Swedish tax agency or older versions kept at local churches. This part of the work was performed by research nurse Karin Nilsson.

Examination of symptoms

Clinical examination

Four affected individuals of the family in paper IV were examined clinically by the author of this thesis or by the main supervisor Andreas Puschmann. As affection status is crucial when investigating potential new genetic causes of a disease, 12 individuals without obvious signs of PD were also examined to decipher affection status or whether some individuals may express mild symptoms due to incomplete penetrance of the presumed genetic cause. Examination was the same for all individuals and included UPDRS and other neurological test such as reflexes, peripheral strength *etcetera* and included ACER assessments and blood-sampling, in part performed by research nurses Christin Karremo and Karin Nilsson.

Examinations of affected individuals in paper III (generations III and IV, see Figure) were performed through clinical appointments to the co-authors of paper III or other physicians. Three affected individuals were also included in a parallel study, examining a tau positron emission tomatography ligand (212).

Medical records

Medical records of all affected individuals in the generations of most interest (generation III in both families and also generation IV for the family in paper IV, see Figures) were acquired and read in full text. In general, the medical records of individuals of older generations (generations I and II in pedigrees) were unavailable, in several instances because to the hospital where the most records were kept was closed in the 1980s. Information regarding these individuals were acquired through interview of living family members.

The affected individuals examined in paper IV were also a part of the PARLU cohort (although one was excluded from the analyses due to too short time of follow-up).

Besides detailed full-text study of the medical records, a digital search was thus performed, see the methods section for the longitudinal cohort studies above.

Genetic analyses

Identification of a disease-causing gene (paper III)

At the time of compilation of clinical data in paper III, the DNA collected from members of the family described in paper III were analysed with whole exome sequencing (WES), for individuals III-2, -3, -6 and IV-9, which identified a *MAPT* mutation in three of these individuals which was then well-defined as disease causing.

Identification of MAPT haplotypes (paper III)

For the four individuals with WES data in paper III, *MAPT* haplotypes were determined by identifying three single nucleotide polymorphisms (SNPs) known to mark *MAPT* haplotype H1 and H2, respectively.

Compilation of a list of gene candidates (paper IV)

The four affected family-members described in paper IV had no mutation that was known to cause PD, neither at screening in the PARLU study in 2007 (211) nor when the four cousins affected with PD were examined with WES in 2015–2017. After collecting DNA from an additional 14 individuals in the family, a list of candidate genes could be compiled. Whole genome sequencing (WGS) analysis was performed for individual III-2 using NEBNext Ultra II DNA Library Prep Kit (New England Biolabs Inc., Ipswich, MA, USA). Apart from the four affected individuals (III-1, -2, -7, and -8) and one clearly unaffected individual (III-12), there were ambiguities in the clinical examination of several family members. One neuropathologically confirmed unaffected individual had no blood sampled and DNA extracted from frozen cerebellum was of low quality, obstructing correct WES analysis. The mutations in the list were therefore firstly filtered by presence of the variants in the four affected in combination with absence of the variants in one unaffected individual (III-12). These individuals constituted five of the eight individuals who were examined by WES in the family (see Figure).

Identification of two top-candidate genetic variants for familial PD (paper IV)

All variants from the filtered list were searched for in WES results from the three additional cohorts, two from Mayo clinic, one with PD probands and one with healthy controls, and one with probands from PARLU (see Table). After this screening in additional cohorts and look-up of mean allele frequencies (MAFs) in two databases on variant frequency: the Genome Aggregation Database – gnomAD (https://gnomad.broadinstitute.org) and SweGene (213), with 129000 and 2000

alleles respectively as well as literature search of the affected genes, two top candidates were identified from the list. The genotypes for the top-candidate variants were then determined in all individuals with DNA using Sanger Sequencing and/or TaqMan genotypic assay analysis. We also performed gene burden analyses for these genes.

Gene burden analyses (paper IV)

The two cohorts from Mayo clinic (case and control) were used to perform the gene burden analyses by examining all variants in the two top-candidate genes, *PGLYRP2* (peptidoglycan recognition protein 2) and *RUNDC3B* (RUN domain-containing protein 3B), that were non-synonymous and had a combined annotation dependent depletion (CADD PHRED) score above 15 (https://cadd.gs.washington.edu). CADD PHRED is a score that predicts deleteriousness of point mutations by combining multiple aspects such as evolutional conservation and simulations of mutation effects. A higher score means higher probability of deleterious effects on the protein.

Frequencies of candidate variants in databases and cohorts (paper IV)

The Mayo Clinic biobank control cohort was searched for individuals carrying either of the 9 identified genetic variants found in the work in paper IV. This cohort consists of 885 individuals of Caucasian descent without history of neurological disease. Mean age in this cohort was 57 ($\pm$15) years and male to female ratio was 49.5% to 50.5%.

The genetic variants were also screened for in two separate familial case cohorts consisting of probands that had PD and also a family history of PD. The Mayo Clinic Florida familial PD cohort consists of 776 individuals with 60.1% males and 39.9% females. The mean age at onset was 62 ($\pm$12) years. Sixty-seven individuals with WES data from the PARLU cohort constituted the other case cohort. The mean age at onset of these individuals of PARLU was 58.2 years, 32.8% were female and 64 of 67 (95.5%) were from the familial history subpart of the cohort.

Systematic review (paper III)

A systematic review of affected individuals with MAPt p.(R406W) was performed by searching pubmed for relevant phrases relating to the causative *MAPT* mutation. These included 18 searches, combining one of three different words for the gene (tau, *MAPT* and microtubule associated protein tau) with one of six different numbers for describing the specific mutation (1216, 1216C>T, 1216C-T, Arg406Trp, R406W and 406). Studies were selected after searching titles and/or abstracts. Included studies were read in full-text and data on demographics, clinical symptoms, CSF markers, neuropathological and radiological findings were compiled on family and individual level (see paper III tables 2, 3 and S2 and S3).

Individuals were identified from descriptions and/or pedigrees to enable compilation of data when there were several studies on the same family. There was also unpublished data compiled after personal communication with colleagues (see table 3 in paper III).

Family studies – Results

Paper III

The results in this paper consist of a description of a new Swedish kindred with the *MAPT* c.1216C>T; p.Arg406Trp (MAPT p.(R406W)) mutation and a systematic review of 66 cases with this mutation described in the literature.

Clinical features in the Swedish family

The median age of disease onset in the family was 55 IQR 51.3–61,8 for individuals in generations III and IV. Median disease duration of the individuals in the family who were deceased but not prematurely diseased due to cancer was 17 IQR 14–26 years. The clinical pictures in the five individuals with available detailed descriptions from onset (III-3, III-4, III-6, IV-9 and IV-16), consisted of a relative dominance of memory impairment early in the disease, such as loss of working memory for three of these individuals. There was one individual with prosopagnosia and another that developed visuospatial difficulties. One patient expressed early personality change with social withdrawal without AD-like feature at onset. Except for this individual, clear behavioural changes were not usually eminent until 5–11 years of disease duration, but eventually emotional liability, aggression or irritability developed in a total of five affected individuals, and another was noted as disinhibited. Language was at some point affected in four individuals and all affected individuals in generation III and IV developed loss of disease-awareness at some point, which included confabulations in three individuals. There were parkinsonian signs in two individuals, but these were noted very late in the disease course at 25 and 24 years after symptom onset for individuals III-4 and -6, respectively. There were also descriptions of individuals in generation II as easily irritated or even aggressive. There might have been some instances of hyperorality as there were descriptions or suspicions of abuse of alcohol or pain killers in five individuals in the family. Individuals III-3, -4, -5 and -6 moved to nursing home after 20, 11, 14 and 24 years, respectively.
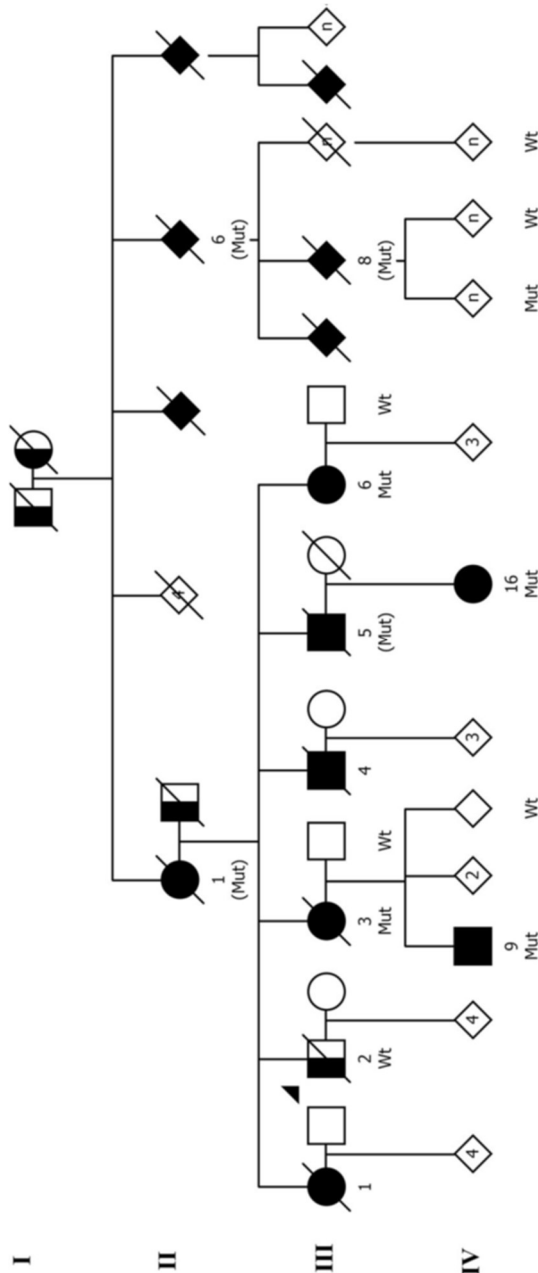
Figure showing pedigree of the Swedish family described in paper III
Fully filled shapes represent development of disease before 71 years of age and semi-filled shapes represent onset of cognitive symptoms after. Males represented as squares, females as circles. Diamond represent that sex was disguised and n that number of individuals were unspecified, both due to confidentiallity reasons. P denotes three neuropathologically examined members. **Mut**, MAPT p.(R406W) detected by genetic testing; **(Mut)**, MAPT p.(R406W) carrier status transposed via genotype of the individual's children; **Wt**: wild type, MAPT p.(R406W) excluded by genetic testing. From paper III (214)

