Cerebrospinal fluid and imaging biomarkers of cognitive impairment in Parkinson's disease

Baseline data from a cohort study in a university hospital based neurological outpatient clinic

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2 Common abbreviations and definitions

A β	amyloid- β
AD	Alzheimer's disease
α -syn	α -synuclein
CA	cornu ammonis
CSF	cerebrospinal fluid
DLB	Dementia with Lewy bodies
ERC	entorhinal cortex
FDG-PET	^{18}F -fludeoxyglucose positron emission tomography
GDS	Geriatric depression scale
GLDS	Global Deterioration Scale
Hippocampal formation	dentate gyrus (DG), cornu ammonis (CA) 1-4, subiculum and presubiculum
LED	Levodopa Equivalent Dose
Lewy pathology	Lewy bodies and Lewy neurites
MCI	mild cognitive impairment
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
MTL	Medial temporal lobe

NBM	nucleus basalis of Meynert
NC	normal controls
o- α -syn	oligomeric α -syn
p- α -syn	phosphorylated α -syn
Parahippocampal region	ERC, PRC and parahippocampal cortex
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PIGD	postural instability gait disorder
PRC	perirhinal cortex
P-tau	phosphorylated tau
QSBB criteria	Queen Square Brain Bank UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria
RBD	rapid eye movement sleep behavior disorder
ROI	region of interest
SCI	subjective cognitive impairment
t- α -syn	total α -syn
T-tau	total tau
UPDRS	The unified Parkinson's disease rating scales of motor function

3 Introduction

3.1 What is the thesis about?

3.1.1 Research problem and need for research

With increasing life expectancy, the numbers of people with neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's disease (PD) increase (1, 2). Although defined as a movement disorder, cognitive impairment and dementia in PD (PDD) and other non-motor symptoms are common (3-5). Both motor and non-motor symptoms represent a large burden to the patients and their families (6), but also socioeconomically (7).

There is lack of knowledge about the pathological mechanisms leading to dementia in PD (8, 9). To date, only symptomatic and no preventive treatment for dementia is available. Recent research suggests that therapeutic intervention should be applied early in the pathological process to prevent development of dementia (10, 11). In addition, an early diagnosis of cognitive impairment may, as for AD (12), have many benefits, including initiate measures to reduce other risk factors for dementia such as hypertension, diabetes and smoking, encourage physical and cognitive training which may delay cognitive impairment, establish medical and caregiving teams to help and support the patient and family members and start planning for the future. To be able to diagnose cognitive impairment at as early a stage as possible and to develop preventive mechanism-based treatments, it is necessary to explore possible early biomarkers of the underlying mechanisms.

Cerebrospinal fluid (CSF) levels of amyloid- β (A β), tau, α -synuclein (α -syn) and neurogranin are thought to reflect the disease processes of synaptic dysfunction (13-17) and neurodegeneration in the brain (18), and specific brain regions as shown by imaging (19-22) may be affected. Whether these potential biomarkers are associated with cognition and may be used as biomarkers for cognitive impairment in early PD is not clarified.

3.1.2 Central research questions

We wanted to explore whether the levels of CSF A β species, tau and α -syn biomarkers are different in early PD patients and non-PD mild cognitive impairment (MCI) patients with

increased risk of developing AD, and whether these biomarkers are associated with cognition in early PD.

Further, we wanted to examine whether the sizes of hippocampal and parahippocampal sub-regions on magnetic resonance imaging (MRI) are smaller in early PD than in normal controls (NC), and whether these sub-regions are associated with the CSF biomarkers and cognition in early PD.

Finally, we wanted to find out whether the levels of CSF A β species, α -syn and neurogranin are associated with ^{18}F -fludeoxyglucose positron emission tomography (FDG-PET) uptake, a marker of cortical synaptic activity, in relevant regions, and whether these regions are correlated with cognition in early PD. Are the levels of CSF neurogranin significantly different in early PD compared to NC, and are there correlations between neurogranin and A β , α -syn and PD motor stage?

3.1.3 Design and patient selection

The papers are based on cross-sectional baseline data from the ongoing prospective longitudinal cohort study *Mechanisms and biomarkers of cognitive decline in Parkinson's disease and dementia with Lewy bodies* at the Department of Neurology, Akershus University Hospital. Patients with early Parkinson's disease with disease duration ≤ 6 years and without dementia and other comorbidities that might contribute to cognitive decline were recruited from the neurological outpatient clinic. For comparison, patients with non-PD MCI were included from the memory clinic, and NCs were recruited from relatives and orthopedic patients in parallel ongoing studies.

3.1.4 Possible implications of the results

The results contribute to the description of early diagnostic biomarkers and to the understanding of the underlying mechanisms of the earliest stages of cognitive impairment and dementia in PD. See *Figure 1: Hypothetical model of pathological mechanisms and biomarkers in Parkinson's Disease cognitive impairment* on page 119 for an overview.

3.2 Background

3.2.1 Parkinson's disease

Definition

201 years ago, in 1817 the London surgeon James Parkinson published *An Essay on the Shaking Palsy* (2, 23), where he described a syndrome today known as Parkinson's disease. Since then, the knowledge of the disease has advanced in the understanding of etiology, pathological mechanisms, clinical symptoms, diagnostics and treatment. PD is a complex, slowly progressive neurodegenerative and multiorgan disorder. It is defined by a clinical motor syndrome of parkinsonism (see below under *Clinical symptoms* on page 11) associated with α -syn deposition and neurodegeneration of the substantia nigra pars compacta leading to dopamine depletion in the basal ganglia (2, 24, 25). In addition, non-motor symptoms including cognitive impairment and dementia, likely due to more widespread neurodegeneration and involvement of different neurotransmitter systems and organs are common (2, 25).

Etiology and risk factors

A definite cause of PD is unknown, but it is multifactorial. There are genetic predispositions in both hereditary (10 % of cases) and idiopathic (90 % of cases) PD (26). Both epigenetic modifications of gene expression in response to the environment and environmental exposures play a role (2, 27). Genetic alterations may include single nucleotide polymorphisms (SNPs) as shown by genome-wide association studies (GWAS) and mutations in the *GBA*, *LRRK2*, *PRKN*, *SNCA*, *MAPT* and *PARK7* and other genes. Epigenetic affection of gene expression may involve methylation, phosphorylation, acetylation and micro-RNAs. Environmental risk factors may be pesticides such as rotenone, head injury and rural living. However, increasing age is the most important risk factor for development of sporadic PD (2).

Pathogenesis and pathology

α -synuclein

α -syn is a 140 amino acids, soluble, random coiled, cytosolic, presynaptic terminal protein monomer, which may be alternatively spliced (6, 27). Its function is unclear, but it is possibly involved in synaptic-vesicle complex formation (28), vesicle transport to the presynaptic terminus and neurotransmitter (including dopamine) release, synaptic plasticity and brain lipid metabolism (27, 29-31).

Pathogenesis

The exact mechanisms of the pathogenesis leading to neurodegeneration in PD are not known, but defects in the regulation of the protein homeostasis and other cellular processes influenced by abnormalities in gene products and environmental factors appear to be involved (2, 6, 27, 30, 32, 33):

- Pathological molecular changes in the α -syn protein (encoded by *SNCA* gene) (and other proteins such as amyloid and tau encoded by microtubular associated protein tau (*MAPT*) gene) leads to abnormal processing and misfolding into soluble oligomers and fibrils, which may be neurotoxic, and aggregation into insoluble fibrils.
- Decreased degradation of misfolded and oligomeric α -syn (o- α -syn) by the ubiquitin-proteasome (affected by the *PRKN* gene encoding parkin) and lysosome-autophagy (affected by *LRRK2* gene encoding leucine-rich repeat kinase 2 and *GBA* gene encoding β -glucocerebrosidase) systems leads to accumulation of toxic α -syn.
- Mitochondrial dysfunction (affected by *PRKN* and *PARK7* mutations and rotenone) and oxidative stress triggers aggregation of α -syn oligomers and fibrils and neuronal injury.
- Intra-axonal aggregation of α -syn causes neurite structural changes (affected by *LRRK2* and *MAPT* genes), abnormalities in intracellular protein and membrane transport (affected by *LRRK2*) (2) and early disruption of axonal transport (32, 34).

- Presynaptic accumulation of abnormal α -syn leads to early synaptic dysfunction (affected by *SNCA* and *LRRK2* genes) (2, 31, 32, 34), neurotransmitter deficiency and postsynaptic dendritic spine loss (17).
- Inflammatory mechanisms have also been implicated by GWAS; PD-implicated genes modulate inflammatory cytokines, play a role in the clearance of foreign pathogens and may modulate reactive oxygen species (33). Neuroinflammation involving activated astrocytes and microglia leads to gliosis within areas of neurodegeneration. Astrocytes and microglia are both involved in clearance of extracellular debris which might aid in the survival of neurons, and microglia release neurotrophic factors, harmful reactive oxygen and nitrogen and pro-inflammatory cytokines and enzymes (2). However, it is unclear whether the neuroinflammation is a causal contributing factor or a secondary effect (2, 30, 33, 35).

Lewy pathology and presynaptic α -syn aggregates

The misfolded, insoluble α -syn fibrils aggregate together with ubiquitin, phosphorylated neurofilaments and other substances in intra-cellular inclusions within the cell bodies (called Lewy bodies) and processes (called Lewy neurites) of neurons in the brain and other parts of the nervous system (2, 6, 27, 30, 32, 36). Lewy bodies were first described by Friedrich Lewy in 1912, and these have been considered to be the pathological hallmark of PD at autopsy. Most of the accumulated α -syn in the Lewy bodies is phosphorylated, but the significance of phosphorylated α -syn (p- α -syn) in the pathogenesis of PD is unclear (29).

The toxic α -syn species and Lewy pathology are proposed to cause neuronal cell death (2, 27). However, it has been suggested that Lewy pathology may be a result of neuroprotective mechanisms (6, 36, 37); Lewy pathology may be found at autopsy in preclinically healthy elderly (6, 24, 27, 34, 38), not all patients with genetic PD have Lewy pathology (6, 17, 24, 34) and it has been difficult to correlate Lewy pathology to neurodegeneration and severity of clinical symptoms (6, 17). Most α -syn aggregates are not localized in the Lewy bodies, but at within presynaptic terminals, and synaptic dysfunction due to presynaptic α -syn aggregates may explain the neurodegeneration with loss of postsynaptic dendritic spines leading to neurotransmitter deficiency (17, 37). Lewy pathology has also been proposed to be a result of a protective mechanism due to axonal retrograde transport of presynaptic α -syn removing α -syn from the presynapses (37).

The Braak theory

The Braak hypothesis suggests a slowly progressive caudal to rostral dissemination of Lewy pathology over several years and describes 6 stages (39, 40):

Stage 1 starting in the peripheral autonomic nervous system, dorsal motor vagal and glossopharyngeal nuclei in the medulla oblongata and the olfactory systems (including the olfactory bulb and olfactory nuclei in amygdala and perirhinal cortex (PRC)). This may explain premotor symptoms such as early autonomic (gastrointestinal, genitourinary, cardiovascular) symptoms and hyposmia (2, 32, 39).

Stage 2 involves pons including the serotonergic raphe nucleus and noradrenergic locus coeruleus, which may contribute to depression and sleep disorders (2, 32, 39).

In stage 3, the pathology spreads to the cholinergic pedunculopontine nucleus in pons, the cholinergic nucleus basalis of Meynert (NBM) in the basal forebrain and to substantia nigra pars compacta in the mesencephalon (2, 27, 32, 39). Loss of more than 50 % of the dopaminergic neurons in the particular vulnerable substantia nigra pars compacta and accompanying dopamine depletion in the nigrostriatal pathways of the basal ganglia causes the parkinsonistic motor symptoms (2, 9, 27, 41). The NBM has a strong connection with the cholinergic pedunculopontine nucleus in the brainstem, and dysfunction in this nucleus contributes to postural instability and gait difficulty in PD (9).

In stage 4, the pathology disseminates further to the diencephalon, thalamus and hypothalamus and the limbic system including amygdala, the temporal mesocortex with the transentorhinal region and CA2 of the hippocampus leading to non-motor symptoms of advanced disease such as executive dysfunction and emotional and behavioral problems (2, 32, 34, 39).

Finally, in stage 5 and 6, the pathology disseminates to multiple neocortical regions, first affecting prefrontal areas and anterior cingulate cortex (stage 5) and subsequently temporal parietal cortex, which may explain cognitive impairment and dementia (2, 8, 32, 39, 42).

It has been hypothesized that the progression of PD may be due to prion-like trans-synaptically spreading of pathological α -syn species between neurons, which may explain the spreading of pathology according to the Braak model (2, 6, 17, 27, 32, 34).

Other pathologies

As described in detail later (in *Pathophysiology of neurotransmitters and cortical neural networks* on page 18), non-dopaminergic neurotransmitters (acetylcholine, noradrenaline, serotonin and glutamate) contribute to different non-motor symptoms, including cognitive impairment and dementia (2, 41).

There is evidence for a genetic overlap between AD and PD (43). Concomitant AD pathology such as extracellular A β plaques and intraneuronal tau-containing neurofibrillary tangles may be found and may act synergistically with and accelerate α -syn aggregation and Lewy pathology and contribute to the cognitive symptoms (2, 30, 34).

Epidemiology

PD is the second most common central nervous system (CNS) neurodegenerative disorder, following AD.