The MAPT p.(R406W) mutation was found in WES of the affected individuals III-3, III-6, and IV-9. The mutation was then confirmed in one additional affected family member and one asymptomatic carrier, imposing that two affected individuals were most likely carriers of this genetic variant (denoted by (mut), see Figure). The proband (individual III-2) was found not to carry the MAPT p.(R406W) mutation. This individual had age at onset several years later than in the family members with the mutation (75 years) and his clinical diagnosis of AD was considered correct.

Possible treatment effects

There was a possible treatment effect of cholinesterase inhibitor in individual III-6 who were reported to regularly use the medication for 17 years. A combination with memantine was attempted but was discontinued shortly thereafter, possibly due to side-effects. When this patient also ceased to medicate with the cholinesterase inhibitor a few years later, she rapidly declined in cognitive functioning. The medication was re-established but the decline in cognitive functioning persisted.
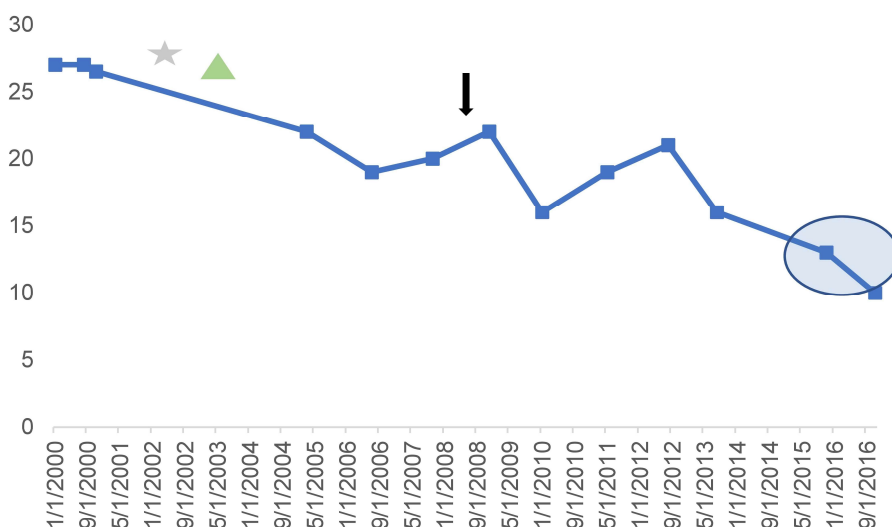
Figure showing repeated MMSE for patient III-6 during 17 years
Start of medication with acethylcholinesterase-inhibitor donepezil 5mg per day marked with gray star. Increased dose to 10 mg a day marked by green triangle. Black arrow showing 3 weeks trial with memantine. Circle corresponds to ceasation of treatment including attempts to re-establish treatment. Dates are manipulated for confidentiallity reasons.

Radiological features

Radiological examination in affected family members revealed atrophy concentrated to the ventral and medial parts of the temporal lobe, eminent from a

widened collateral sulcus in all patients and with additions of atrophy of the parahippocampal gyrus in three patients in the family. There was one individual with only temporal atrophy but all the others had additions of primarily frontal and/or parietal atrophy. Hippocampus was found atrophic in one family member.

Neuropathological features

The neuropathological pictures of three individuals from the family were coherent with PSP. Immunohistochemical staining revealed a dominance of 4R tau isoforms, albeit 3R tau was also present in all three (see Figure 3 in paper III).

Individual III-1 had died from adenocarcinoma only two years after onset of memory problems and the neuropathological examination of this individual was performed on sparse material that had been archived for more than 20 years. There was nonetheless neurodegeneration in the midbrain and the neocortex with abundant tau pathology, predominantly of the 4R isoform.

Individuals III-3 and -6 had both neuronal degeneration in the frontal lobes but also more severe degeneration in the temporal lobes with marked hippocampal atrophy. The third and fourth ventricle was expanded due to atrophy of the surrounding areas, including central nuclei and brainstem. Affection was particularly obvious in the basal and/or medial parts of the temporal lobe, including the enthorinal region and parahippocampal gyrus. Tau pathology was marked in the regions with degeneration with NFTs and white-matter affection. Tau pathology was especially marked in the thalamus, insular cortex and subthalamic nucleus of individual III-3. The amygdala, basal ganglia and nucleus coeruleus in the brainstem were also markedly degenerated in these patients.

Glial affection was also present in all three individuals examined. Besides from tufted astrocytes found in all three individuals, III-3 also showed glial plaques and III-6 showed glial tau affection in the white matter. Interestingly, there were co-pathological additions in both individuals III-3 and III-6 with concomitant TDP-34 pathology and hippocampal LBs, respectively.
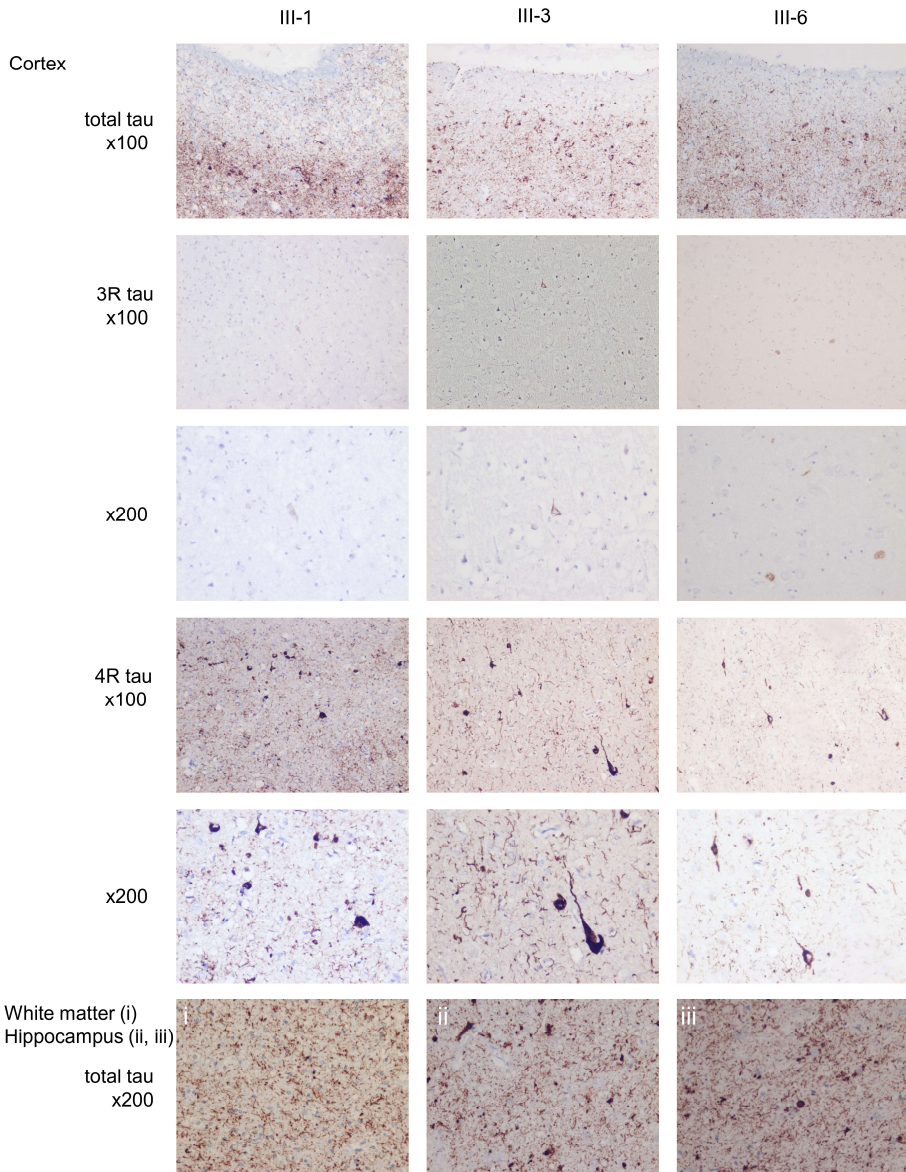
Figure illustrating neuropathological findings of three individuals from the family in paper III
Photomicrographs from brains of three family members. Individuals,immunohistochemistry, enlargements, and locales as indicated. The three patients had abundant tau pathology in the cortex and white matter, with a marked predominance of four-repeat (exon 10+) tau isoform immunoreactivity. 3R tau Three microtubule binding repeat (exon 10−) tau isoform. 4R tau Four microtubule binding repeat (exon 10+) tau isoform. From paper III (214)

Systematic review

Clinical descriptions

The systematic review part of paper III identified 66 patients with the MAPT p.(R406W) mutation previously reported in the literature; 63 heterozygote and three homozygote cases. The median age at onset for MAPT p.(R406W) heterozygotes was 56 IQR 54–60 years and median duration was 13 IQR 12–21 years. Consistent with the findings in the family, memory impairment was the most prominent clinical symptom followed by behavioural changes and language impairment. Nine patients with heterozygote mutation (14%) were reported to have developed parkinsonism.

Radiological descriptions

In the literature, temporal atrophy was reported in all but three of the 23 MAPT p.(R406W) patients with their radiological pictures described, with additions of frontal atrophy in four and parietal atrophy in one patient. The temporal atrophy was commonly described as medial and/or anterior but the parahippocampal gyrus was only described in one patient in the literature, specified as atrophied in that patient. There was hippocampal atrophy in 9 patients.

Neuropathological descriptions

There were 9 additional MAPT p.(R406W) cases with neuropathology described in the literature. The neuropathological diagnoses varied but one other study found PSP to be the correct interpretation, two others found mostly NFTs without clear signs corresponding to PSP, summarizing the picture as NFT-dementia and "features of PSP/CBD/NFT-dementia". All studies had found both 3R and 4R tau but three studies found more abundant 3R tau than 4R tau (215-218).

Paper IV

The main results of this paper consist of a detailed description of a new Swedish kindred with unknown cause of inherited PD and a synthetised list of candidate genetic causes. We describe the clinical picture of four affected individuals that were directly examined, of whom two were neuropathologically confirmed as PD. One other family member in generation III had also developed PD but was not examined. Synthetisation of the genetic results was presented as a list of rare genetic mutations, of which two were highlighted as top-candidates to be disease-causative.

Clinical phenotype

After 14 years of continuous follow-up of the family, the clinical subtype had solidified as that of typical PD, as in similar to sporadic PD. There was, however, an unusual high amount of cognitive decline in affected individuals (III-1, -2, -5, -6, and -19) and also other individuals with cognitive decline in the family (see Figure).

Motor symptoms

Onset of symptoms in the four individuals with PD who were examined were at mean (range) 61.8 (56–67) years of age. All had good effect of levodopa treatment and eventually experienced ceasing medicine effect at end of dose interval after 8.8 years (3–12). All four cousins experienced dystonia and two individuals developed symptoms of PISA-syndrome or camptocormia. Three of the individuals developed dyskinesias and two eventually had DBS surgery. All four affected family members had resting tremor at some stage, but one developed mild tremor and one did not have tremor until late in the disease course. Other supportive symptoms suggesting that the clinical phenotype was caused by spread of α-synuclein, were that three of the individuals had vivid dreams which included clear symptoms of RBD in two, and that all four developed orthostatic hypotension. All individuals had difficulties with wakeups or insomnia and three of them experienced excessive daytime sleepiness. Individual III-19 was not examined directly in paper IV but moved to nursing home and was noted to have tottering gait and bilateral rest tremor according to medical records and was thus considered affected.

Cognitive symptoms in clearly affected individuals

All members in generation III who were considered affected by PD also developed cognitive decline considered to be of dementia dignity. This level of severeness was, however, not formally diagnosed in III-6, who had clinical diagnosis of MCI but the cognitive functioning of this individual deteriorated shortly before dying. All individuals developed confusions and for individuals III-19 and III-5 fluctuating cognitive symptoms were a large part of the symptomology. Hallucinations developed in all affected individuals in generation III, including III-19 (not directly examined), and where in the four examined cousins reported after mean of 12.5 (10–15) years of disease duration.

Cognitive symptoms in other individuals in the family

Three individuals in the family had had some kind of traumatic brain injury or intracranial haemorrhage (III-12, -13 and -18). III-12 had isolated problems with word-finding and/or fluency that appeared after intracranial haemorrhage some decades before examination. He was otherwise completely unaffected, and his genetic status was therefore used in the filtering of genetic variants. III-18 had brain

injuries at birth and was partially hemiparetic and had neglect but showed no signs of parkinsonism at examination.

Individuals, III-3 and III-13 showed mild signs of bradykinesia or uncertainties at motor examination and were therefor difficult to determine as affected or unaffected. They both developed dementia, III-3 AD and III-13 dementia not otherwise specified. Individual III-15 had similar ambiguities in motor findings but developed no cognitive nor any motor symptoms during repeated telephone follow-up. III-10 had a clinical diagnosis of AD and had no apparent motor dysfunction when examined. She was therefore primarily considered unaffected, although clearly affected by another neurodegenerative disease which we considered was sporadic AD.

Figure showing pedigree of the Swedish family in paper IV
Males represented as squares, females as circles. Diamond represent that sex was disguised and n that number of individuals were unspecified, both due to confidentiallity reasons.

Genetic results

Initial genetic analyses identified 9 filtered variants (see Table) after 5 variants in *MUC4* and *MUC6* was disregarded, since the three mutations in *MUC4* were represent an indel and *MUC6* has a known polygenic nature, with many different variations reported to induce false-positive WES results and been proposed to be black-listed from genetic analyses (219). WGS analysis of individual III-2 did not not find any copy number variants in the regions of interest nor any genetic variant previously associated with PD.

Chromosome	Gene names	HGVS p.	American cohort, WES (n=776)	American controls WGS (n=885)	PARLU cohort WES (n=67)	European Non-Finnish MAF (129000 alleles)	SweGene MAF (2000 alleles)
7	MDH2	p.Val139Ile	**1**	**9**	**2**	**0.00458**	**0.01300**
7	PTPN12	p.Ser684Leu	0	0	0	0.00088	0.00750
7	RUNDC3B	p.Asp315Asn	1	0	0	0.00008	0
7	CASD1	p.Ala2Gly	0	0	0	0.00002	0
7	DLX6	p.Gln54Pro	0	0	0	0.00019	0
15	CGNL1	p.His322Asp	**2**	**3**	**0**	**0.00149**	**0.00300**
19	C19orf57	p.Asp630Glu	**6**	**15**	**0**	**0.00917**	**0.00950**
19	PGLYRP2	p.Pro263Leu	0	0	0	0.00002	0
19	F2RL3	p.Val351Leu	0	0	0	0.00031	0.00100

Top genetic candidates

Three of the 9 genetic variants after initial filtering were identified in healthy carriers in the Mayo Clinic biobank control cohort. The MAFs of the variants in *MDH2, CGNL1* and *C19orf57* were 1.02%, 0.34% 1.69%, respectively which was considered too high for a cause of a very rare disease.

Of the remaining six variants, two top-candidate genetic variants were then identified:

I) The *PGLYRP2* c.788C>T p.(Pro263Leu) rs773808477 (PGLYRP2 p.(P263L)) variant was selected after literature search, which showed that this gene and its gene-family had previously been associated with PD in humans.

II) The *RUNDC3B* c.943G>A p.(Asp315Asn) rs760764597 (RUNDC3B p.(D315N)) variant was not found in the controls but in one PD proband from the Mayo Clinic Florida familial PD cohort. This proband had a family history of PD from his grandmother.

Co-segregation of disease

Since the genetic variants were filtered on the four affected cousins and one unaffected individual (III-12) the genetic variants thus co-segregated with the affection status of these individuals. In the other family-members, however, the co-segregation of disease was sub-optimal for the two top-candidate genetic variants. One individual, with no signs of parkinsonism nor cognitive decline on examination, and one individual with clinical diagnosis of AD carried the RUNDC3B p.(D315N) variant. The PGLYRP2 p.(P263L) variant had several carriers, at least two were completely unaffected on examination. However, all three individuals with ambiguities in motor-findings (III-3, -13, -15) had this variant.

Family studies – Discussion

Unique features of MAPT p.(R406W) patients (paper III)

Clinical picture

The clinical picture in the family studied and in MAPT p.(R406W) patients reported previously in the literature, can be summarized as a slowly processing dementia with memory impairment as the most commonly reported symptom. Thus, the (initial) clinical picture was in many ways similar to AD, although FTD symptoms were developed later for most patients. AD was also the most commonly reported clinical diagnosis in the literature and in the Swedish kindred.

One individual was a phenocopy

The similarity to AD was also demonstrated in that individual III-2 was a phenocopy. He was diagnosed with AD and did not have the mutation found in the other family-members, but rather developed more typical disease progression of AD. This should be considered a typical case of a phenocopy, as in an individual without the mutation but with similar/the same phenotype as individuals with a disease-causing mutation. Interestingly, there have been observations that phenocopies are relatively often apparent in families with monogenetic disease-causing variants, which could imply that shared factors such as similar genetic background and/or environmental exposure can lead to increased risk of several different neurodegenerative disorders (220). The fact that the initial genetic investigation in the family was performed on this individual complicated the picture and postponed the identification of the causative gene in the family by decades. The increased level of complexity inferred by phenocopies highlights that genetic investigations should involve a large number of individuals of the affected family and needs to be unbiased and consider that anyone in a family might be a phenocopy, even the proband.