Prevalence

The prevalence of PD is approximately 1.1-2.6 ‰ (44), and increases with age from 0.4 ‰ in the forties, 1.1 ‰ in the fifties, 4.3 ‰ in the sixties, 1.1 % in the seventies to 1.9 % in those over 80 years (45). There is a higher prevalence in the group of 70-79 years old individuals in Europe, North America and Australia (1.6 ‰) compared to Asia (0.7 ‰) (45).

Incidence

The incidence of PD per year is reported to be approximately 0.1-0.2 ‰ (44, 46, 47), which is consistent with an incidence of 0.1 ‰ in a Norwegian study (48). The incidence increases with age with an incidence of 3.5 ‰ in the age group above 60 years (44) and peak incidence in the 70-80 years old (46).

Some studies have found higher prevalence and incidence rates in men compared to women, while others not have found this (44-48).

Clinical symptoms

The four cardinal parkinsonistic motor symptoms of PD are bradykinesia, muscular rigidity, rest tremor and usually later postural instability (2, 49) leading to the characteristic parkinsonian gait and appearance. There is usually a unilateral onset with persisting asymmetry (49). There is a substantial variation in motor symptoms between patients (24). Two major motor subtypes have been described; tremor (8 % of cases) and non-tremor akinetic-rigid (26 % of cases) dominant PD. The latter is possibly associated with a faster rate of progression, but most patients have a mixed condition (66 %) (2, 24, 50).

Pre-motor symptoms may occur more than 10-20 years before the onset of motor symptoms, and these may include autonomic symptoms such as constipation, erectile dysfunction, rapid eye movement sleep behavior disorder (RBD), excessive daytime sleepiness, hyposmia and depression (2, 24, 25, 51). Other non-motor symptoms such as pain, fatigue and MCI may also appear early in the disease.

The disease progresses over time with increasing motor symptoms such as postural instability, freezing of gait, falls, dysphagia and speech dysfunction, autonomic dysfunction such as postural hypotension, urinary incontinence and constipation, apathy and eventually dementia (2). Anxiety, insomnia, concentration difficulties, restless legs syndrome, swallowing problems, dribbling of saliva, nausea, nocturia, bowel incontinence, excessive sweating and double vision are other non-motor symptoms (25). Manifestations such as fluctuations, dyskinesia, impulse control disorders, hallucinations and psychotic symptoms may also be related to the medical treatment (2, 25).

Diagnostic criteria

The gold standard for diagnosis of definite PD is the neuropathological detection of Lewy pathology and dopamine neuronal loss in the substantia nigra pars compacta at autopsy in combination with a clinical syndrome of parkinsonism and absence of any symptoms suggestive of atypical parkinsonism (2, 24).

For clinical practice and research, several diagnostic criteria have been proposed (24). The Queen Square Brain Bank (QSBB) UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (49) have been recommended from the European Federation of

Neurological Societies (EFNS) and the European Section of the International Parkinson and Movement Disorder Society (MDS-ES) (51). The QSBB criteria requires bradykinesia in addition to either rigidity, rest tremor or postural instability, none of the exclusion criteria and at least three supportive criteria such as unilateral onset, response to levodopa or hyposmia for the diagnosis of definite PD. Recently, MDS has published new clinical diagnostic criteria (52).

Diagnostic biomarkers

Single photon emission computed tomography (SPECT) with radioligands targeting the dopamine transporter (DAT) and PET with specific radioligands measure the level of presynaptic dopaminergic nerve terminals projecting from substantia nigra pars compacta to the striatum, and can be used to differentiate PD and atypical parkinsonism from disorders without neurodegeneration in substantia nigra pars compacta (2, 51). ^{18}F -FDG-PET measuring the resting brain glucose metabolism may show reduced frontal metabolism and increased metabolism in the lentiform nucleus (putamen and globus pallidus), which may discriminate from atypical parkinsonism. Structural MRI may be useful in differential diagnosis showing abnormalities suggestive of atypical parkinsonism. (51)

In the CSF, several potential early biomarkers are being explored, including α -syn, A β and tau (2), in relation to cognitive impairment (as discussed later in *Cerebrospinal fluid biomarkers* on page 29). For t- α -syn, studies in PD have shown lower or overlapping levels with other synucleinopathies compared to controls, and thus cannot be used to discriminate the diseases. Most studies have found a slight reduction in A β 42 in PD, but this is also seen in other neurodegenerative diseases such as AD and dementia with Lewy bodies (DLB), Tau is usually found to be unchanged in PD. A combination of CSF biomarkers interacting in the pathogenesis might increase diagnostic accuracy, but there is a need for validation of the biomarkers and longitudinal studies. (29) Other possible diagnostic biomarkers of PD are under investigation, and we recently described reduced levels of the postsynaptic spine-protein neurogranin (53).

Medical therapy

As there are no disease modifying or neuroprotective drugs, the treatment is symptomatic and the goal is to improve function and quality of life (2, 54).

Antiparkinsonian medications enhance intracerebral dopamine concentrations and stimulate dopamine receptors through various mechanisms (2): Levodopa passes the blood brain barrier and is converted to dopamine. Dopamine and dopamine agonists (DA) activate nigrostriatal dopamine receptors. Monoamine oxidase B (MAO-B) inhibitors and Catechol-O-methyltransferase (COMT) inhibitors block the degradation of dopamine and levodopa. (54)

Bradykinesia and rigidity respond to dopaminergic medications, while tremor has a variable response (2, 54). DA is usually given in patients under 60 years to delay the need for levodopa, which may cause motor complications such as dyskinesia and motor fluctuations. Older patients are more prone to develop psychiatric adverse effects of DA such as hallucinations, and levodopa, which provide the best effect, is preferred. (54) There is usually a good response to levodopa treatment for 5 years or more (49, 51).

In patients with motor fluctuation between “on” periods with good response to medications and “off” periods when response from medications wears off, DA, MAO-B inhibitors, COMT inhibitors and in advanced disease a Duodopa (levodopa-carbidopa) infusion pump into the duodenum or subcutaneous infusion of the DA apomorphine provide more stable dopamine concentrations. Late-stage disease responds poorly to dopaminergic treatment, and this may be due to abnormalities in other neurotransmitter systems and non-dopaminergic brain regions as well. (2, 54)

Deep brain stimulation (DBS) of the subthalamic nucleus or globus pallidus internus is effective and relevant in moderate to severe PD with response to levodopa, but with distressing fluctuations and dyskinesia (2). However, attention must be paid to possible worsening of cognitive impairment (31).

There is a need for developing disease-modifying drugs that slow or stop the neurodegeneration (2, 54). Gene therapy and cell transplantation may be other alternative future possibilities (2).

3.2.2 Cognitive impairment and dementia in Parkinson's disease

Definition

Impairment of cognitive functions is common at all stages of PD, and range from mild impairment to severe dementia. MCI in PD (PD-MCI) is a condition of insidious subjective cognitive decline which can be detected on cognitive tests, not severe enough to significantly affect functional independence, and caused by the underlying neurodegenerative process (55). With gradual increasing cognitive decline in several domains leading to significant loss of functional independence more than one year after the onset of parkinsonism, the condition is defined as PDD (56).

Etiology and risk factors

As for PD, the underlying cause of cognitive impairment is only partly known, but ageing is an important factor (41, 56). Most cases of PDD are sporadic, but there is a familial association between dementia and PD, suggesting a genetic contribution (8, 57). Several genes linked to PD (as described above in *Etiology and risk factors* on page 6) have been associated with increased risk of cognitive impairment and dementia in PD, including *GBA*, *MAPT* and *SNCA* (6, 8, 9, 31, 41, 57).

The APOE $\epsilon 4$ allele (involved in lipoprotein transport), which is a strong risk factor for AD, has been associated with cortical A β and α -syn pathology and increased risk for cognitive impairment and dementia in PD, especially in those with comorbid AD pathology (6, 8, 41).

Other potential risk factors for development of PDD are MCI (especially if affecting multiple domains), lower education, longer disease duration, late onset and increasing motor severity, predominance of rigidity and postural instability with gait disorder (PIGD), depression, visual hallucinations and psychosis, RBD and olfactory dysfunction (6, 8, 56, 57).

Mechanisms: Lewy and AD pathology and synaptic dysfunction

The neuropathology of cognitive impairment and dementia in PD is heterogeneous, involving Lewy and AD pathology (58), early synaptic dysfunction and loss of neurotransmitters and cortical neural networks (8, 41).

Lewy pathology

Lewy pathology comprising Lewy bodies and Lewy neurites in limbic and neocortical areas has been suggested as the cause of neurodegeneration leading to dementia (6, 9, 31, 41), but as previously discussed (in *Lewy pathology and presynaptic α -syn aggregates* on page 8), Lewy pathology may also be a result of neuroprotective mechanisms (6, 36, 37). The Braak theory proposes the spread of Lewy pathology from the brainstem to the forebrain, limbic system and finally neocortex (39). This correlates with the development of cognitive impairment and dementia in PD (6, 8, 41, 57), although not all patients with PD-MCI have limbic or neocortical Lewy pathology, and not all with cortical Lewy pathology will develop dementia (6, 9, 41, 57).

AD pathology

Amyloid precursor protein (APP) is linked to synaptic vesicle docking, priming and exocytosis at the presynaptic terminals, and is essential for synaptic function and plasticity (28). APP is endocytosed from the cell surface in the neurons into endosomes where it is enzymatically cleaved by β - and γ -secretases into different species of A β , which are released to the extracellular fluid (14, 18, 59). A β peptides, especially A β 1-42 (A β 42) and to a lesser degree A β 1-38 (A β 38) and 1-40 (A β 40), are prone to misfold and aggregate into soluble A β oligomers and insoluble A β fibrils in A β plaques, which both are neurotoxic and disrupt synaptic plasticity (14, 18, 59, 60). Intraneuronal A β may also cause synapse dysfunction (61). Mutations in the genes encoding the secretases or APP increase the production of A β 42 (59). The accumulation of A β in AD has also been linked to decreased clearance from the brain parenchyma by the glial-lymphatic (“glymphatic”) system, which is driven by fluid transport through aquaporin-4 water channels. The “glymphatic” system may also be involved in reduced clearance of α -syn (62). A β turnover decreases from approximately 40 years of age, increasing the likelihood of protein misfolding and deposition (63). Increased A β brain deposition is associated with shorter time to PDD (64).

The tau protein binds and stabilizes axonal microtubules involved in axonal transport of among other synaptic constituents (18). Hyperphosphorylation of tau proteins leads to reduced binding affinity for and dysfunction of microtubules (18) and promotes tau aggregation into neurofibrillary tangles (65). Neurofibrillary tangles are pairwise, helical protein filaments in the cytoskeleton of neuronal cells (18) contributing to neuronal cell death (15).

A β plaques and tau-containing neurofibrillary tangles, the pathological hallmarks of AD, are associated with PDD, and to a lesser degree with PD-MCI (6, 9, 31, 41, 66, 67). AD pathology is found in the limbic system in PD without dementia, and A β plaques are also found in striatum and more widely distributed in the neocortex in PDD (57). Up to 50 % of patients with PDD may fulfill the pathological criteria for co-morbid AD, which is associated with an older age of onset of PD (6, 41).

Synaptic dysfunction

As previously described (in *Lewy pathology and presynaptic α -syn aggregates* on page 8), it has been suggested that synaptic dysfunction due to α -syn aggregation at the presynaptic terminal, and not Lewy pathology, causes the neurodegeneration with loss of postsynaptic dendritic spines and neurotransmitter deficiency in PD (17, 37). Synucleins are likely involved in and regulate presynaptic vesicle endocytosis (68). There is evidence for reduced endocytosis in early PD prior to aggregation of α -syn pathology, supporting the hypothesis that synaptic dysfunction occurs prior to neuronal loss (69). Accumulation of (oligomeric) α -syn may mediate the early neurodegeneration by disrupting synaptic vesicles and SNARE-dependent synaptic vesicle fusion (70, 71) and by impairing neurotransmitter release leading to postsynaptic dendritic spine loss (17, 37), synaptic dysfunction and loss of synaptic markers such as synaptophysin (37, 70) and neurogranin. In vivo imaging of synaptic function has shown striatal presynaptic dysfunction and dopaminergic deficiencies in PD and PDD (72).

A β , especially A β oligomers, and phosphorylated tau (P-tau) may disrupt synaptic plasticity in PD as in AD, leading to synaptic loss and subsequently neuronal network alterations and cognitive dysfunction (13-16, 59, 73). Experiments on transgenic mice have shown hippocampal synaptic dysfunction related to increasing levels of soluble A β at A β levels too low for plaque deposition (74). It has been hypothesized that increased presynaptic activity

leads to increased endocytosis that recycle the vesicular membrane (including the synaptic vesicle-related proteins synaptophysin and synaptotagmin) from the cell surface after neurotransmitter release, and that more APP is endocytosed as well, resulting in increased A β production and release into the interstitial fluid. A β , especially oligomers, released from a neuron might be able to inhibit the activity in the same neuron, and thus modulate synaptic activity (14).

Neurogranin, a substrate of protein kinase C, is concentrated below the postsynaptic dendritic spine plasma membrane (75). It is involved in signal transduction (76), plays a role in synaptic plasticity and may interact with α -syn (77). This putative interaction decreases in PD along with decreased phosphorylation of neurogranin, a process thought to be involved in learning and memory. The cellular (pre- versus postsynaptic) location of interaction is so far undetermined. Thus, neurogranin may be involved in pathways leading to dementia. (77) A post-mortem study suggests that loss of synaptic markers reflecting synaptic degeneration predicts cognitive decline in Lewy body diseases (78).

Protein degradation by the ubiquitin-proteasome (UPS) and lysosome-autophagy systems is crucial for the function of neurons and for remodeling of synapses as part of synaptic plasticity, the process of modifying synaptic connections in response to prior activity underlying learning. Proteasomal degradation may control synaptic vesicle cycling and limit vesicle release. Defective presynaptic proteolysis in AD and PD may affect both A β , tau and α -syn metabolism (15); A β may impair lysosomal degradation, and deficient autophagy of APP may contribute to increased levels of pathogenic A β . Tau impairs the proteosomal degradation leading to decreased degradation of P-tau and increased formation of neurofibrillary tangles. Pathogenic α -syn may inhibit proteasomes and lysosomes, impairing its own degradation.

Interactions and synergistic effects between α -syn, A β and tau pathologies

Cortical A β plaques and neurofibrillary tangles correlate with limbic and neocortical Lewy pathology (6, 8, 30, 41, 57, 79-81), and the mixed cortical pathology were greater in patients with PDD (79). Two small neuropathological studies in PD-MCI have shown coincidence between Lewy bodies and A β plaques (66, 67). A β , tau and α -syn are aggregated in the same brain regions and cells, and both tau and α -syn are found in both neurofibrillary tangles and Lewy bodies (34). Transgenic mouse models with the combination of α -syn and tau or

amyloid pathology have shown greater neurodegeneration than AD pathology alone (6, 82). There are also similarities in the processing and metabolism of α -syn, A β and tau (15, 28, 83-85).

These observations suggest shared underlying mechanisms and synergistic effects (31). In vivo and in vitro studies show that α -syn, A β and tau, in particular oligomeric forms, may interact by synergistic pathways, each enhancing pathological modifications and aggregation of the other (6, 30, 34, 41, 82, 86-93). Intracellular A β 42 and α -syn cooperate to produce toxic α -syn oligomers (71).

Pathophysiology of neurotransmitters and cortical neural networks

There is a loss of limbic and cortically projecting dopamine, acetylcholine, noradrenaline, serotonin and glutamate neurons in PD (41) and dysfunction of cortical neural networks with subsequent cognitive impairment and dementia (9). In PD-MCI neurotransmitter dysregulation rather than neurodegeneration has been proposed to play the most important role, especially dopamine depletion in the frontostriatal pathway (41) .

Frontostriatal, mesocortical and mesolimbic dopaminergic networks

Cognitive impairment in early PD is associated with impaired nigrostriatal dopaminergic function (Braak stage 3) leading to abnormal cognitive processing in the frontostriatal dopaminergic network with reduced prefrontal and parietal activity (9, 57) and subsequent executive dysfunction (concerning set shifting and working memory) (9).

Lewy pathology and loss of mesocortical dopaminergic network neurons from the ventral tegmental area (VTA) in the mesencephalon to neocortical (prefrontal, insula and cingulate) regions in PDD contribute to executive impairment such as reduced cognitive flexibility (9, 41).

The mesolimbic dopamine network neurons project from the medial substantia nigra and VTA to the limbic regions, nucleus accumbens and hippocampus and are involved in reward behavior, impulsivity and cognition (41). Degeneration of neurons from the medial substantia nigra is associated with PDD (41, 57).

Nucleus basalis of Meynert and the cholinergic network

Lewy pathology is present in the NBM in the basal forebrain in early PD (Braak stage 3) (9). This correlates with the degeneration of the cholinergic network neurons in the NBM projecting to the entire neocortex, limbic regions, hippocampus and nucleus accumbens, which is commonly seen in advanced PD and PDD, and is associated with cognitive impairment in several domains (6, 9, 41): Cortical cholinergic dysfunction is associated with attention deficits, which contribute to deficits in executive functions such as working memory, rule-switching and response inhibition. Dysfunction in this network may impair encoding of memories and visuospatial functions as mentioned in the next section. Dysfunction in cholinergic projections to the posterior visual cortices may contribute to visuoperceptual dysfunction and visual hallucinations in PDD. The cholinergic network also plays a central role in arousal and REM sleep, thus cholinergic dysfunction may lead to impaired vigilance, cognitive fluctuations, visual hallucinations and RBD. In addition, PIGD may be associated with cholinergic dysfunction. (9)

The posterior visual processing network

Lewy pathology is found in both parietal and temporal limbic cortices involving the posterior visual processing network. Reduced activation, dysfunction in cholinergic projections as mentioned in the previous section and atrophy in these cortical areas are probably associated with impairment in visuospatial and visuoperceptual functions and visual hallucinations in patients with PD, PD-MCI and PDD (9).

Locus coeruleus and the noradrenergic network

Degeneration of noradrenergic network neurons associated with Lewy pathology in locus coeruleus (Braak stage 2) is common in PDD. The noradrenergic neurons project to the forebrain and also influence the substantia nigra and thalamus pathways affected in PD, and NBM and the limbic and subcortical-cortical pathways affected in PDD (9, 41).

Noradrenaline release in the prefrontal cortex facilitates attention and cognitive flexibility, and degeneration of this system may lead to deficits in executive functions such as rule-shifting, response inhibition and working memory, and may also play a role in the generation of visual hallucinations (9).

Raphe nucleus serotonergic network

Serotonin neurons project from the raphe nucleus in the brain stem to the (frontal) cortex, nucleus accumbens and hippocampus. There is found early Lewy pathology in the raphe nucleus (Braak stage 2), and there may be a degeneration of serotonin neurons in end-stage PD and reduced serotonin innervation in PDD, but it is not clear whether this relates to cognitive impairment (41).

Cortico-subthalamic glutamatergic pathway

There are glutamatergic efferents from cortex and thalamus to the subthalamic nucleus and striatum (94). Glutamate is the main excitatory neurotransmitter in the CNS, released from presynaptic glutamatergic neurons and stimulating postsynaptic glutamate receptors. Glutamate transporters in the synapses are important for glutamate uptake as excessive glutamate stimulation induces toxic effects on the CNS including dopamine neuron death in substantia nigra, parkinsonism and cognitive impairment. Glutamate transporters are involved in regulating learning, memory and motor behavior. There is evidence of dysfunctional glutamate transporters in PD, but it is unclear whether this is a cause or a consequence of PD (95).

The cortico-subthalamic glutamatergic pathway probably works by turning on the basal ganglia circuitry, and cortico-subthalamic overactivation could induce parkinsonian symptoms (54, 96). Dopamine reduces glutamatergic activity in the nucleus subthalamicus (94). The nigrostriatal dopaminergic pathway modulate corticostriatal glutamatergic transmission in striatum (97) and is implicated in learning and neural plasticity (94).

The therapeutic effect of DBS targeted to the subthalamic nucleus is partly mediated by glutamatergic cortico-subthalamic synaptic depression (98). Glutamate dysfunction has been associated with cognitive impairment, and the N-methyl D-aspartate (NMDA) glutamate receptor antagonist memantine is used to treat AD (99).

The frontoparietal network

The frontoparietal network encompasses prefrontal and posterior parietal cortices and is affected in PD-MCI and PDD. Lewy pathology has been shown in these areas in PDD. FDG-PET and MRI have shown progressive hypometabolism followed by atrophy corresponding to

executive deficits in PD-MCI and PDD. This suggests a progressive degeneration leading to loss of functional connectivity within the frontoparietal network associated with attentional dysfunction in the development of PDD. Dysfunction in this network may also contribute to visual hallucinations. (9)

The dual-syndrome hypothesis

The dual-syndrome hypothesis suggests that dopamine deficiency in the frontostriatal pathway causes stable MCI due to affection of executive functions in early PD, while widespread α -syn and Lewy pathology and Lewy pathology in the NBM with dysfunction in temporal and posterior cortical cholinergic networks lead to more rapid cognitive decline in memory and visuospatial functions, and development of dementia (8, 9, 100-102).

Pathophysiology of the medial temporal lobe network

Anatomy

The medial temporal lobe (MTL) structures (hippocampus, parahippocampal region and amygdala) are involved in memory storage and retrieval (9), and much of our knowledge concerning hippocampal structure and function described here is based on studies in monkeys and rodents (103). The hippocampal formation is a 5 cm long C-shaped structure in the MTL consisting of dentate gyrus (DG) with the innermost layer cornu ammonis (CA) 4, CA 3-1, and the subicular complex consisting of subiculum and presubiculum. The parahippocampal region consists of entorhinal cortex (ERC, corresponding to Brodmann area 28 and 34), perirhinal cortex (PRC, Brodmann area 35 and 36) and parahippocampal cortex. (103, 104) The transentorhinal cortex is the medial part of PRC (Brodmann area 35) (105).

The hippocampus receives extrinsic input from various cortical regions, orbital frontal cortex, amygdala, retrosplenial cortex, the septal region, thalamus, supramammillary region and brainstem (locus coeruleus, raphe nuclei and VTA) (103, 104). Sensory information from cortical regions project directly or through PRC and parahippocampal cortex to the lateral and medial ERC and subiculum (103).

The perforant pathway projects from the superficial input layers II and III of ERC to DG, CA3, CA1 and subiculum (103, 106). All the hippocampal subfields are connected in the polysynaptic pathway in an unidirectional route (106); DG is connected to CA3 by mossy

fibers, Shaffer collaterals project from CA3 and CA2 to CA1, and CA1 project to and forms a transitional zone with subiculum (103, 106). CA1 and subiculum projects to presubiculum and back to the deep layers V and VI of ERC (106).

The ERC projects back to PRC and parahippocampal cortex, which project to orbital and medial prefrontal cortices respectively (103, 104). Most of the cortical regions have reciprocal connections with the ERC, which influence temporal, parietal and frontal regions (103). There are also direct cortical output projections from the subicular complex (to association cortices, PRC, parahippocampal gyrus, cingulate gyrus, medial orbitofrontal cortices, the septal complex, nucleus accumbens, retrosplenial cortex, thalamic nuclei, mammillary nuclei, orbital and medial prefrontal cortex and amygdala) and from CA (to association cortices, the septal complex, nucleus accumbens, prefrontal cortex and amygdala) (103, 104). The hippocampal formation also has projections to striatum. Commisural projections between presubiculum and contralateral ERC may represent the major link between the two hippocampi (103).

Physiology

CA1, CA3, subiculum, presubiculum and ERC are involved in memory, spatial information and navigation (106):

The ERC conveys and associates spatial and non-spatial information about external stimuli essential for declarative memory and the function of the hippocampal formation (106, 107). The hippocampal formation processes and retrieves details of this information (106).

CA3 may be essential for encoding and storing of episodic memories (106). The projections between CA1 and subiculum and the parahippocampal region are involved in sensory processing and integration of information. The projections between CA (mainly CA1) and the subicular complex (mainly subiculum) and amygdala, nucleus accumbens, medial and orbital prefrontal cortex are involved in affective and social learning, memory, emotion and motivation. (104)

The projections between subiculum and presubiculum and the mammillary bodies, retrosplenial cortex and anterior thalamic nuclei are important for episodic memory and recollective-based recognition and spatial learning and also involves the head-direction system (104). Head-direction cells in presubiculum, ERC and the mammillary bodies, which

provide information about the head's direction in the environment, are important for place- and grid cell functioning. Place cells in CA1, CA3 and (pre)subiculum provide information about past, present and future specific locations in an environment. They connect to grid cells in medial ERC, which provide information about one of several specific locations (106). Place cells, grid cells and head-direction cells play different roles in location and spatial memory (107).

The projections between the parahippocampal region, including PRC, and the prefrontal cortex and medial dorsal thalamic nucleus are important for familiarity-based recognition and memory retrieval and visual recognition memory (104).

Pathology

In Braak stage 4, the Lewy pathology is distributed to the temporal mesocortex with the transentorhinal region and CA2 of the hippocampus, and in stage 5 Lewy pathology also extends into CA1 and CA3 (39). According to this, Lewy pathology is found in CA2-3 in PD without dementia and even more in PDD (108-110) and in transentorhinal cortex and ERC in PDD (111-114).

A β plaques and depositions are found in CA1, CA2 and ERC in PDD (113-116). There are also neurofibrillary tangles and tau, initially in the transentorhinal cortex, but also in ERC in non-demented patients (79, 103, 117), and CA1 and CA2 in PDD (114, 115).

Other pathologies

The role of cerebrovascular disease in cognitive impairment in PD is undetermined, but cerebral amyloid angiopathy has been associated with both PD, PDD and Lewy and AD pathologies (41). Other mechanisms may involve neuroinflammation (8) (as previously described under *Pathogenesis and pathology* on page 7). Increased microglial activation is thought to lead to cell death (31), but whether hippocampal sclerosis (gliosis and loss of neurons in CA1 and subiculum) is associated with PDD is unclear (41).

Epidemiology

PD-MCI

The frequency of MCI among patients with early PD is about 15-19 %, which is a twofold increase compared to the rest of the population in the same age groups (118, 119).

Approximately 20-40 % of all PD patients without dementia have MCI, with the highest proportion in advanced PD (3, 4, 31, 120). There is an increased risk for developing dementia in PD-MCI (3, 5), although some remain stable, 20-60 % develop dementia within 2-5 years (3).