Similarities to another genetic tauopathy

Interestingly, there has recently been another mutation found which substitute the amino acid on 406th position of tau and causes a similar clinical picture, the MAPT

p.(R406G). The individual reported with this genetic variant also showed marked memory loss and inferior temporal atrophy, although the disease onset at 63 years was slightly later than most patients in the kindred of paper III (221). This individual also developed behavioural changes and preference for sweet food without insight of his own disease, similar to several patients described within the systematic review of MAPT p.(R406W). The findings of several mutations at this amino-acid-position and that mice with MAPT p.(R406W) also develop neurodegenerative disease suggests this section of the tau protein to be intricately involved in pathogenetic mechanisms of tauopathies (222).

Ventromedial temporal lobe atrophy was a marked feature

Atrophy in the ventromedial temporal lobe was shown as a prominent radiological sign with widened collateral sulci of all the affected family members. This observation differed somewhat from the medial temporal atrophy ratings used in normal practice, which focuses more purely on hippocampal atrophy as commonly observed in AD pathology (223).

Affection of the entorhinal area was also confirmed in the neuropathologically examined patients III-3 and -6, where the collateral sulcus was prominently widened and parahippocampal area was markedly degenerated. As the entorhinal region is where NFT pathology is detectable also in healthy individuals in the entity PART. and very early in AD, we suggested that this region is the starting point of tau pathology in several different tauopathies, and a hot-spot for further mechanistic and imaging studies (134, 218). PART is a state where tau accumulates in this area without other neurodegenerative disease features, which leads one to speculate that this is an area prone to tau aggregation which can be harmless unless certain pathophysiological mechanisms are present to modify the process.

Furthermore, we note that the findings of tau pathology in healthy individuals of PART constitute a parallel between AD and PD, since also incidental LB pathology have been described in different regions of healthy brain, including the olfactory bulb, brainstem/medulla oblongata and the amygdala (224). This findings could perhaps be considered a variant of the synucleinopathies not leading to clinical disease, similar to how PART might be related to tauopaties. Continued research on these hotspots for neurodegenerative processes in AD, PD/DLB and some FTDs, might untangle some of the age-related susceptibility to neurodegenerative diseases and substantiate the work of *authentic subtypes* of these disorders.

Neuropathological picture of PSP with predominantly 4R tau

Tau isoform ratios

Expression of both 4R and 3R tau in patients with the MAPT p.(R406W) mutation was in agreement between the Swedish kindred and 6 other families with neuropathological reports on tau isoforms. This finding is expected given the position of the mutation on exon 13 of *MAPT* is not considered involved in the alternative splicing of exon 10 (215-217, 225, 226) (and one additional family from Mayo Clinic, see paper III). However, 4R tau was clearly more marked than 3R tau in the three Swedish brains which contrasted with previous literature, where three different families had been reported with the opposite, 3R more prominent that 4R tau (215-217). The reason for this discrepancy is unknown but three hypotheses are suggested here.

1) The genetic background of patients could affect tau isoform expression and/or tau deposition into NFTs and thereby the relative abundance of the tau isoforms. We examined *MAPT* haplotypes in the Swedish individuals without finding an explanation, which suggests other factors to be responsible (H1/H2 haplotypes found in individuals III-3, III-6 and IV-9, see Supplementary Table 1 from paper III).

2) There could be a maturation process of the NFTs in the patients. There have been reports of a continuum between 4R as a major inclusion of pretangles and 3R tau to be increasingly abundant in fully formed NFTs and ghost tangles (227). That research was foremost performed on AD brains but it was noted that there could potentially be disparities between tau fibril ultrastructure and isoform composition between different tauopathies.

3) Differences in methodology could be responsible for the diverging observations. Albeit we used the same 3R tau antibody (RB3) as the authors who found stronger 3R staining, we used a different 4R tau antibody, TIP-4RT-P01 instead of an RB4 antibody previously used (215-217). Differences in antibody binding to the R406W-mutated tau could thus theoretically be responsible for the isoform-ratio discrepancies. The dissimilar binding capacities could in turn be affected by different post-translational manipulations of the tau protein, such as glycosylation or factors affecting cell membrane binding affinity, and could be directly or indirectly modified by the MAPT p.(R406W) mutation. For instance, antibody affinity could be influenced by the changed form of the protein due to the amino acid exchange and/or change in amount or pattern of tau phosphorylation.

Diverging neuropathology in MAPT p.(R406W) patients

There were ambiguities in the pathological diagnoses reported for MAPT p.(R406W) patients in the litterature. Our neuropathological examinations established a diagnosis of PSP in these patients based on the distribution and qualities of tau pathology in neurons and glial cells (i.e. tufted astrocytes). This finding was fully consistent only with the results of one study in previous literature (225), although one other study reported features of CBD as well as PSP (228). Interestingly, the nature of the astrocytic changes that are a part of the distinction between PSP and CBD has been reported to be modified by tau phosphorylation and other post-translational changes to the protein. Effects of tau phosphorylation could be involved in the discrepancies in the reports, since the p.(R406W) mutation is situated close to the amino acid position 404, a major and early site of abnormal tau phosphorylation (229). Varying propensities of tau such as phosphorylation patterns, might in theory give rise to slightly different pathological changes. It has been reported that some *MAPT* mutations lead to specific appearance of astrocytes, similar to, but with slight characteristic differences from, tufted astrocytes (230). Such pathological *mimicry* could increase the variation in diagnoses between examiners without there being large disparities in pathological pictures.

New potential causes of PD (paper IV)

We identified 9 different gene-variants as potential candidates for causing the disease in the described family. Among these we highlighted *RUNDC3B* and *PGLYRP2* as top-candidate genes for hereditary PD, based on new and previously established connections to PD, respectively.

PGLYRP2 gene variant as potential cause

The *PGLYRP2* gene had previously been associated with PD in humans in the context of several SNPs in the gene increasing the frequency of PD in two different cohorts (231, 232). Also, *PGLYRP2* knock-out mice were found to have changed motor-behaviour and increased anxiety as well as α-synucleinopathy in the frontal areas of female mice brains (233, 234). The *PGLYRP2* gene codes a N-acetylmuramoyl-L-alanine amidase protein (PGLYRP2 or PGRP-L) that cleaves bacterial peptidoglycans.

The main theory of how the *PGLYRP2* gene could be associated with risk of developing PD could be connected to the well-established main function of the *PGLYRP* gene family, namely, to cleave bacterial cell wall components. Dysfunction of the PGLYRP2 protein could thus lead to imbalance in bacterial gut flora and subsequent pathological induction such as abnormal inflammatory

response. Interestingly, bacterial overgrowth in the small intestines have been observed in PD patients, a finding giving some slight support to this hypothesis (235). An abnormal constitution of bacteria *per se* could thus potentially be associated with PD, but there are also several associations made between PD and bowel wall inflammation, both on its own and in combination with bacterial invasion. Some associations are described here:

❖ PD patients have in a small study been shown to have more enteric inflammation than controls (236).

❖ Inflammation of the bowel wall has been shown to increase α-synuclein accumulation in animal models (237, 238).

❖ Risk of PD is increased in IBD-patients (239, 240).

❖ IBD-associated gene-variants have been shown to increase PD-risk (241).

❖ Some PD risk variants are *vice versa* associated with the IBD, Crohn's disease (242). *PGLYRP2* variants have also been associated with Chron's disease (243).

❖ Leakiness of gut epithelium, increase of bacterial invasion and markers of inflammation and/or endotoxin damage was found in PD-patients (244), and also found to colocalize with α-synuclein pathology (245).

❖ In mice, knock-out of any of the four *PGLYRP*-genes have been shown to increase sensitivity to epithelial injury and inclination to develop IBD. Furthermore, *PGLYRP2* and *PGLYRP3* knock-out mice had the largest change in microbiota and highest cell activating capacity in that study (246). The study on human PGLYRP SNPs also suggested that change in gut microbiota and leakiness of the colon mucosa constitute common mechanisms between IBD and PD (231).

Interestingly, IBD is hypothesized to increase risk of PD through upregulation of the LRRK2 protein (242), the gene harbouring the most common cause of monogenetic PD, LRRK2 p.(G2019S) (64). LRRK2's effects on bowel wall inflammation are thought to be mediated through nucleotide-binding oligomerization domain 2 (NOD2), which binds specific sites on bacterial proteoglycans. Interestingly, PGLYRP2 cleavage of proteoglycans destroys this binding site, suggesting that a lack of function of PGLYRP2 could induce bowel wall inflammation through increased NOD2 signalling, as in *LRRK2*-associated PD. *NOD2* was also established as a new PD candidate in a recent Genome-wide association study (53).

It should be noted that this proposed mode of PGLYRP2-associated pathogenicity is generally in line with the brain-gut hypothesis of PD, described in the introduction. The gene was found to be mostly expressed in the liver of adults, which fits with the known functions indicated above (247). However, the protein was also

found to be expressed during fetal development in mice brains in one study (233). An alternative theory could thus be that the PGLYRP2 protein have direct implications in brain-development that could facilitate the start of PD pathology many years later, or impose a vulnerability for other genetic and/or environmental factors that causes PD. There could also be different functions of the PGLYRP2 protein in adults and during fetal development that both add to PD development.

RUNDC3B gene variant as potential cause

The *RUNDC3B* gene is located on the short arm of chromosome 7. Its exons overlap with the gene ATP-binding cassette -B1 (*ABCB1*). The functions of the RUNDC3B protein is relatively unknown, but it is reported to be similar to the protein coded by *RUNDC3A*, which has been shown to interact with a protein of the Ras superfamily, replication associated protein 2, which has functions in regulating differentiation and motility of cells (248). *RUNDC3B* methylation has been associated to different forms of cancers, predominantly lymphoid malignancies but also lung and breast cancer and was found to have a methylated promoter involved in the circadian clock of cells (248, 249).

RUNDC3B share several exons with the *ABCB1* gene (https://genome.ucsc.edu). This gene encodes a protein (multi-drug resistance 1; MDR1) that is an integral part of the blood-brain barrier and functions as a pump for xenobiotics and cytokines in the brain (250). Mutations in *ABCB1* were reported to lead to recurrent encephalopathy (251). Disrupted functioning of this protein was in one study associated with a 100-fold increase of concentrations of the neurotoxic pesticide ivermectin (250). Any direct affection of the RUNDC3B p.(D315N) mutation on the expression or functioning of MDR1 was not examined in this thesis. Since the RUNDC3B p.(D315N) mutation is 70 kbp from the *ABCB1* gene, a direct affection is not very probable, but the variant could in theory mark inheritance of a haplotype or a larger part of the chromosome that could be associated with functioning of the MDR1. This is an untested link to PD but it is mentioned here since it explains a potential way for mutations in the region to affect PD risk via environmental toxins.

Other variants as potential cause

Variants not identified in the control group
Of the 9 candidates remaining after filtering of the genetic results, 6 were not identified in controls. Although *PGLYRP2* was previously associated with increased risk of PD, the identified CASD1 p.(A2G) variant had similar rarity in GnomAD as the PGLYRP2 p.(P263L) variant (0.002%). These two variants showed the lowest MAF in GnomAD of the 9 variants on the list. The other top candidate RUNDC3B p.(D315N) was almost as rarely reported in GnomAD with 0.008% MAF. The other

three variants (PTPN12 p.(S684L), DLX6 p.(Q54P) and F2RL3 p.(V351L)), albeit still rare variants, were at least 10 times as commonly reported in GnomAD.

Variants identified in the control group

Although not commonly reported in the GnomAD, three genetic variants (MDH2 p.(V139I), CGNL1 p.(H322D) and C19orf57 p.(D630E)) showed the highest MAFs of the nine filtered variants reported in gnomAD and was also found in individuals of the control group. The MAFs found in the control group were, furthermore, about two times as high than what was reported in GnomAD, and the findings were interpreted as clearly rebutting a causative effect of these genetic variants.

Genotypes did not fully co-segregate with PD

Reduced penetrance

Co-segregation with disease in paper IV was not in line with a high penetration of the proposed genetic causes, as individuals without PD was found to carry the top-candidate genetic variants. Another study on several families with marked family history of PD also found genetic variants, proposed to be associated with PD, to be present in both affected and unaffected individuals and for some candidates also identified in unrelated PD cases, similar to our findings of RUNDC3B p.(D315N) (252). A reduced penetrance of causative genes, such as found with the LRRK p.(G2019S) variant, could explain the distribution of genotype and phenotypes in these families and the family studied in paper IV (253, 254).

Oligo- or polygenetic cause

As stated in the introduction, monogenetic causes of PD have been established in relatively low percentage of cases with family history. Large studies have instead identified genetic linkage to other regions, not found to hold monogenetic causes, and poly or oligogenetic inheritance of low-risk PD genes could explain some of the missing inheritance of PD (113, 115). There are, however, no well-established oligogenetic combinations of risk-alleles that have shown to infer a risk for PD while their monogenetic genotypes don't. However, the affected individuals studied in paper IV all had the identified genetic variants listed as potential candidates in combination and contributory actions of some or several of these genes remains a possibility. The identification of one asymptomatic carrier with both the PGLYRP2 p.(P263L) and RUNDC3B p.(D315N) variants, is, however, indicative that the combination of exactly the two highlighted variants might not result in clinical disease. Even though the aim in studying this family was to find a direct causing gene, and despite that simulated impact of the genes were large (CADD PHRED scores > 15), we might have found candidate genetic variants with somewhat lower impact, that may hence need other risk-factors to lead to symptomatic disease, such

as combinations of environmental exposure and/or a genetic background of low-risk variants.

Environmental cause

Clinical symptoms coherent with an environmental cause

As mentioned in the introduction, development of PD has been associated with pesticide exposure and rural living / own-drilled wells as water supply. This association and the considered propagation of pathology starting from the dorsal motor nucleus of vagus in a large proportion of patients with PD, has inspired hypotheses of PD pathogenesis such as the brain-gut hypothesis and the brain first vs body first PD hypothesis. The latter, more nuances hypothesis implies environmental factors to be more important in some PD-patients and perhaps not a relevant risk-factor in others (3, 29, 87). Three of the affected individuals in paper IV had symptoms that could indicate RBD and none had reports on tremor before other motor-symptoms. This could fit with early brain stem pathology as suggested in the body-first PD, implying a peripheral initiation of pathology, which then spread to the brainstem and leads to clinical symptoms associated with this brain area.

Furthermore, at least 15 of the 17 individuals examined in the family were either raised at relatively rural conditions, with drilled wells as water supply, or had farming as occupation, in parts of Sweden where fruit farms are relatively common. All five of the clearly affected individuals in the family were either farmers or raised in rural conditions with drilled wells for water-supply. Although interactions between environmental and genetic factors are generally not well-characterized in PD, there has been such reports, as in a small study indicating a clear gene-environmental interaction of mutations affecting the liver enzyme CYP2D6 (255). Individuals in that study who had the risk genotype were not at increased risk for developing PD if not exposed to pesticides. Gene-environmental interactions could theoretically be relevant for this family and explain some of the missing co-segregation of disease with the top-candidate genotypes.

Possible links to environmental factors was discussed above for the RUNDC3B p.(D315N) –a theoretical impact on the nested gene *ABCB1*– and the PGLYRP2 p.(P263L) –impact on gut microbiota and inflammation–. An unexplored potential pathogenic mechanism was also found for the variant in the Cingulin-like 1 gene, *CGNL1* c.964C>G p.His322Asp rs112079075 variant (CGNL1 p.(H322D)). This genetic variant could potentially be associated with increased toxic exposure and/or gut leakiness since it encodes the protein paracingulin, which has a major role in the maturation of primordial adherent junctions to tight junctions in epithelial cells (256). The epithelium of mucosal linings in the bowel or olfactory bulb is related to early accumulation of α-synuclein pathology, often many years before clinical

diagnosis (257). This gene was also found to be expressed in the brain including subthalamic nuclei, suggesting other functions of the protein as well (258). Although disregarded due to presence of the variant in controls, the MAF in GnomAD was somewhat lower than the other disregarded variants and was discovered in a similar rate between controls and PD probands (3 vs 2), whereas the other two disregarded variants were more common in controls than individuals with PD. Since there are no data on exposure for environmental risk factors for individuals in gnomAD, a gene-environmental interaction also for this variant could not be excluded by our findings.
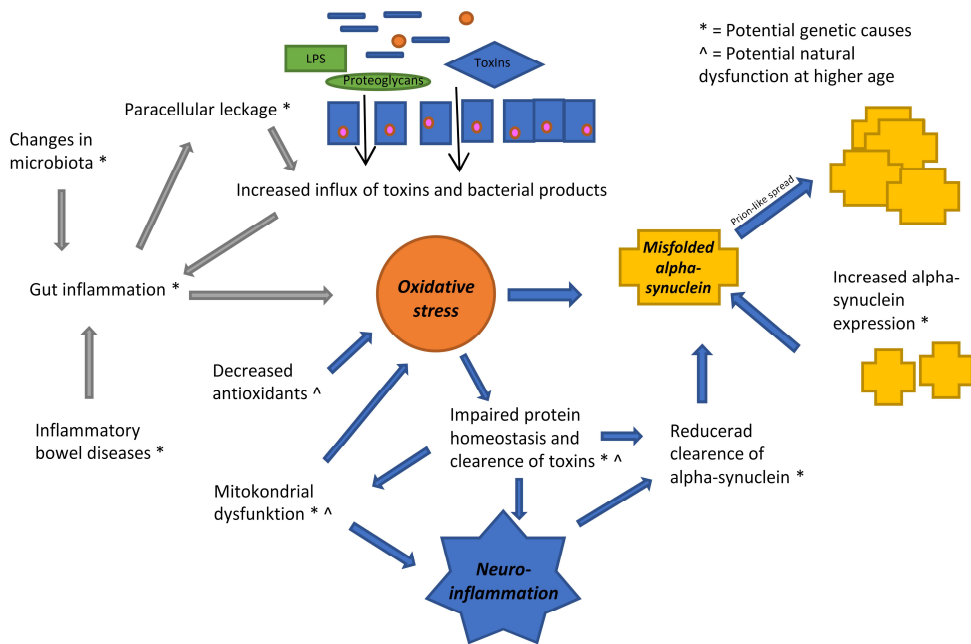

Figure compiling some pathogenic mechanisms mentioned in this thesis

General considerations (paper III and IV)

Identifying rare diseases correctly

The ongoing expansion of known genetic factors

Genetics is a rapidly advancing research field and the number of genetic variants associated with clinical diseases or subtypes of diseases is constantly expanding. As instanced in the clinical examination of the family studied in paper III, a causative mutation might not be known at the time of clinical genetic examination but established later. The MAPT p.(R406W) mutation was one of the first described mutations in the *MAPT* gene, but it still passed more than 10 years after the disease in the family was known to physicians in Lund until the MAPT p.(R406W) mutation was discovered (259). Digital records of WES data are an important mean to keep up with the nowadays rapidly expanding discoveries of new mutations, as logistic infrastructures now enable search through existing clinical WES results when new mutations with similar disease-phenotypes are found. Such methods are now being implemented into clinical practice, leading to an optimization of the otherwise resource-consuming process of genetic screening tests (49). If WES had been performed as a clinical analysis earlier, the delay in identifying the genetic variant causing the disease in the family in paper III likely would have been avoided. There are, however, important aspects of data security to keep in mind since genetic information is both comprehensive and sensitive. Handling genetic information is specially regulated in the European Union, including Sweden, since WES-data can not only inform of ones known diseases, it can also inform on several personal traits not mentioned in medical records and include risk factors or disease-causing factors both known today and identified in the future.