PDD

Patients with PD have about 4-6 times increased risk of developing dementia compared to non-PD subjects (5, 8, 56). The point prevalence of dementia in PD is about 30 % and the cumulative prevalence is about 80 % after 20 years (5). The incidence of dementia in PD is approximately 10 % (5, 56). The mean time from onset of PD to development of dementia is about 10 years, although there are wide variations (5, 31).

Clinical symptoms and diagnostic criteria

PD-MCI

Subjective cognitive impairment (SCI) where the patient, family members or health personnel register cognitive decline, but the patient performs within the normal range on cognitive tests, may precede MCI (31).

Cognition is affected already in newly diagnosed, untreated PD, including attention, psychomotor speed, executive functions such as set-shifting, planning and abstract reasoning, visuospatial abilities and memory functions such as verbal recall and visual memory (121).

We and others have shown that the profile of cognitive impairment in PD is characteristic and differs from that of AD. Cognitive domains that are relatively more affected are attention and working memory, psychomotor speed, executive, memory and visuospatial functions (55, 122), while AD patients show earlier problems in memory, semantic verbal fluency and language (121).

Non-amnestic single domain MCI (3) with executive, attentional or visuospatial dysfunctions or memory impairment is most common, while language is less affected (120). Recall is more affected than recognition suggesting difficulties with spontaneous retrieval affected by prefrontal executive impairment (9). Verbal fluency may be affected, phonemic more so than semantic fluency in the earliest stages (121). According to *The dual-syndrome hypothesis* (described on page 21), dopaminergic frontal executive dysfunction related to disrupted dopaminergic frontostriatal network is relatively stable, while there is a more rapid cognitive decline in cholinergic posterior-cortical memory and visuospatial functions related to cortical pathology with development of dementia (8, 9, 120, 122, 123).

The Movement Disorder Society has proposed detailed diagnostic criteria. There should be a gradual subjective decline reported by patient, caregiver or clinician. Level 1 requires in addition impaired performance on a scale of global cognitive functions validated for use in PD (for example Montreal Cognitive Assessment, MoCA (124)) or on at least two tests in a limited neuropsychological test battery. Level 2 requires impairment on at least two neuropsychological tests within the five cognitive domains (attention, executive, memory, visuospatial and language). The cognitive impairment is not sufficient to interfere significantly with functional independence, and other reasons that better explain the cognitive decline must be excluded (55, 57).

PDD

Attention, which may fluctuate, executive functions, such as verbal fluency, concept formation, set shifting, working memory, cognitive flexibility, response inhibition, and visuospatial perception and construction are impaired in PDD, probably to a greater degree than in AD in mild to moderate stages (9, 56, 57). Bradyphrenia (impaired mental speed) is common (125). In addition, verbal and visual short-term memories are also impaired, but probably less widespread and severe than in AD, and recognition may be less affected than recall in early and middle stages (9, 56, 57). In advanced stages, there are also difficulties with recognition, especially visual recognition memory, suggesting additional MTL memory storage dysfunction. This is in accordance with the dual-syndrome hypothesis mentioned above, suggesting that executive dysfunction due to dopamine depletion in the frontostriatal pathway causes stable MCI, while widespread cortical cholinergic dysfunction contribute to cognitive impairment in PDD. (9)

There is also probably less affection of language functions such as aphasia, anomia and spontaneous speech in PDD compared to AD (56, 57), but it could be associated with difficulties in phonemic verbal fluency related to executive dysfunction as mentioned above (6, 9), and semantic verbal fluency related to affection of the temporal lobe as well (121).

Insight is initially preserved in PDD, but most patients will lose it after a few years (9). There is an increased morbidity and mortality, and life is shortened by about 4 years (125).

The Movement Disorder Society has proposed clinical diagnostic criteria for PDD (56). There must be a slow progression with deficits in at least two of the four cognitive domains attention, executive, memory and visuospatial functions severe enough to significantly affect activities of daily living such as personal care, housekeeping, social and occupational activities. The diagnosis of PDD should be made when dementia develops more than one year after the onset of the parkinsonian motor symptoms, otherwise patients will fulfill criteria for DLB (31). DLB is associated with early fluctuating cognition and visual hallucinations (57).

Neuropsychiatric symptoms

Depression, anxiety and apathy are common in PD (56) and PDD (125), and these symptoms may also affect the ability to attention, important for memory and executive functions (121).

Approximately 30-40 % of patients with PD have depression, with a similar percentage of anxiety or apathy. The symptoms may fluctuate in relation to off-periods. Depression may be reactive to the diagnosis, but is also a consequence of the disease due to dopaminergic, serotonergic and noradrenergic mechanisms. Symptoms of depression are low mood, lack of interest or pleasure, altered appetite, sleep disturbances, weight change, psychomotor slowness, reduced memory and loss of energy, some of the symptoms which also can be seen in PD without depression. Anxiety, which may be related to depression, may present as panic attacks, generalized anxiety or (social) phobias (126).

Apathy and fatigue are related to basal ganglia and frontal-subcortical disturbances, and are associated with depression and cognitive impairment, but can also overlap with motor symptoms such as bradykinesia and bradyphrenia. Apathy comprises reduced interest, motivation and emotions and indifference. Fatigue involves physical lack of energy, tiredness and exhaustion, and is associated with attentional difficulties. (126)

Hallucinations, especially visual ones, are quite common, affecting more than half of the patients with PDD and are together with psychosis risk factors for developing dementia in PD (9, 56, 57). Visual hallucinations often involve persons at night, and the patient may have insight that it is not real (126). In contrast, delusions are less frequently seen in PDD compared to AD and DLB (56), and may appear as the patient believes the spouse is another person or unfaithful or persecutory delusions. Psychotic symptoms are related to cognitive impairment, depression, anxiety and sleep disturbances, cholinergic deficits and dopaminergic treatment (126). The pathophysiology probably involves pathological changes and Lewy bodies within cortical visual processing pathways (99).

Treatment

Information about the disease, avoidance of aggravating factors such as drugs that may worsen cognitive function (for example anticholinergic agents) and disturbances in the environment, treatment of co-existing illness that may worsen cognition and cognitive and physical activities may be important to preserve the function as long as possible (9, 99, 127, 128). Dopaminergic drugs have shown conflicting positive and negative effects on cognitive functions in PD and PDD (9, 127), but may improve cognition in the earliest stages, while in advanced stages with on-off motor fluctuation cognition may be impaired (121).

There is currently no neuroprotective or disease-modifying treatment, but in PDD symptomatic treatment with acetylcholinesterase inhibitors should be considered (6, 31, 99, 127, 129). Acetylcholinesterase inhibitors block the degradation of acetylcholine, decrease the cortical cholinergic deficit and increase prefrontal and parietal cerebral metabolism (129). Both rivastigmine and donepezil have documented effects on global assessment, cognitive function, behavioral disturbances and activities of daily living in PDD (9, 130-132). There is currently not sufficient evidence to support the use in PD-MCI (127, 130). Rivastigmine may be more efficient (127, 132), but mild to moderate adverse effects such as transient increasing tremor were more frequently seen (130, 132). Other frequent adverse effects are nausea and vomiting (99). Rivastigmine and donepezil may also decrease the mortality (130-132). Acetylcholinesterase inhibitors may also improve psychomotor slowing, apathy and anxiety and reduce visual hallucinations and delusions (125, 127).

There may be glutamatergic overactivity in PDD. Memantine, an NMDA receptor antagonist reducing the concentration of glutamate, slightly improved global assessment, but not Mini-Mental State Examination (MMSE), in one meta-analysis and could not be recommended to be used in PDD (132).

Other possible treatments with different points of attack such as different neurotransmitters or immunotherapies targeting A β , α -syn or tau are under investigation (31).

Treatment of concomitant neuropsychiatric symptoms such as depression, anxiety, apathy and psychosis is also important. In mild depression, information and cognitive behavioral therapy may be sufficient. Optimization of dopaminergic treatment with for example Pramipexole may improve depressive symptoms as well (126), but increases the risk for hallucinations. Selective serotonin reuptake inhibitors (SSRIs) such as paroxetine or citalopram and serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine are possible alternatives in treating depression (25), apathy and anxiety (125). Tricyclic antidepressants should be avoided due to their anticholinergic effects. In case of hallucinations or psychosis, one should try to reduce dopaminergic treatment, especially dopamine agonists, MAO-B inhibitors and anticholinergics. (57) Information, distraction, environmental adaptations and treatment of concomitant diseases are important (126). Atypical antipsychotic drugs which are less likely to exacerbate parkinsonism or cause malignant neuroleptic syndrome such as quetiapine are often used first line, while clozapine needs monitoring for agranulocytosis (25, 99). Quetiapine and clozapine may also be used for agitation (125).

3.2.3 Potential biomarkers of cognition in Parkinson's disease

Definition

A biomarker is defined as an objectively measurable indicator of normal or pathological biological processes or pharmacological responses to a therapeutic intervention (133). Biomarkers may have diagnostic or prognostic potentials and may link biochemical and molecular pathogenesis to clinical manifestations (30). A biomarker should possess high sensitivity and specificity, be reliable and reproducible and preferably easy to perform and inexpensive (60). There is a need for early diagnostic and prognostic biomarkers of cognitive impairment in PD.

Cerebrospinal fluid biomarkers

The CSF is in close contact with the extracellular space of the brain, and about 20 % of the CSF proteins are released by cells of the CNS (30, 134). CSF biomarkers are therefore believed to reflect pathological changes in the brain (18). For instance, decreased CSF A β 42 and increased total tau (T-tau) and P-tau representing A β plaques, neuronal damage and neurofibrillary tangles respectively, are established biomarkers of AD even before the development of dementia (60, 135, 136) and may predict cognitive decline and dementia in cognitively normal elderly (60). Since α -syn-related Lewy pathology, A β plaques and tau neurofibrillary tangles are found in the cortex of patients with cognitive impairment in PD, changes in the CSF levels of α -syn, A β and tau are potential biomarkers.

α -synuclein protein

The levels of total α -syn (t- α -syn) have been measured in the CSF. While most studies have shown lower levels in PD and other synucleinopathies, others have found similar levels as in controls (18, 29-31, 137-139). The inconsistent findings may be due to heterogeneity of patients and controls, methodological differences and blood contamination, which increases the concentration of t- α -syn in the CSF as α -syn is abundant in red blood cells (29).

Soluble oligomeric α -syn (o- α -syn), which may be toxic to neurons, and phosphorylated α -syn (p- α -syn) have been found to be elevated in the CSF in PD patients compared with controls, but the results are inconsistent (18, 29, 30, 137-140).

Studies examining whether CSF α -syn species are associated with cognitive impairment in PD have been inconsistent (18, 31, 140), and both low and high values of t- α -syn have been associated with cognitive decline in PD (31). This is thoroughly reviewed in *CSF T- α -syn and cognition* on page 95.

Amyloid- β peptides

CSF A β 42 levels are reduced years and even decades before development of cognitive decline and dementia in AD (141, 142).

Although some studies have found similar levels of A β 42, most studies have found a slight decrease in A β 42 in PD and even lower levels in PD patients with cognitive impairment and dementia compared with controls (18, 29, 138, 140, 143), but not as low as in AD (138).

Lower levels of A β 42 have also been associated with cognitive impairment in different domains and may predict cognitive decline in PD (18, 29-31, 138, 140). This is carefully reviewed in *CSF biomarkers in relation to cognition in PD without dementia* on page 36 and *CSF A β species and cognition* on page 93.

CSF concentrations of A β 38 and 40, which are less prone to aggregate, have also been found to be reduced in de novo PD and associated to cognition (144).

Phosphorylated tau and total tau proteins

Tau is released into the extracellular space, may be increased in the CSF after neuronal damage (18) and is a marker of ongoing neuronal degeneration (65). P-tau measured in the CSF is usually phosphorylated at position Thr181 (30). Most studies have reported no changes in CSF P-tau or T-tau levels in PD without dementia compared to controls, although others have found decreased or increased levels (18, 29, 138). P- and T-tau levels are usually found to be elevated in PDD compared to PD and controls, while other studies have found similar levels (18, 29, 143). Only a few studies have found associations between tau levels and cognitive impairment, while others not have found this (18, 29, 140) as described thoroughly in *CSF biomarkers in relation to cognition in PD without dementia* on page 36 and *CSF T-tau and P-tau and cognition* on page 94.

Neurogranin

In AD, increased CSF levels of neurogranin are thought to reflect synaptic degeneration with loss of integrity of postsynaptic dendritic spines in the cortex and hippocampus and may be used as a synaptic biomarker in prodromal AD (75, 145-147). To our knowledge, only two studies have assessed the levels of CSF neurogranin in PD with conflicting results. The study in longer-lasting PD did not find any difference from controls (145), while the other study found increased neurogranin levels in drug-naïve PD, associated with reduced cognition (MoCA) and more advanced motor stage (148).

Structural MRI

Structural MRI is used to detect cerebral atrophy, and hippocampal, medial temporal lobe or parieto-occipital atrophy on MRI are validated biomarkers for AD-MCI and ADD (135, 136).

Methods

Volumetric region-of-interest (ROI) analysis measures specific anatomic structures according to an a priori hypothesis. This may be done manually, which is the gold standard, but time-consuming and with rater variability, or by automated segmentation protocols, for instance hippocampal segmentation with FreeSurfer software. Cortical thickness analysis using FreeSurfer is sensitive to early grey matter changes. Voxel-based morphometry (VBM) is an automated technique analyzing the entire grey matter by comparing tissue volume or concentration differences on a voxel by voxel basis.