A change in nomenclature

Many patients with MAPT p.(R406W) in both the Swedish family and reviewed in the literature had a diagnosis of AD, suggesting that much data on clinical progression in R406W-associated disease was unfortunately not accessible for clinicians, patients, and next-of-kin, due to misdiagnosis. Increased information about the disease imposed by this genetic variant might increase the probability of correct identification of carriers. Neurodegenerative taxonomy can, however, be complicated. There was an ongoing change of terminology at the time of publication of this study. The proposal was regarding a name change of the clinical disease, from FTDP-17 to familial FTLD-tau, put forward shortly after paper III was published (123).

We added some thoughts about the MAPT p.(R406W) mutation and the resemblance of genetical factors to clinical disease in a letter to the editor (124). The main points made were that updated classifications based on advancing knowledge

is logical, and could thus increase correct taxonomy used by physicians, but that it is nevertheless important to remember that the classification groups are in some way arbitrary until the knowledge is increased in the future. We should thus be vigilant not to adhere too much similarity between similar but in part different potential sub-diseases when grouping them together as the causative mechanisms are most likely not understood well enough today to enable correct classification, which should in the future optimally be based on cause and not only on symptoms. Future changes in nomenclature enabling further disease classifications when mechanistic pathways are clearer, should thus be encouraged.

Methodological considerations

When suggesting new candidates for disease causing mutations, as in paper IV, connections to disease must be verified. There are several ways to investigate if a mutation is potentially pathogenic and preferably, several of these modes would inarguably speak in favor of pathogenicity for a mutation to be considered disease-causing, apart from crucial replication by separate observations. Here follow some key points.

Filtering based on rarity in healthy subjects

One of the key elements in genetic research is to establish that a mutation found in individuals with supposed monogenetic disease is extremely rare in healthy controls. Normally large datasets available online are used nowadays, such as gnomAD used in paper IV.

Filtering based on prediction software

There are several types of genetic prediction software evaluating whether the protein corresponding to a given mutation would likely be affected and to what degree. Most prediction software is based on a combination of several datasets and aspects of the protein such as evolutional conservation of the amino acids encoded, 3-demensional structure, databases on earlier experiments or specific types of predictions or simulations. Examples are many but include POLYPHEN-2 and SIFT. Another one is CADD score, which was the mainly used prediction tool in paper IV. This score include a raw and a PHRED variant, were the PHRED is a broader reference for all available nucleotides at the position, and the raw score is more directly angled towards deleteriousness.

Filtering based on tissues expressing the gene

Another mode to dictate probability of mutations to lead to disease is in what tissues the encoded protein is normally expressed. However, if based on an incomplete picture of pathogenesis this method can be less applicable. In other words, since much is unknown about the pathogenicity in PD, a genetic reason for PD-associated neurodegeneration might or might not be considered inevitably associated with protein expression in the brain. Indeed, the definition of PD has been expanded from a motor-only disorder affecting limited brain circuits, to a very broad clinical disease that could hypothetically be of systemic nature. Genetic associations to genes expressed in the gastrointestinal tract or affecting uric acid levels are examples that would perhaps have been considered not relevant for PD some decades ago but are relevant suggestions today.

Filtering based on previous literature

The *PGLYRP2* gene identified in the family of paper IV, was previously described as both mechanistically associated with motor, behavioural and pathological neurodegenerative aspects in mice and with risk of PD in humans. Filtering a gene candidate on associations with the disease phenotype has advantages in that it can be based on independent observations already made. In this case however, the observations in humans were made for other *PGLYRP2* SNPs than the variant found in paper IV, which decreases the usefulness of this confirmation. On the other hand, the different *PGLYRP* genes have been associated with enough variability in behaviour among humans to enable hypothesizing an association with PD, as showed by the Goldman *et al* study (231). That an increased risk for PD was confirmed for several SNPs in *PGLYRP2* and several other SNPs in the other three *PGLYRP* genes expressed in humans, give some ground for extrapolation. Further studying of the PGLYRP2 p.(P263L) point-mutation is needed to assess if there really are associations with neurological changes in humans.

Pattern of inheritance

The genetic analyses and the investigation of co-segregation of disease in paper IV were based on the hypothesis that the mode of inheritance in the family was mendelian. Thus, filtering of potential variants in paper IV was made on presumptions of high impact genetic effects (selected individuals having and not having the disease phenotype, high CADD scores and low MAFs in healthy individuals). Interpretation of patterns of inheritance can be largely affected by the presence of reduced penetrance and/or phenocopies which could in turn obscure both correct interpretation and replications of the findings.

As the family described in paper IV was in many instances exposed to environmental factors, the genetic causes might have been lower risk variants that would have been filtered out in the process. The notion that several individuals where asymptomatic carriers up in ages above 75, furthermore increases the

probability that the inheritance could instead be complex even if we have identified the correct genes.

Ethical considerations (paper III and IV)

Disease awareness

If a relatively distant relative has a disease, one might not reflect about the personal risk of having the same gene. With the familial studies of paper III and IV we were therefore at risk to raise individual concerns about whether the family members contacted were themselves at risk to be affected. For individuals closely related to affected individuals, the risk to rise concern is likely lower since they are more probably aware of the disease and likely already reflecting about the genotypes as they know of their relatively close family relation. For several of these individuals increased knowledge of the disease might be wanted, as they could have been pondering about disease cause and affection status for a long time.

Individuals more remotely related to affected individuals could instead be more likely to never think about personal risk to have inherited the disease or might be unaware about a familial presentation of the disease. Knowledge and opinions about disease and potential research is of course dependent on individual traits and how prone the family is to discuss the disease. Theoretically, the kindred in paper IV, that had lesser spread of clinical disease in comparison to the family studied in paper III, could have been more at risk of experiencing negative effects by the research project making them aware of the disease.

To minimize revealing unwanted details, individuals who were potentially unaware of the studies we performed were primarily contacted by a mail that was imprecise for which individuals were affected. If there was no answer we had ethical approval to make a phone call, but we still tried to use somewhat imprecise descriptions. Perhaps a bit surprising, only one individual asked, fully denied participating in the work in paper IV, and the impression of the author is that almost all individuals contacted in both families were very positive to the study and to help potentially identifying a genetic cause. The family members thus likely found the potential gain of information about the disease to be much larger than any discomfort induced by the study, and all family members seemed relieved that we wanted to perform a research project concerning their family.

Unaffected carriers

As with most genetic diseases, we were at risk of finding unaffected carriers of mutations that are at risk of developing the disease. Such situations are important to tackle with care. Although penetrance is seldom 100%, notifying an individual that they are at risk to develop an uncurable neurodegenerative condition can impact their lives considerably. Some individuals might have the opinion that it would be best not to know about carrier status, and some might find it crucial to know if they were at risk, as it might enable them to plan and live their lives differently than if they would not know about the likely disease-development in the future. To enable asking the family members about their opinion without raising concern, we typically included this question in the general information about the study, consent *etcetera*, at inclusion before starting any examination. Furthermore, pedigrees were masked for sex of several individuals and also modified to prevent identification of carriers from the pedigrees. There is likely no perfect solution to this delicate situation, but the question was not interpreted as troublesome for the research participants when being asked and this procedure thus seems a relevant way to handle potential unaffected carriers.

Mutations of unknown significance

The ethical considerations involving unaffected carriers become even more complex when studying genetic variants that have potential to be, but that are not yet confirmed to be, disease causing. This was the situation for the family studied in paper IV and highlights a not so uncommon ethical problems with genetic research. To minimize the risk of terrible misinterpretations of the information, (either on the individual or the researchers end) one must be very cautious about how to inform study participants about the proposed impact of the genetic variants studied. An individual without medical background could perhaps easily overinterpret or misinterpret the importance of the findings if enough details is not communicated about the known/unknown impact of the genetic variant. The timing of when to receive this information could also be important, as receiving test results of a genetic variant of unknown significance close to a family member being diagnosed might rise more concerns than at a later stage. This is a potential problem with direct-to-consumer genetic tests, speaking for genetic counsellors to be involved in the process of genetic tests, until effects of consumer-based tests are properly evaluated and/or regulated (260, 261).