MRI atrophy and associations with cognitive impairment in PD

In early PD with normal cognition, cortical atrophy is usually not observed with VBM, but cortical thickness analyses may be more sensitive, and widespread cortical thinning has been described in frontal, temporal, parietal and occipital lobes. In longer-lasting PD with normal cognition, VBM also shows some cortical atrophy. (21) Small hippocampal volume may predict development of MCI and dementia in PD (31, 149).

In PD-MCI, VBM has shown grey matter atrophy in frontal, temporal and parietal lobes and also hippocampal atrophy has been reported (21, 150, 151). Using FreeSurfer, frontal, parietal, temporal and occipital atrophy predicted cognitive impairment in early PD (152) and parietotemporal atrophy was associated with worse cognition (153). Parietotemporal and hippocampal atrophy were also associated with and predicted cognitive impairment in PD (154). Regional atrophy has been correlated with relevant cognitive impairments (21, 31), for example using ROI techniques and visual rating scales, hippocampal atrophy has been associated with impairment of verbal memory in PD without dementia (19, 20). Hippocampal volume has been correlated to memory in PD and PD-MCI (150). Using VBM, semantic fluency and executive dysfunctions have been associated with gray matter atrophy in frontal and temporal areas, while visuospatial and -perceptual dysfunctions mainly have been associated to temporoparietal cortical atrophy including parahippocampus (19, 155).

In PDD, VBM has shown widespread cortical atrophy with bilateral gray matter loss frontally, temporally and occipitally, with less marked changes parietally (21, 150, 151). Both ROI analyses and VBM have found hippocampal, limbic (ERC, amygdala and anterior

cingulate gyrus), caudate nucleus, putamen and thalamus atrophy in PDD (20, 21, 156). In ROI analyses, hippocampal atrophy has been correlated with impaired memory (21).

Across all stages of PD, ROI analyses have shown atrophy of the substantia innominata, where the cholinergic NBM is located (21), and the atrophy has been correlated with poorer attention, executive function and verbal fluency (157).

Longitudinal studies have described higher rates of atrophy in PDD compared to controls, but results in PD without cognitive impairments have been conflicting (20, 21).

Another study emanating from the same project as this thesis, found that the PD patients had reduced white matter integrity underlying the temporal, parietal and occipital cortex as compared with controls using diffusion tensor imaging (DTI). White matter integrity changes underlying the prefrontal cortex correlated with executive and visuospatial abilities, suggesting that also white matter changes are related to cognitive impairment in PD (158).

MRI atrophy and associations with CSF biomarkers in PD

Few studies have assessed cortical atrophy in relation to CSF biomarkers in PD. Using VBM grey matter volume loss in areas within mainly frontal, temporal and parietal lobes were found to be associated with lower A β 42 and higher P-tau and T-tau in PD with and without dementia. Losses within these areas were also associated with poorer cognition in memory, naming, fluency and visuospatial functions. (159) A longitudinal study in non-demented PD patients assessing cortical thickness with FreeSurfer showed that low baseline A β 42 was associated with a thinner parietal cortex at baseline, and prospectively associated with parietal and occipital thinning (160). Both lower A β 42 and cortical thinning in frontal and anterior cingulate at baseline were predictors of development of dementia. Dementia-converters had significantly more longitudinal thinning in frontal, temporal, anterior cingulate and parietal areas. Using the same cortical thickness analysis, lower t- α -syn, but not o- α -syn, was found to be associated with frontal cortical thinning in PD patients without dementia, while higher tau correlated with posterior cortical thinning in PDD (161).

Enlargement of the lateral ventricles has been used as a measure of atrophy in AD and has also been reported in both PD-MCI and PDD (21), and is associated with poorer cognition (162). Radial distance thickness analysis of the lateral ventricles and hippocampus has shown

associations between lower CSF A β 38, 40 and 42 and T-tau and lateral ventricular enlargement, but not hippocampal atrophy, in early PD patients with and without MCI (163).

¹⁸F-fludeoxyglucose positron emission tomography (FDG-PET)

FDG-PET measures the resting cerebral glucose metabolism using a radioligand, reflecting local neuronal activity and synaptic density and activity (102), as shown by the strong correlation with the synaptic protein synaptophysin (164). Cortical hypometabolism could reflect either local affection at the cortical synapses or a diaschisis effect resulting from affection of different neurotransmitter networks.

There is widespread cortical hypometabolism, most prominently in frontoparietal and occipital cortices, probably even in early stages of PD and in patients without cognitive impairment before the development of substantial atrophy (22, 165).

In non-demented PD patients, hypometabolism in frontal, parietal, occipital and temporal areas is associated with cognitive impairment including memory, visuospatial and executive dysfunctions. There is more widespread hypometabolism in multiple-domain compared to single-domain PD-MCI. (166-168) A longitudinal study found hypometabolism in occipital (especially visual association cortex) and posterior cingulate cortices at baseline in those who converted to dementia (169).

There is progressive hypometabolism with increasing disease duration and severity (22) and in PDD similar to, but with a more pronounced occipital hypometabolism and less marked hypometabolism in MTL compared to AD (167, 170).

3.2.4 Choice of research questions and central variables

With this background introduction in mind, there is a lack of knowledge of the mechanisms leading to the development of cognitive impairment in PD and a lack of early predictive biomarkers. Therefore, we wanted to explore the relationship between cognition, CSF, MRI and PET biomarkers in early PD patients.

CSF biomarkers in relation to cognition

As described in *Cerebrospinal fluid biomarkers* on page 29, α -syn, A β and tau dysmetabolism in the cortex are probably involved in the development of dementia in PD, suggesting partially common underlying mechanisms with AD and possible synergistic effects. At the start of this project, very few studies had actually examined whether there is a relationship between these neuropathologies and cognitive impairment at an early stage of PD (see *CSF biomarkers in relation to cognition in PD without dementia* on page 36). Therefore, we wanted to explore whether CSF A β species, T-tau, P-tau and t- α -syn, believed to reflect the pathological processes in the brain, are associated to cognition in early PD patients and thus may be potential early biomarkers of dementia.

Since there may be overlapping pathological pathways in PD and AD, we also wanted to examine whether it is possible to distinguish a CSF biomarker profile of early PD from non-PD MCI with increased risk of developing AD as this has not been described before.

Hippocampal and parahippocampal sub-regions in relation to CSF biomarkers and cognition

As described under *Pathophysiology of the medial temporal lobe network* on page 21, both α -syn, A β and tau pathologies have been found in the hippocampus and parahippocampal region respectively. MRI has shown atrophy in these areas related to cognitive impairment in PD patients with and without dementia. Few studies have assessed whether hippocampal and parahippocampal sub-regions are smaller and related to cognition in early PD (as described in *Hippocampal and parahippocampal sub-regions in relation to cognition and CSF biomarkers in PD* on page 44). To date no one has examined the relationship between the hippocampal and parahippocampal sub-regions and α -syn, A β and tau pathology as reflected by the CSF biomarkers in PD. Therefore, we wanted to explore whether the hippocampal and parahippocampal sub-regions at MRI are smaller, and whether these structures are associated to the CSF biomarkers or cognition in early PD and thus may be used as early biomarkers of cognitive impairment.

Cortical glucose metabolism in relation to CSF A β , t- α -syn and neurogranin and cognition

FDG-PET studies have shown widespread cortical hypometabolism, probably also in early PD, and this has been related to cognitive impairment in several domains as described in *¹⁸F-fludeoxyglucose positron emission tomography (FDG-PET)* on page 33. CSF A β and possibly also t- α -syn levels, presumed to reflect the A β and α -syn metabolism in the brain, have also been associated to cognitive impairment in PD as described in *CSF biomarkers in relation to cognition in PD without dementia* on page 36. Both A β , α -syn and neurogranin are located at the synapses and may be related to synaptic activity and dysfunction as reflected by cortical glucose metabolism as described in *¹⁸F-fludeoxyglucose positron emission tomography (FDG-PET)* on page 33. Thus, we hypothesize that synapse dysfunction is a common link between cortical hypometabolism and the synaptic markers CSF A β , t- α -syn and neurogranin.

To our knowledge, no one has examined the relationship between cortical hypometabolism and A β , t- α -syn or neurogranin. Therefore, we wanted to examine whether regional cortical hypometabolism as measured by FDG-PET may be associated with CSF A β , t- α -syn or neurogranin. Further, we wanted to examine whether there are correlations between regions with the synaptic markers associated FDG-PET uptake and cognition, relating the synaptic markers to development of cognitive impairment through reduced glucose metabolism.

Finally, we wanted to investigate whether the level of CSF neurogranin as a marker for postsynaptic dendritic spine integrity is changed in early PD. We also wanted to explore whether there are correlations between neurogranin and A β , α -syn and clinical relevance with PD motor stage, as this may contribute to the understanding of synaptic dysfunction.

3.3 Literature review

This is a presentation of the most relevant literature published at the beginning of the project including primary studies with similar research questions as in the current project. Literature searches were carried out using PubMed.

3.3.1 CSF biomarkers in relation to cognition in PD without dementia

Inclusion and exclusion criteria

Studies exploring the relationship between the CSF biomarkers A β , T-tau, P-tau or t- α -syn and cognition in PD patients were considered for this review. Searches were made in PubMed with the research words “((parkinson) AND (cerebrospinal fluid OR CSF)) AND (cognition OR cognitive)”. In addition, other relevant studies were also included. Only studies including PD patients without dementia at baseline and both cross-sectional and longitudinal studies were included, as we were interested in early and potential predictive biomarkers of cognitive impairment.

Relevant publications at the start of the project

At the startup of this project in 2011, only 4 studies assessing the relationship between CSF biomarkers and cognition in PD had been published (see *table 1* on page 38): One study found association between lower A β 42 and poorer phonetic fluency, but not memory, in the PD group, and no association in the control group (171). The Norwegian ParkWest study was the largest and only study in early PD, and it showed associations between A β 38, 40 and 42 and verbal memory, but not semantic fluency (144). The only longitudinal study did not find any association at baseline, but lower A β 42 was associated with decline in attention, memory and conceptualization at mean 1.5 years follow-up (172). The fourth study found correlation between A β 42 and processing speed, but this was not longer significant after adjusting for age (173). None of these four studies found associations between T-tau or P-tau and cognition.

Conflicting results

The studies were partially conflicting, but most of them suggested that lower A β was associated with poorer cognition (144, 171, 172). Only one study did not find any association after adjusting for age, but this included patients with the mean longest education (173) which may mask early cognitive impairment. The different results may be due to differences between patient cohorts, the CSF biomarker analysis methods and neuropsychological tests.

Limitations

Limitations of the studies were the relatively small sample sizes (171-173), cross-sectional design (144, 171, 173), short follow-up in the longitudinal study (172), recruitment from Movement disorders units (171, 172) instead of population-based recruitment (144), no assessment of associations between CSF biomarkers and cognition in healthy controls (144, 172, 173) and no pathological confirmation of clinical diagnosis in any of the studies. Only one of the studies assessed patients with early, unmedicated PD and A β 38 and 40 (144), and none of them measured t- α -syn.

Potential contribution of current project

We wanted to examine whether CSF biomarkers are associated to cognition at an early stage of PD before development of dementia. We would clarify whether CSF A β 42 is associated with memory functions, and whether t- α -syn is related to cognition in PD. In addition, we wanted to assess whether it is possible to distinguish a CSF biomarker profile of early PD from non-PD MCI with increased risk of developing AD.

For this purpose, we established a longitudinal cohort study, recruiting early PD patients from the neurological outpatient clinic at Akershus University Hospital, the only public clinic in the area so that a large majority of relevant patients would be referred here. At baseline, all included patients were supposed to undergo lumbar puncture for collection and analysis of CSF and neurological and neuropsychological examinations. Statistical analysis would be carried out with adjustment for potential confounders.

Our aim was that the current project would contribute to the knowledge of whether CSF biomarkers may be used as early predicting biomarkers of cognition and to the understanding of the early neuropathological processes in the brain of PD patients developing cognitive impairment. This knowledge is important if preventive or at least early disease-modifying treatments are to be developed.

Table 1: Studies on CSF biomarkers and cognition in PD without dementia

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Compta et al., 2009 (171)	20 PD; QSBB criteria	37 % female; 69.1 years old; Education 8.6 years; Disease duration 10.7 years; UPDRSIII (off) 32.7; H&Y (off) 3; LED 1046.5; MMSE 27.9	Cross-sectional; Movement Disorders Unit	A β 42 (538.6), T-tau (220.3), P-tau (28.7): ELISA Innotest, Innogenetics	MMSE, verbal fluency, WAIS III digits subtest, RAVLT, BNT, VOSP (age and gender adjusted, 1.5 SD)	Lower A β 42 in patients with impaired phonetic fluency (MWU) and correlated with poorer phonetic fluency (Spearman); No associations in the matched control group (N = 15)
Siderowf et al., 2010 (172)	45 PD > 60 years; QSBB criteria	13% female; 73 years old; 68 % college education; Disease duration 11 years; H&Y (on) I 13%, II 64 %, III 20 %, IV-V 2 %	Cross-sectional and longitudinal, mean follow-up 1,5 years; Movement Disorders Center	A β 42 (224), T-tau (52), P-tau (18): Multiplex xMAP Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics	DRS-2	No association between CSF biomarkers and baseline cognition (adjusted for disease duration); Lower baseline A β 42 associated with more rapid cognitive decline in DRS-2 (mixed linear models, adjusted for age, disease duration, baseline H&Y); Low A β 42 associated with declines in attention, conceptualization and memory
Alves et al., 2010 (144)	109 newly diagnosed, unmedicated PD; Gelb criteria (174)	34.9 % female; 67.2 years old; Education 11.3 years; Disease duration 2.2 years; UPDRSIII 21.2; H&Y 1.8; MMSE 27.8	Cross-sectional; Population based incident cohort, the Norwegian ParkWest study	A β 38 (484), A β 40 (5973), A β 42 (362): Electrochemiluminescence using A β triplex assay, Meso Scale Diagnostics; T-tau (224), P-tau (58): ELISA Innotest, Innogenetics	CVLT II, semantic verbal fluency, MMSE, SCW, VOSP silhouettes (age, gender and education adjusted, verbal, visuospatial and attentional-executive z-scores)	Associations between A β 38, 40 and 42 and memory impairment (multiple regression analysis, adjusted for age, education, gender, disease duration, UPDRS III, MADRS)
Leverenz et al., 2011 (173)	22 PD; QSBB criteria	22.7 % female; 68.7 years old; Education 16.5 years; UPDRSIII 21; H&Y 2.5; MMSE 28	Cross-sectional	A β 42 (544.4), T-tau (136.6) : Luminex MAP, Biosource, InVitrogen	Logical Memory II, Category Fluency, Digit Symbol, TMT A and B	Associations between processing speed test and A β 42, but not after adjusting for age (Spearman correlation, linear regression adjusting for age and gender)
Compta et al., 2013 (160)	27 PD (21 with neuropsychological tests at follow-up); QSBB criteria	30 % female; 69 years old; Disease duration 10 years; UPDRSIII (off) 29; H&Y (off) II (15 %), III (66 %), IV-V (19 %); MMSE 28; 41 % developed dementia	Cross-sectional and longitudinal follow-up 18 months	A β 42 (569.7), T-tau (247.1), P-tau (30.2) : ELISA, Innogenetics	MMSE, RAVLT, phonetic fluency, semantic fluency, BNT, VOSP (1.5 SD, normative cutoffs)	Low A β 42 associated with lower phonetic fluency at baseline (MWU); Lower baseline A β 42 predicted dementia (binary logistic regression adjusted for gender, age, disease duration, H&Y, UPDRSIII, MMSE, visual hallucinations, PIGD); Low baseline A β 42 prospectively associated with poorer verbal recall and recognition and object-decision (Least squares mean differences, adjusted for baseline values)

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Kang et al., 2013 (175)	63 early, unmedicated PD; H&Y I/II; Positive DaTScan	38.1 % female; 62 years old; Education 16.4 years; Disease duration, median 0.4 years; UPDRSIII 22.6; H&Y 1.65; MoCA 27.2	Cross-sectional; Multicenter (PPMI)	A β 42 (228.7), T-tau (46.1), P-tau (21.0): Multiplex xMAP Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics; t- α -syn (1082): ELISA, Covance; Hemoglobin: ELISA, Bethyl Laboratories	MoCA, WMS-III LNS, HVLT-R, SDMT, semantic fluency	No associations between CSF biomarkers and cognitive tests
Zhang et al., 2013 (176)	403 early, unmedicated PD; H&Y I/II; MMSE $\geq$ 23; QSBB criteria	45.6 % female; 60.9 years old; Disease duration 2 years; UPDRSIII 16.3; H&Y 1.6; MMSE 28.9	Cross-sectional; DATA TOP multicenter clinical trial	A β 42 (236.1), T-tau (47.0), P-tau (23.3) (frozen for more than 20 years): Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics	MMSE	No correlations between CSF biomarkers and a global cognitive test (general linear models, adjusted for age and gender)
Wennström et al. 2013 (177)	38 PD; Gelb criteria	47 % female; 64 years old; H&Y 2.3; MMSE 28.5	Cross-sectional; Neurology and Memory clinics	t- α -syn: ELISA, Invitrogen	MMSE	No correlation between t- α -syn and MMSE (Pearson correlation)
Yu et al., 2014 (178)	26 PD with normal cognition/ 36 with cognitive impairment (33 MCI/ 3 PDD); QSBB criteria	50/ 47.2 % female; 57.4/ 61.1 years old; Education < 6 years 19.2/ 52.8 %, 6-12 years 61.5/ 33.3 %, > 12 years 19.2/ 13.9 %; Disease duration median 2/ 3 years; H&Y median 2/ 2	Cross-sectional; Department of Neurology	A β 42 (432/ 416), T-tau (96.0/ 135.2), P-tau-S396 (71.1/ 76.5): ELISA, CUSABIO ; P-tau (77.2/ 61.6) : ELISA, Invitrogen	MoCA, MMSE	PD-CI: MoCA is negatively correlated with T-tau and P-tau-S396 (Spearman correlation)
Alves et al., 2014 (64)	104 newly diagnosed, unmedicated PD (14.4 % MCI); QSBB and Gelb criteria	31.7 % female; 66.6 years old; Education 11.3 years; Time since diagnosis 1.5 months; UPDRSIII 20.9, H&Y 1.8; MMSE 28.0; 19.2 % developed dementia	Longitudinal follow-up 5 years; Population based cohort, the Norwegian ParkWest study	A β 38, A β 40, A β 42: Electrochemiluminescence using A β triplex assay, Meso Scale Diagnostics; A β 42, T-tau, P-tau: ELISA Innotech, Innogenetics	MMSE, CVLT II, semantic verbal fluency, Stroop interference, SCW, VOSP silhouettes, Cube subtests	Baseline A β 42 was reduced in those who developed PDD compared to those who did not; Low baseline A β 42 predicted increased risk for PDD (Cox proportional hazard models, likelihood ratio tests and multivariate Cox regression, adjusted for baseline age and MCI)

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Parnetti et al., 2014 (179)	44 PD; QSBB criteria	38.6 % female; Median 66 years old; Disease duration median 3 years; UPDRSIII 25.4; H&Y 1-2.5; MMSE median 27; MoCA median 25.5	Longitudinal, median follow-up 2 years	A β 42 (693), T-tau (146), P-tau (19.5): ELISA Innostest, Innogenetics; t- α -syn (22.2), o- α -syn (4838): ELISA, Santa Cruz Biotechnology. Excluded samples with erythrocyte count > 500/ mm ³	MMSE, MoCA	Baseline A β 42 correlated negatively with decline in MMSE and MoCA (Spearman correlation); Low baseline A β 42 was associated with a higher rate of decline on MMSE and MoCA (multivariate linear regression, adjusted for baseline values and follow-up duration); No associations in the age-matched control group with other neurological conditions (N = 25)
Stewart et al., 2014 (180)	350 early, unmedicated PD in phase I/ 266 medicated PD in phase II; H&Y I/II; MMSE $\geq$ 23; QSBB criteria	37/ 35 % female; 60.9/ 62.6 years old; Disease duration 2.1/ 3.8 years; UPDRSIII 15.9/ 30.2; H&Y median 1.5/ 2; MMSE 28.9/ 28.7	Cross-sectional and longitudinal, average follow-up 1.8 years; DATATOP multicenter clinical trial	t- α -syn (0.63/ 0.47) (frozen for more than 20 years): Luminex, Austin; Hemoglobin: ELISA, Bethyl Laboratories. Excluding samples with hemoglobin > 200 ng/ mL	MMSE, SRT-Total and-Delayed, SDMT, NDT	Cross-sectionally lower t- α -syn was associated with reduced verbal recall in the start of phase I (partial correlation, adjusted for age, gender, education, study drug); t- α -syn at baseline of phase I did not predict cognitive outcome in phase I (Linear mixed models, adjusted for age, gender, education, drugs, performance at baseline); Lower t- α -syn at the beginning of phase II predicted better preservation of cognitive function in verbal learning and memory and visuospatial working memory (Linear mixed models, adjusted for age, gender, education, drugs and performance at baseline of phase II)
Yarnall et al., 2014 (181)	67 newly diagnosed PD; QSBB criteria	64.5 years old; Education 13.5 years	Cross-sectional; Community and outpatient clinics; ICICLE-PD	A β 42 (930.5), T-tau (130.4), P-tau (47.8): Innostest, Innogenetics; A β 40 (10.2) : hAmyloid β 40 Elisa ABETA GmbH; t- α -syn (110.2): ELISA; Sample free of visual contamination by blood	MMSE, MoCA, Cognitive Drug Research computerized battery, Power of Attention score, PRM, SRM, PAL, Tower of London task, phonetic and semantic fluency (1.5 SD)	Correlation between A β 42 and visual memory and MoCA (linear regression model, adjusting for age and education)

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Liu et al., 2015 (182)	403 early, unmedicated PD in phase I/ 305 medicated PD in phase II	45.6/ 49.8 % female; 60.9/ 62.8 years old; Disease duration 2/ 3.8 years; UPDRSIII 16.3/ 29.9; H&Y median 1.5/ 2; MMSE 28.9/ 28.8; QSBB criteria	Cross-sectional and longitudinal, average follow-up 2/ 4.3 years; DATATOP multicenter clinical trial	A β 42 (236.1/ 232.2), T-tau (47.0/ 47.2), P-tau (23.3/ 23.6) (frozen for more than 20 years): Luminex platform using INNO-BIA AlzBio3 immunoassay, Innogenetics	MMSE, SRT-Total and-Delayed, SDMT, NDT	No cross-sectional correlation of CSF biomarkers and cognition at the beginning of phase I/ II (Partial correlation, adjusting for age, gender and education); No associations between baseline CSF biomarkers and cognition during phase I; Higher P-tau at the beginning of phase II predicted decline in verbal learning and visuospatial working memory/ processing speed during phase II (linear mixed models, adjusted for gender, education, age, MMSE, UPDRS total, cognitive score, follow-up time).
Hall et al., 2015 (183)	42 PD; Gelb criteria	45.2 % female; Median 67.5 years old; Education median 12 years; Disease duration median 7 years; UPDRSIII median 20; H&Y median 2.25; MMSE median 29; LED 647.5	Longitudinal follow-up 2 years; Swedish BioFinder study	A β 42 (616), T-tau (422), P-tau (48.2), t- α -syn (50.5): xMAP Multiplex assay (Luminex); Hemoglobin: ELISA, Bethyl Laboratories, for t- α -syn excluding samples with hemoglobin > 1000 ng/ L (1 ng/ mL)	MMSE, ADAS 1-3, AQT, animal fluency	Higher baseline t- α -syn associated with decline in cognitive speed over 2 years; Lower baseline A β 42 associated with decline in delayed memory recall over 2 years; (Multivariate models adjusted for age, gender, disease duration, LED)
Buddhal et al., 2015 (184)	77 PD; QSBB criteria; CDR < 1	42.9 % female; Median 66.9 years old; Education median 15.9 years; Disease duration median 4.9 years; UPDRSIII off median 23.2; LED median 810.9	Cross-sectional	A β 42 (780.8), T-tau (218.2), P-tau (0.178): Innotech ELISA, Fujirebio; t- α -syn (1852.8): Covance ELISA kit; Hemoglobin: ELISA, Bethyl Laboratories, no correlation between hemoglobin and t- α -syn (	MMSE; CVLT-short form, logical memory, digit symbol, digit span, BNT, JOLO, spatial relations test, TMT, verbal fluency, SCW (age-adjusted, domain and global Z scores)	A β 42 positively correlated with MMSE (Spearman correlation)
Compta et al., 2015 (161)	21 PD	33 % female; median 68 years old; Disease duration median 10 years; UPDRSIII median 28; H&Y off median 3; MMSE median 28	Cross-sectional; Movement disorders unit	t- α -syn (387): ELISA, Invitrogen; o- α -syn (0.182 A at 405 nm): ELISA; Not measured hemoglobin	MMSE, RAVLT, phonetic and semantic fluency, working memory index, BNT-15, VOSP (abnormal scores > 1.5 SD below normative data)	Low t- α -syn correlated with dysfunction in phonetic fluency and visuospatial function (Spearman correlation)