Insights from studying different neurodegenerative disorders

This thesis encompassed different conditions with parkinsonism and cognitive decline and was thus titled using the term PD and related disorders, as in a wider meaning than the parkinsonian disorders of PD/DLB and APS. Since the pathobiological processes underlying these, in many ways similar conditions, are still not adequately identified and explained, an important way to moving the field forward would be to highlight factors that show interplay between the diseases. One should note that factors with pathogenetic influence can be more closely associated to modified prognosis than to differences in which symptoms that are expressed, as symptoms are direct effects of the neuronal dysfunctions irrespective of cause.

Even though the conclusions in this section should be considered theoretical extrapolations and hypotheses, since these diseases are not directly assessed in this thesis, there are some overlapping factors that should be mentioned:

❖ The enthorinal cortex is involved early in MAPT p.(R406W) disease. This is a feature also present in AD and also in the PDC-Guam, another taopathy with AD-like dementia and marked parkinsonism, which is a mixture of tau, TDP-43 and α-synuclein pathology (262). This topographical similarity could imply that the modes of disease initiation share a common ground. Thus, even though these disorders are categorized as different diseases due to different clinical and neuropathological features, they might develop pre-phases or early disease at similar locations in the brain, e.g., due to neuronal vulnerability or some common cellular mechanism particularly important there.

❖ Glial pathology was found in one of the two individuals with neuropathological examination in paper IV, and typically microglia constitute an important neuroinflammatory response in PD (154), a feature correlated with NFT deposition in PDC-Guam (263). A reduced function of brain barrier tight junctions has been observed in AD (264) and PDC-Guam (262). If the *CGNL1* mutations found in paper IV would be associated with tight junction dysfunction and neuropathological processes, the increased environmental exposure due to decreased endothelial barrier integrity could be a common ground for several variants of PD with potential environmental causes.

❖ Patients with the MAPT p.(R406W) mutation has both biochemical and clinical findings similar to AD, and the mutation is known to affect phosphorylation properties, an event that take place early in AD related tauopathy (265). More recent findings on tau phosphorylation can thus potentiate earlier findings, such that the p.(R406W) mutation is differently

phosphorylated than tau proteins from other *MAPT* mutations (266). An interesting finding was recently made on this topic. The relative abundance of tau relative to tau phosphorylated at threonine 217 was found to be in favor of normal tau in 4R tauopathies but AD patients and patients with MAPT p.(R406W) had larger proportion of tau phosphorylated at threonine 217 (267). Although the number of MAPT p.(R406W) patients in that study were very small compared to those in the AD and 4R tauopathy group (5 compared to 80 and 74, respectively), and this phosphorylation site is relatively far away from the 406th amino acid, these findings highlight the role of post-translational changes such as phosphorylation and concur with our observations that the MAPT p.(R406W) mutation show different biological properties compared to several other *MAPT* mutations.

The highlighted similarities between different neurodegenerative disorders could in theory mean that categorization of umbrella terms of different neurodegenerative disorders and subtypes of specific diseases should in a not-so-distant future be made on the basis of pathogenetic similarities or means of disease initiation or propagation rather than the resulting proteinopathy or symptoms. A potential of subtyping PD in such a way is implied with the recently suggested brain vs body first PD (3, 87). This interesting approach, with similar pathogenic mechanism spreading in different ways from different points of initiation giving rise to different clinical courses, could thus perhaps be extrapolated to other neurodegenerative disorders, as proposed with R406W-associated FTD-tau and AD in paper III.

Implications of the findings and future perspectives

In paper IV we compiled a list of new candidate genetic variants to cause PD with cognitive decline. There is a need of future studies to validate the association to PD for one or several of these mutations.

New proposed causes to familial PD are important since they can

- ❖ Identify rare diseases in families that would otherwise have no explanation for the familial accumulation of disease.

- ❖ In a longer run identify new pathogenic mechanisms, and enable more detailed examinations of established pathogenic mechanisms, increasing likelihood of understanding the mechanisms relevant for sporadic PD.

Since there have been associations between PD and different kinds of cancer (268, 269) and the RUNDC3B p.(D315N) mutation was highlighted as a top-candidate variant in the family of paper IV, there could be incitements to continue exploring effects of PD on genetic risk-factors for different malignancies.

In-depth descriptions of the clinical and neuropathological phenotype of patients with rare genetic causes to neurodegenerative diseases, such as MAPT p.(R406W), can increase awareness of the specific condition and help to identify these diseases in the clinic. When putting a focus on the few clinical differences from the clinical picture of AD, it is more likely that clinicians think about differential diagnostic possibilities and note any divergencies from the supposed clinical trajectories, (similar to what is regularly done in PD–APS).

We furthermore identified a need for a more detailed radiological signs associated to some genetic tauopathies since radiological standard examinations of MAPT p.(R406W) patients, might not have allowed separation of hippocampal atrophy and other atrophy-patterns of the medial temporal lobe. Better radiologic patterns and/or more detailed descriptions made more common in clinical use could reduce diagnostic misinterpretations and increase awareness of the rare genetic forms of neurodegenerative diseases

We identified a radiological sign with ventromedial temporal lobe atrophy with a markedly widened collateral sulcus in the Swedish family with MAPT p.(R406W) mutation and suggest that this sign should be further assessed to establish potential associations to some familial tauopathies.

We suggest that further mechanistic studies be made on differences in 4R and 3R tau deposits, and other varying biological features of tau, such as disparities between tau fibril ultrastructure, in different tauopathies. We propose that special focus could be put on post-translational changes of R406W-mutated tau, such as phosphorylation.

Strengths and limitations

When the 9 candidate genes were filtered out in paper IV, after searching two genetic databases and assessing risks with genetic prediction tools, we added searches in three separate cohorts and also studied the literature for previously known associations between the genes and PD, to further substantiate the findings.

Furthermore, one of the databases was based on Swedish genomes and one of the cohorts was based on the same population as the family studied, increasing the relevance of examining these populations for the mutations of interest.

We studied rare genetic variants, and the number of affected individuals were few. In paper III this was, however, in part balanced by a comprehensive systematic review which solidified the picture of the disease.

In paper IV, the incomplete co-segregation of disease of the two proposed top-candidate genetic variants impose uncertainties of the variant's pathogenicity and

underline the need for other studies to confirm or dismiss the proposed associations between the variants and PD. There were also uncertainties regarding some individuals' clinical phenotype. Even though these individuals were generally elderly and contact with the family was withheld during 4 years after examinations, this could have been a too short observation period to observe all potential phenotype conversions to PD.

Also, albeit efforts were made to meet large part of the family in paper IV, there were individuals that were not contacted in the study, which potentially could have improved the variants' co-segregation with disease.

Conclusions – Familial studies

After studying 8 individuals in a Swedish family and 63 individuals from the literature, we summarized the clinical picture of MAPT p.(R406W) patients as AD-like with behavioural changes and language impairments in several individuals but parkinsonism only in a few.

We proposed that radiological picture of some familial tauopathies could be differentiated from that of AD and other neurodegenerative disorders, due to specific changes in the medio-ventral temporal lobe.

Widening of the collateral sulcus was suggested as such a radiological sign in R406W-associated disease.

We found R406W-associated disease to be associated with a PSP 4R tauopathy and discuss reasons for discrepancies in neuropathological descriptions patients with this genetic variant.

We constructed a list of 9 candidate genetic variant to cause PD with cognitive decline in a Swedish kindred with no known genetic cause.

We highlighted two of the genetic variants as top-candidates, due to their associations with other patients with PD and discuss their potential relationship to PD.

We found discordant co-segragation of disease with the proposed top-candidate variants in the family and discuss reduced penetrance an gene-environmental interactions to hypothetically be affecting the results.

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To identify all factors that has led to this thesis and inspired me during the years I was working with it, is likely beyond me. I can, however, reflect about some that I find important for my learning and personal development.

I am incredibly grateful to my supervisor *Andreas Puschmann*, who has always been very optimistic and on point. I have very much enjoyed working close with him and receiving so much support through the years. It is interesting to think about that chance made me meet him one day as a young medicine student in one of Lunds neurology wards in 2011. Without him telling me about his research that week, this thesis would likely not have been made, since it led to master essay, later published (270) and continued projects that led to this thesis.

I am also very grateful to my wife *Maria Ygland Rödström*; without her support I would not have been able to complete this thesis. She made very valuable contributions by helping me understand several aspects of the statistical analyses performed in paper I and II and has been a huge support during the time this thesis was written. I proposed to her, and we got married during the work of this thesis, and now share a happy home that will soon include two MD PhDs and a soon-to-be born daughter.

I like to thank the co-workers of all the papers, for interesting and teaching interactions, but special thanks go out to *Niklas Mattsson-Carlgren* who assisted with statistical suggestions and important evaluations of the text. My co-supervisor *Oskar Hansson* has been an inspiring person to interact with, and was also a co-authour in paper II and III, to which he gave important input.

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My *family* has always been supportive, and I thank them for being there both before, during and after the work with this thesis. A few mathematical discussions with two of the engineers in the family and some English words facilitated several parts of the writing, but foremost my family has contributed emotional support and much

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Lund was a fantastic city to study in and the city and the university at its center will always have a very special place in my heart. Also the sometimes strenuous and hectic workdays at my clinical working-place at the *healthcare station in Staffanstorp* was in retrospect a crucial part that drew me further into academia and made me cherish some of the many upsides with being a researcher. Other factors instead made the work possible to complete, and include meeting many wonderful collegues and patients at *Sårcentrum, Södersjukhuset* in Stockholm, and singing with friends in the student choir *Ultraljud*. Important mentions goes also out to *Tumba Judo* and *Hashidate Aikido club* that, during my adolescence, introduced me to regular physical activity, a habbit that *Gerdahallen* in Lund vastly improved as I personally experienced the important connection between motor and cognitive functioning.

Appendix

Appendix 1 SCS classification algorithm from supplement of paper I (170)
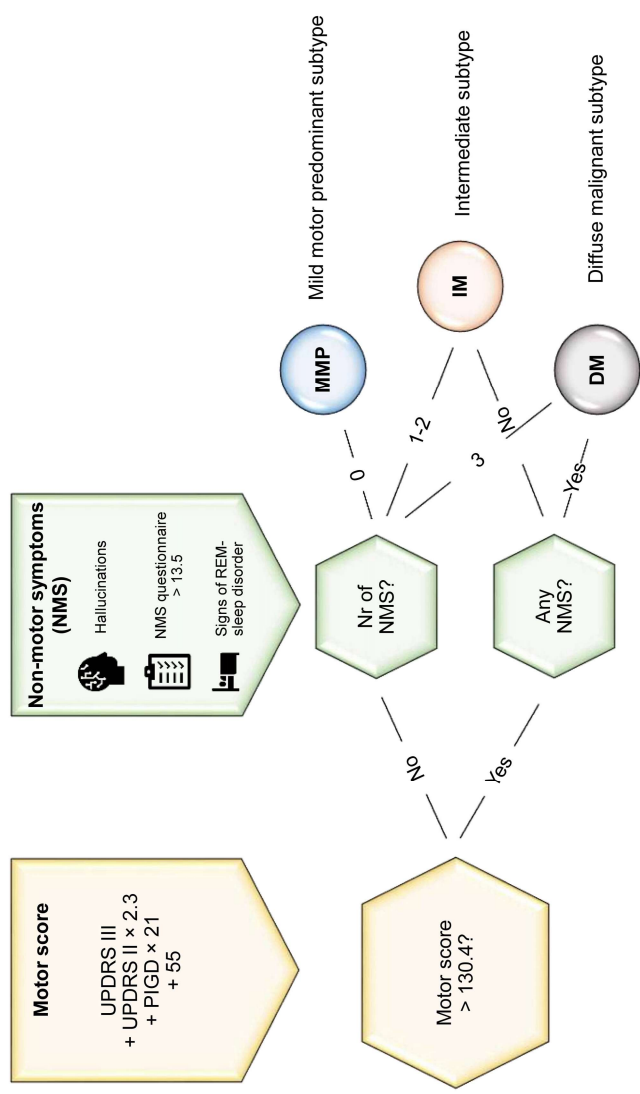
Appendix 2 Protocol for digital search of medical records and variable definitions.

Variable	Variable type	Comment	Input	Search terms (Swedish)	Added terms
Age of onset	Contineous	Onset of patient-experienced symptoms, should be PD-symptoms. If contradictions Medical records>baseline investigation>follow up, or either that is specified with 1-2 years if others give larger timespan	Numerical (year)	Började, diagnos	
Deceased	Dicotomic	Has duration of death = 1 otherwise 0	Yes=1 No=0 Missing=-999		
Duration at death	Contineous	Year of onset - year of death	Numerical (year)		
Duration at Bl investigation	Contineous	Year of onset - date of examination	Numerical (year)		
BL_UPDRS-total	Contineous	Investigated in on, maximal point if range, parenteses are ignored	Numerical, Missing= -999		
TD/Undefined/PIGD	Categorical	See reference	TD/UI/PIGD Missing= -999		
TD/Undefined/PIGD paper 2	Contineous	See reference	0=TD, 1=UI, 2=PIGD Missing= -999		
SCS group	Categorical	See methods	MMP/IM/DM Missing= -999		
SCS group paper 2	Contineous	See methods	0=MMP, 1=IM, 2=DM Missing= -999		
BL_H&Y_modified	Categorical		1, 2, 2.5, 3, 4 or 5		
Any ortostatism	Dicotomic	BP drop below 20/symtoms at investigation, anamnestic problems or medication with orstanorm, gutrone or effortil	Yes=1 No=0 Missing=-999	ton, (låg), tryck, ortostati	
At onset: tremor (resting) in limb	Dicotomic	The first symtom as described by the patient, if other symptom ensued 2 weeks later or more, ignore that	Yes=1 No=0 Missing=-999	Read first entries in full-text	
At onset: Other tremor?	Dicotomic		Yes=1 No=0 Missing=-999	Read first entries in full-text	
At onset: bradykinesia	Dicotomic	The first symtom as described by the patient, if other symptom ensued 2 weeks later or more, ignore that	Yes=1 No=0 Missing=-999	Read first entries in full text	
At onset: Gait disorder	Dicotomic	The first symtom as described by the patient, if other symptom ensued 2 weeks later or more, ignore that	Yes=1 No=0 Missing=-999	Read first entries in full-text	
At onset: Stiffnes or ache in limb	Dicotomic	The first symtom as described by the patient, if other symptom ensued 2 weeks later or more, ignore that. Also includes complex motoraction and clumbsiness	Yes=1 No=0 Missing=-999	Read first entries in full-text	
Resting tremor	Categorical	Any resting tremor	Yes=1 No=0 Missing=-999	Skak, trem	
Hallucinations	Dicotomic	Feeling of prescense, noices etc is also regarded as yes, medication with neurolepticsis regarded as yes	Yes=1 No=0 Missing=-999	halluc, syn	
Hallucinations when	Contineous	Duration at first onset of hallucinations	Numerical (year), Missing= -999		

Variable	Variable type	Comment	Input	Search terms (Swedish)	Added terms
Delusional		Clearly has misconcepted reality at any time. Could be considered "paranoid" if experiencing threats while delusional.	Yes=1 No=0 "Hotfulla=paranoid" Missing=-999	Föreställ, tror, paranoi	
Dementia	Dicotomic	Treated with memantine or AcEi, Dementia diagnosis or obvious severe memory impairment that affects daily living, or MMT<23 if had better testresults before and patient or relative note problems related to cognition	Yes=1 No=0 Missing=-999	minne, demen, glömsk	
Duration when demented	Continous	The first time where E_dementia is fullfilled	Numerical (year), Missing= -999		
DLB?	Dicotomic	Cognitive problems present within 1 year of motor onset. Not if only subjective problems that did not detoriate shortly after	Yes=1 No=0 Missing=-999		
Nursing home at what duration	Continous	Defined as living at nursing home at least 25% of the time and not moving back to own home at fultime, any residence with health care staff employed. Not fullfilled if within 2 months of death!	Numerical (year), Missing= -999	Boende, social	
>= Walker use at what duration	Continous	Defined as the first time needing "rollator or wheelchair at most times outdoors or sometimes indoors (or when patient becomes bedridden)" and did not clearly improve to not having to use it	Numerical (year), Missing= -999	rollator, förflytt, (går, gång)	
Bedridden/H&Y 5 at what duration	Continous	First notion of confinement to wheelchair or bed and the patient cannot walk without personal support afterwards	Numerical (year), Missing= -999	rull, förflytt, säng.	
Symptoms suggestive of RBD	Dicotomic	Profound moving in sleep and/or talking/shouting/singing	Yes=1 No=0 Missing=-999	Rem-	REM-sömn, Remsöm, dröm
Dyskinesias	Dicotomic	Any notion that the patient has dyskinesias/hyperkinesia	Yes=1 No=0 Missing=-999	rör, kine	
End of dose wearing off	Dicotomic	Any report that medications stop working before the next dose (not unpredictable offs)	Yes=1 No=0 Missing=-999	glapp, effekt	
Konfusion	Dicotomic	Mentioned in medical records or described as period of severe cognitive dysfunction	Yes=1 No=0 Missing=-999	Förvirr, konfus	
Dystonia ever?	Dicotomic	Any musclespasm without other cause (including camptocormia, early morning dystonia in calves or other or when walking)	Yes=1 No=0 Missing=-999	Dyston, kramp	cam, (felstäl)
Insomnia	Dicotomic	Problem falling asleep or several wake-ups	Yes=1 No=0 Missing=-999	Sömn, sov, (natt)	
EDS	Dicotomic	Involuntary or more than one planned sleep per day or sleeping three or more hours daytime regularly! Not only sleepiness or fatigue without accually sleeping.	Yes=1 No=0 Missing=-999	trött, somn	dagtid (somnar, sover)
Symptoms suggestive of RLS	Dicotomic	Diagnosis mentioned in medical record or symptoms of parestesias that are worsened/only present at night and/or improved with motion or L-dopa	Yes=1 No=0 Missing=-999	(stick), domn, restless, rastlös, parestesi, RLS	kryp
For re-examination only					
ACE-R	Continous		Numerical (year)		
Partner for majority of the time	Categorical	At least 50% of the time. Any friend or partner living in the same house.	(1=no partner, 2=partner but living apart, 3=married or living with someone 4= some part of the time		

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Prognostic subtypes in Parkinson's disease and related disorders

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This thesis focuses on long-term progression of patients with Parkinson's disease and familial variants of neurodegenerative disorders with Parkinsonism and affected memory. The vision is to one day be able to account for the different processes underlying neurodegeneration in every-day clinical appointments.

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