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Terrelonge et al., 2016 (185)	286 newly diagnosed, unmedicated PD without CI within 2 years of diagnosis, not required medication within 6 months from baseline; Positive DaTScan	No CI/ CI (12.9 %) at 2 years: 35.3/ 29.7 % female; 60.6/ 61.8 years old; Education 15.8/ 15.3 years; Disease duration 6.9/ 7.7 months; UPDRSIII 8.7/ 9.8; GDS 5.2/ 4.9	Longitudinal 2 years; Multicenter (PPMI)	No CI/ CI at 2 years: Aβ42 (380.4/ 343.8), T-tau (44.1/ 40.9), P-tau (16.2/ 14.9): Multiplex xMAP Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics; t-α-syn (1897.3/ 1689.2): ELISA, Covance; Hemoglobin: ELISA, Bethyl Laboratories	HVLT-R, JOLO, LNS, semantic fluency, SDMT (CI defined as at least 2 test scores > 1.5 SD below normative mean)	Patients with CI at 2 years had significantly lower mean baseline Aβ42 than those without CI; Lower baseline Aβ42 were associated with higher odds for CI at 2 years (Multivariable logistic regression, adjusted for age, gender, disease duration, education, motor severity, depression, corrected for multiple comparisons with Bonferroni-Holm correction)
Bäckström et al., 2015 (186)	99 early, incident, drug-naïve PD (40 PD-MCI); QSBB criteria	41.4 % female; Median 71.3 years old; Disease duration median 16 months; MMSE median 29; 35.4 % developed PDD	Longitudinal 5 to 9 years follow-up; Population-based, university hospital	Median Aβ42 (695), T-tau (236), P-tau (38): ELISA, Innogenetics; t-α-syn (732): ELISA, Invitrogen, adjusted for hemoglobin levels (ELISA, Bethyl Laboratories)	MMSE, SRT, BVMT, Digit span backwards, TMT-B, WCST, semantic fluency, JOLO, BNT (CI defined as at least 2 test scores > 1.5 SD below normative mean)	Low Aβ42 at baseline was related to future PDD (Cox proportional hazards regression model, adjusted for age, gender, disease duration, baseline MCI status, corrected for multiple comparisons)
Skogseth et al., 2015 (187)	414 early, untreated PD (140 PD-MCI)	33 % female; 61.3 years old; Education 15.6 years; Disease duration median 4.0 months; UPDRSIII median 20	Cross-sectional; Multicenter (PPMI)	Median Aβ42 (367.2), T-tau (40.9), P-tau (12.2): Multiplex xMAP Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics; t-α-syn (1709.9): ELISA, Covance; Hemoglobin: ELISA, Bethyl Laboratories	JOLO, HVLT-R, semantic fluency, LNS, SDMT, MoCA (z-scores, domain and global scores)	Lower t-α-syn was associated with reduced performance on executive-attention domain and composite cognition (Multiple linear regression adjusted for age, education, gender, UPDRSIII)
Hall et al., 2016 (188)	63 PD; Gelb criteria	Baseline/ follow-up: 33.3 % female; 64.7 years old; Disease duration 5.5 years; UPDRSIII 19.2/ 17.7; MMSE 28.5/ 28.0; LED 565/ 767	Longitudinal follow-up 2 years; Swedish BioFinder study	Baseline/ follow-up: Aβ42 (415/ 434), T-tau (81/ 86), P-tau (40/ 44), t-α-syn (1763/ 1830): AlzBio3 immunoassay, Fujirebio; T-α-syn: Covance assay; Hemoglobin assay, Bethyl Laboratories, hemoglobin < 200 ng/mL	MMSE, ADAS 1-3, verbal or letter fluency test	Increase in P-tau over 2 years correlated with worsening letter fluency executive functioning (Linear regression, adjusting for age, LED)

Study	Patient selection	Descriptives	Study design	CSF biomarkers (mean/ median ng/ L) and technique	Cognitive tests	CSF biomarker associations with cognition
Caspell-Garcia et al., 2017 (152)	423 newly diagnosed, untreated PD (9-22 % with MCI); Positive DaTScan	34.5 % female; 61.7 years old; Education 18 % < 13 years, 81.3 % 13-23 years, 0.7 % > 23 years; Disease duration 6.7 months; UPDRSIII 20.9; GDS ≥ 5 in 13.9 %; CI in 15-38 % and dementia in 3-6 % after 3 years	Longitudinal follow-up up to 3 years; Multicenter (PPMI)	A β 42, T-tau, P-tau: Multiplex xMAP Luminex platform using INNO-BIA AlzBio3immunoassay, Innogenetics; t- α -syn: ELISA, Covance; Hemoglobin: ELISA, Covance	JOLO, HVLT-R, semantic fluency, LNS, SDMT, MoCA	Lower A β 42 at baseline predicted clinical diagnosis of cognitive impairment (Univariate and multivariable analyses)
Lerche et al. 2018 (189)	415 PD (19 PD with 10 year follow-up); QSBB criteria	37 % female; 66 years old; Disease duration 6 years; UPDRSIII on 26; H&Y 2; MoCA 25;	Cross-sectional and longitudinal follow-up up to 10 years; ward or outpatient clinic for PD at a university hospital	A β 42, T-tau, P-tau: Innostest ELISA, Fujirebio	MoCA; MMSE	Lower A β 42 associated with lower MoCA at baseline, 2 and 4 years; Low (<300 ng/mL) A β 42 at baseline developed cognitive impairment more frequently and earlier; 67 % of patients with normal cognition and low A β 42 at baseline, developed cognitive impairment during follow-up, compared with 41 and 37 % of patients with intermediate (599-300 ng/mL) and high (600 ng/mL) A β 42. (Multiple linear regression, Kaplan-Meier survival curves and Cox proportional hazards models)
Goldman et al. 2018 (190)	115 PD; QSBB criteria; 3 parkinsonian signs (bradykinesia, rigidity and resting tremor) disease duration ≥ 4 years; onset 50-75 years; response to dopamine	37.4 % female; 68.2 years old; Education 17 years; Disease duration 8.3 years; UPDRSIII off 39.1; H&Y 2.2; MoCA 26.8; LED 740.5	Cross-sectional; BioFIND study USA	A β 42 (296.3), T-tau (37.2), P-tau (16.4): INNOBIA Alz Bio3 kits, Fujirebio; t- α -syn (1466.3): ELISA, BioLegend; Hemoglobin (157 ng/mL): ELISA, Bethyl Laboratories, no correlation between hemoglobin and t- α -syn	MoCA total and subdomain scores	A β 42 correlated weakly with MoCA recall scores (Spearman rho)

Values are given in mean or median unless otherwise stated.

Abbreviations: ADAS = Alzheimer's Disease Assessment Scale; AQT = A Quick Test of Cognitive Speed; BVMT = Brief Visuospatial Memory Test; BNT = Boston Naming Test; CDR = Clinical Dementia Rating; CVLT = The California Verbal Learning Test; DATATOP = deprenyl and tocopherol antioxidative therapy of parkinsonism; DaTScan = Dopamine transporter scan; DRS-2 = Mattis Dementia Rating Scale; ELISA = enzyme-linked immunosorbent assay; GDS = Geriatric Depression Scale; HVLT-R = Hopkins Verbal Learning Test-Revised; H&Y = Hoehn & Yahr; ICICLE = Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation; JOLO = Benton Judgment of Line Orientation; LED = Levodopa Equivalent Dose; LNS = Letter Number Sequencing; MADRS = Montgomery and Aasberg depression rating scale; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; MWU = Mann-Whitney U; NDT = New Dot Test; PIGD = postural instability gait disorder; PPMI = The Parkinson's Progression Markers Initiative; PRM = Pattern recognition memory; PAL = Paired associates Learning; QSBB = Queen Square Brain Bank; RAVLT = Rey Auditory Verbal Learning Test; SCW = Stroop Color Word; SDMT = Symbol Digit Modalities Test; SRM = Spatial Recognition memory; SRT = Selective Reminding Test; TMT = Trail Making test; UPDRSIII = The unified Parkinson's disease rating scales of motor function, part III motor examination; VOSP = Visual Object and Space Perception; WAIS-III = Wechsler Adult Intelligence Scale; WCST = Wisconsin Card Sorting Test; WMS-III LNS = Wechsler Memory Scale third edition, Letter-Number Sequencing test;

3.3.2 Hippocampal and parahippocampal sub-regions in relation to cognition and CSF biomarkers in PD

Inclusion and exclusion criteria

Studies comparing the sizes of hippocampal or parahippocampal sub-regions in PD and controls and exploring the relationship with CSF biomarkers or cognition in PD patients were considered for this review. We performed searches in PubMed using the search terms “(parkinson) AND (hippocampus OR hippocampal OR perirhinal OR entorhinal) AND (MRI OR magnetic resonance) AND ((cerebrospinal fluid OR CSF) OR (cognition OR cognitive) OR (control))”. In addition, other relevant studies were also included. As few studies have assessed this relationship in PD, all studies including PD patients with and without dementia were included.

Relevant publications at the start of the project

Before the startup of this project in 2011, only 3 studies assessing the relationship between hippocampal or parahippocampal sub-regions and cognition in PD had been published (see *table 2* on page 46). All 3 studies were cross-sectional examining PD, PDD and controls.

Two studies in longer-lasting PD assessed the head, body and tail of hippocampus. One of the studies found that hippocampus and hippocampal head volumes were smaller in PDD and in PD > 70 years compared to controls, and that left hippocampal head volumes correlated with verbal and visual recall in PD, but not in controls (191). The other study found that when analyzing all the PD patients together (PD, PD with visual hallucinations and PDD), but not PD alone, there was gray matter density loss in left and right hippocampal head and left tail compared to controls, and hippocampal head gray matter loss correlated with learning impairment. 26 % of PD and 78 % of PDD patients had hippocampal gray matter density loss, predominantly in the head, compared to controls (192).

The only study in early PD patients assessed entorhinal cortex (ERC), and not hippocampal subfields. They found that patients with PDD, but not PD, had significantly smaller total ERC volume compared to controls and that right ERC was smaller compared to both controls and PD. In addition, total ERC correlated with learning memory in PD (193).

Conflicting results

Although they used different patient selections, neuropsychological tests and MRI techniques on 1.5 Tesla (T) MRI scanners, both studies found that the hippocampus and hippocampal head were smaller in PD patients with or without dementia compared to controls, but the hippocampal head correlated with different memory functions (191, 192).

Limitations

All the 3 studies were cross-sectional, not population-based and recruited relatively small groups. VBM (192) is possible less sensitive than volumetric MRI (191, 193) and cortical thickness measures. Manual ROI segmentation is dependent on operator skills, neuroanatomical boundary selections and number of imaging slices (191, 193). Only one of the studies assessed the association between the hippocampal sub-regions and cognition in controls (191). None of the studies evaluated the CA1-4, dentate gyrus (DG), subiculum or presubiculum of the hippocampus or perirhinal cortex (PRC), or the relationship between hippocampal and parahippocampal sub-regions and CSF biomarkers.

Potential contribution of current project

We wanted to analyze the volume of the hippocampal subfields subiculum, presubiculum, CA1, CA2-3, CA4-DG, the hippocampal tail and the thickness of the parahippocampal structures PRC and ERC in early PD patients compared to controls. Further, we wanted to explore the relationship with cognition (especially memory functions) and CSF biomarkers (A β 38, 40 and 42, T-tau, P-tau and t- α -syn), as this has never been done before.

In addition to lumbar puncture for CSF analysis and neuropsychological tests as previously mentioned, the patients and controls included in our cohort study had 3 Tesla (T) MRI scans followed by automated segmentations and measurements of the hippocampal subfields volumes and parahippocampal region thicknesses (ROIs) using FreeSurfer image analysis. Statistical analysis was carried out with adjustment for potential confounders.

We hoped that the current project could clarify whether hippocampal and parahippocampal sub-regions are affected already at an early stage of PD, are related to cognition and amyloid, α -syn or tau pathologies as reflected by CSF biomarkers, and in that way are potential early biomarkers of cognitive impairment.

Table 2: Studies on hippocampal or parahippocampal sub-regions and cognition in PD with or without dementia

Study	Patient selection	Descriptives	Study design	MRI and segmentation technique	Cognitive tests	MRI in PD vs controls and associations with cognition
Bouchard et al., 2008 (191)	44 PD/ 13 PDD/ 44 age, gender and education matched controls, $\geq$ 65 years; QSBB criteria	40.9/ 30.7/ 40.9 % female; 71.1/ 71.9/ 71.1 years old; Education 14.1/ 14.1/ 14.8 years; Disease duration 8.4/ 10.3 years; UPDRSIII 16.8/ 21.7/ 1.9; H&Y 2.2/ 2.3; MMSE 28.3/ 25.9/ 28.4; LED 659.5/ 696.4;	Cross-sectional; Movement Disorders Clinic and advertisements	1.5 T Siemens Sonata machine, T1-weighted 3D MPRAGE sequence, manually segmentation of the head, body and tail volumes of the HC using the DISPLAY software and the protocol reported by Malychin (194), normalized by ICV	MMSE, DRS, FAB, CVLT-II, BVM-T-R (adjusted for age and or gender)	Smaller mean (and right) HC and HC head volumes in PDD and in PD > 70 years compared to controls (ANOVA, Dunnett's test post hoc); Left HC head volumes positively correlated with verbal and visual recall in PD, no correlations in controls (Pearson's or Spearman's correlation)
Ibarretxe-Bilbao et al., 2008 (192)	19 PD/16 PD-VH/ 9 PDD/ 56 age and education matched controls; QSBB criteria	57.9/ 68.8/ 44.4/ 50 % female; 72.5/ 73.5 /69.8/ 73 years old; Education 7.8/ 7.6/ 8.6/ 7.9 years; Disease duration 10.9/ 12.9/ 13.1 years; UPDRSIII 24.7/ 29.7/ 42.8; H&Y 2.5/ 3.2/ 3.8; MMSE 28.2/ 26/ 15.7/ 28.7	Cross-sectional; Parkinson's disease Movement Disorders Unit	1.5 T GE Nvi/Cvi 8.4 machine, axial 3D IR Prep Inversion Recovery Prepared SPGR sequence, VBM on SPM2 using the procedure by Mechelli (195), visual inspection of HC head, body and tail (ROI)	MMSE, RAVLT	All PD together: Gray matter density loss in left and right head and left tail; PD-VH: Gray matter loss in left and right head; PDD: Gray matter loss in the left HC and right head and tail; 26 % of PD, 31 % of PD-VH and 78 % of PDD had gray matter loss in HC (88 % had head predominance) compared to controls (Two sample T-tests compared to controls, corrected for multiple comparisons); PD-VH and all PD together: HC head gray matter loss correlated with verbal learning impairment (SPM2 correlation analysis, corrected for multiple comparisons)
Kenny et al., 2008 (193)	31 PD, 30 PDD, 37 age-matched controls; > 65 years; QSBB criteria	25.8/ 36.7/ 45.9 % female; 75.2/ 71.9/ 75.4 years old; Disease duration 3.7/ 6.5 years; UPDRSIII 26.7/ 36.1/ 1.4; MMSE 26.7/ 19/ 28.1	Cross-sectional; Old-age psychiatrists, geriatricians and neurologists in hospital- and community-dwelling populations	1.5 T GE Signa scanner, whole-brain T1-weighted 3D, volumetric analysis using MIDAS software on a Sun UltraSparc-60 micro-computing system, ROI manual segmentation of ERC volume, normalized by ICV	MMSE, CAMCOG	PDD: 14.7 % smaller total ERC volume compared to controls, right ERC smaller compared to controls and PD (Analysis of variance and post hoc Tukey); PD: Total ERC correlated with learning memory (Pearson correlation)

Study	Patient selection	Descriptives	Study design	MRI and segmentation technique	Cognitive tests	MRI in PD vs controls and associations with cognition
Goldman et al., 2012 (196)	14 PD with normal cognition/ 12 PDD with memory impairment / 14 age-, gender- and education-matched normal controls; QSBB	35.7/ 16.7/ 50 % female; 70.6/ 75.8/ 72 years old; Education 15.2/ 14.2/ 15.9 years; Disease duration 9/ 12.5 years; UPDRSIII 21.1/ 37.8/ H&Y 2/ 3; LED 915.3/ 800.2; MMSE 28.9/ 21.9/ 29.3	Movement Disorders clinic	1.5 T GE Signa scanner, 3D Fourier transform spoiled gradient recalled pulse sequence, ROI manual segmentation of ERC volume using Analyze software, normalized by ICV	MMSE, CERAD, digit span, symbol digit modalities test, category naming of animals, BNT-15, Similarities, JOLO (z-scores)	ERC volumes were smaller in PDD compared to PD and controls and in PD compared to controls (ANOVA, post hoc Sheffe's test, controlling for age); PD and PDD together: Verbal memory predicted volume of ERC (Linear regression)
Beyer et al., 2013 (197)	114 PD/ 29 PD-MCI, newly diagnosed, unmedicated; Gelb criteria	40.4/ 37.9% female; 65.8/ 70.6 years old; Education 11.3/ 11.4 years; UPDRSIII 21/ 24.2; H&Y 1.8/ 2; MMSE 28.3/ 26.9	Cross-sectional; Population-based; Multicenter (Norwegian ParkWest study)	1/ 1.5 T Phillips Intera/ Siemens Symphony, T1-weighted 3D, intensity and spatial normalization to the ICBM53 brain atlas, automated HC segmentation with AdaBoost and radial distance mapping for each structure thickness (CA1-4, DG and subiculum)	MMSE, CVLT-II, VOSP Silhouettes and Cube subtests, animal fluency, SCW (corrected for age and education by regression)	PD and PD-MCI together: Associations between lower verbal long delay free recall and atrophy in left and right CA1, CA3 and subiculum, verbal short delay free recall and CA1 and subiculum, verbal recognition and right CA1 and subiculum (Linear regression ,adjusting for imaging center); PD: Associations between lower verbal long delay free recall and atrophy in right CA1, CA3 and subiculum
Pereira et al. 2013 (198)	18 PD/ 18 PD-VH (83.3 % MCI in PD)/ 18 age- and gender-matched controls; QSBB criteria	66.6/ 66.6/ 66.6 % female; 73.8/ 73.7/ 73.6 years old; Education 8.4/ 7.5/ 7.7 years; Disease duration 12.8/ 12.9 years; UDPRSIII 25.4/ 28; H&Y 2.9/ 3.1; MMSE 27.9/ 26.1/ 27.9; LED 671.2/ 702.8	Cross-sectional; Outpatient Movement Disorders Clinic	1.5 T GE Signa scanner, T1-weighted 3D, axial SPGR sequence, automated segmentation and volumetry of the HC subfields CA1, CA2-3, CA4-DG, subiculum, presubiculum, fimbria and the HC fissure using FreeSurfer software according to Van Leemput (199) and Hanseeuw (200), adjusted by ITV	RAVLT, (adjusted by age, education and gender by linear regressions)	CA2-3 and CA4-DG were smaller in both PD and PD-VH, while subiculum and whole HC was only reduced in PD-VH compared to controls (multivariate ANOVA, post hoc Bonferroni); Correlations between verbal learning and CA2-3, CA4-DG and whole HC volumes (Pearson correlation) in all PD patients together, but no correlations in the controls
Pagonabarraga et al., 2013 (201)	26 PD/ 26 PD-MCI/ 20 PDD/ 18 controls; QSBB criteria	45/ 41/ 48/ 47 % female; 71.5/ 73.3/ 76.9/ 68.2 years; Education 9/ 9.2/ 7.1/ 10.4 years; Disease duration 7.3/ 6.8/ 9.6 years; UPDRSIII 24/ 21/ 29; H&Y 2.2/ 2/ 2.8; LED 791/ 700/ 860	Cross-sectional; Outpatient Movement Disorders Clinic	3 T Philips Achieva, 3D-MPRAGE of the whole brain, automated cortical thickness measurement of ERC using FreeSurfer software, visual inspection and manual correction	PD-CRS	Thinning of ERC in PDD (left and right) more than in PD-MCI (left) more than in PD and controls (One-way ANOVA with post-hoc Bonferroni); No correlations between ERC and neuropsychological tests (Pearson correlation, controlling for age, education and disease severity)

Study	Patient selection	Descriptives	Study design	MRI and segmentation technique	Cognitive tests	MRI in PD vs controls and associations with cognition
Tanner et al., 2015 (202)	40 PD (9 memory impaired) / 40 controls; DRS-2 raw score > 130, QSBB criteria, H&Y 1-3	20/ 17.5 % female; 67.8/ 68.2 years old; Education 16.3/ 16.8 years; Disease duration 7.5 years; UPDRSIII 17.6/ 2.8; LED 686/ 1.0	Center for Movement Disorders	3 T Siemens Verio, T1-weighted, volumetric segmentation with FreeSurfer 5.3, left ERC volume manually traced in ITK-SNAP, correcting for total ICV	DRS-2, P(r)VLT, WMS-III, TMT-A, WAIS-III Digit Symbol, SCW	PD had 11 % less left ERC volume compared to controls, and PD with memory impairment had smaller ERC than PD without (t-tests); Left ERC volume was not associated with verbal memory composite score, but larger left ERC volume was associated with logical memory retention in both PD and controls (linear regression, adjusted for age)
Foo et al., 2017 (203)	54 PD / 11 PD-MCI; Gelb criteria; H&Y < 3; age 50-90 years	75.9/ 54.6 % female; 63.4/ 69.5 years old; Education 10.9/ 9.7 years; Disease duration 4.7/ 4.1 years; UPDRSIII 19.0/ 15.3; H&Y 2.1/ 2; LED 621.2/ 633.9; 12 PD converted to PD-MCI	Cross-sectional and longitudinal follow-up after 18 months; tertiary neurology centre	3 T GE scanner; T1-weighted MPRAGE; Automated volume segmentation of the hippocampus with FreeSurfer 6.0 into 13 subfields (alveus, parasubiculum, presubiculum, subiculum, CA1, CA2-3, CA4, GC-DG, HATA, fimbria, molecular layer, hippocampal fissure, hippocampal tail), corrected for age, gender, education and ICV	MMSE; MoCA; ADAS-Cog; 10-point clock drawing; FAB; digit span; colour trails 1; fluency (z-scores, composite summary index for each cognitive domain)	PD-MCI had lower volumes in the left fimbria and right CA1 and HATA compared to PD (ANCOVA and post-hoc Bonferroni); Baseline right CA1 volumes correlated with baseline attention (Partial correlation) PD-MCI converters showed greater atrophy in the right CA2-3 (ANCOVA and post-hoc Bonferroni); Decreasing baseline volumes of GC-DG, right CA4 and left parasubiculum and HATA predicted conversion to PD-MCI (Logistic regression)
Caspell-Garcia et al., 2017 (152)	160 (110 at 2 years follow-up) newly diagnosed, untreated PD (with or without MCI); Positive DaTScan	35.6 % female; 61.0 years old; Education 23.8 % < 13 years, 76.3 % 13-23 years, 0 % > 23 years; Disease duration 6.87 months; UPDRSIII 20.9; GDS ≥ 5 in 15.6 %	Longitudinal follow-up at baseline, year 1 and 2; Multicenter (PPMI)	3 T Siemens Healthcare, 3D-MPRAGE sequence for brain anatomy, measurements using FreeSurfer 5.1, volume and thickness for 34 regions, left and right values averaged, adjusted for total ICV	JOLO, HVLT-R, semantic fluency, LNS, SDMT, MoCA	Baseline decreased ERC volume associated with decline in MoCA over time (Univariate and multivariable analyses)

Values are given in mean/ median unless otherwise stated.

Abbreviations: ADAS-Cog = Alzheimer's Disease Assessment Scale-cognitive subscale (word-list delayed, recognition recall, figure copy test, Maze test, comprehension of test instructions); BFRT = Benton's facial recognition test; BNT = Boston Naming Test; BVM-T-R = Brief Visuospatial Memory Test-Revised; CA = cornu ammonis; CAMCOG = Cambridge Cognitive Examination; CERAD = Consortium to Establish a Registry for Alzheimer's disease; CVLT = California Verbal Learning Test; DaTScan = Dopamine transporter scan; DG = dentate gyrus; DRS = Dementia Rating Scale; ERC = entorhinal cortex; FAB = Frontal Assessment Battery; GC-DG = granule cell layer of dentate gyrus; GE = General Electric; GDS = Geriatric Depression Scale; HATA = hippocampus-amygdala-transition-area; HC = hippocampus; H&Y = Hoehn & Yahr; HVLT-R = Hopkins Verbal Learning Test-Revised; ICV = intracranial volume; ICBM53 = International Consortium for Brain Mapping brain; JOLO = Benton Judgment of Line Orientation; LED = Levodopa Equivalent Dose; LNS = Letter Number Sequencing; MDRS = Mattis Dementia Rating Scale; MIDAS = Medical Information Display and Analysis System; MMSE

= Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; MPRAGE = Magnetization prepared rapid acquisition gradient echo; PD-VH = PD with visual hallucinations; PPMI = The Parkinson's Progression Markers Initiative; P(r)VLT = Philadelphia (repeatable) Verbal Learning Test; QSBB = Queen Square Brain Bank; ; RAVLT = Rey Auditory Verbal Learning Test; ROI = region-of-interest; SCW = Stroop Color Word; SDMT = Symbol Digit Modalities Test; SPGR = Spoiled Gradient-echo; SPM = Statistical Parametric Mapping; 1.5/3 T = 1.5/3 Tesla; TMT = Trail Making test; UPDRSIII = The unified Parkinson's disease rating scales of motor function, part III motor examination; VBM = Voxel Based Morphometry; VFDT = visual form discrimination test; WAIS-III = Wechsler Adult Intelligence Scale; WMS-III = Wechsler Memory Scale 3rd Revision

3.3.3 Cortical hypometabolism in relation to A β , t- α -syn and neurogranin and cognition in early PD

Inclusion and exclusion criteria

Studies exploring the relationship between cortical glucose metabolism and A β , t- α -syn and neurogranin in PD were considered for this review.

We performed searches in PubMed using the search terms “(parkinson) AND (glucose metabolism OR FDG OR fludeoxyglucose OR fluorodeoxyglucose) AND (PET OR positron emission tomography) AND (amyloid OR A β OR abeta OR synuclein OR neurogranin)”.

Relevant publications at the start of the project

To our knowledge, no other studies have assessed the relationship between glucose metabolism and CSF A β species, α -syn or neurogranin in PD.

Potential contribution of current project

Both the levels of CSF A β species and cortical glucose metabolism have been shown to be related to cognitive impairment, even in early PD. Our project aimed to examine whether there is a relationship between CSF A β species, α -syn or neurogranin, cortical glucose metabolism, cognition and PD motor stage in the likely absence of A β plaques in early PD patients as no others have done this to date. This would have implications for the understanding of mechanisms for cognitive impairment.

In addition to lumbar puncture for CSF biomarkers, MRI and neuropsychological tests, the patients and controls in our cohort study had FDG-PET scans for measure of glucose metabolism at baseline. Statistical analysis was corrected for potential confounders and multiple comparisons.

This project may add to the understanding of the early neuropathological processes and clarify whether A β , α -syn or neurogranin dysmetabolism may contribute to cortical hypometabolism and cognitive impairment in the absence of A β plaques in early PD, and thus may be potential early biomarkers of synaptic dysfunction and neurodegeneration.

3.4 Presentation of the research questions

With this literature review in mind, there were seven main research questions that we wanted to answer in the three papers:

Paper 1: Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

1. Are the levels of CSF A β 38, 40 and 42, T-tau, P-tau and t- α -syn biomarkers significantly different in early PD patients and non-PD MCI patients with increased risk of developing AD?
2. Are the levels of the CSF biomarkers associated with neuropsychological tests in early PD?

Paper 2: Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

3. Are the volume or thickness of total hippocampus, hippocampal (subiculum, presubiculum, CA1, CA2-3 and CA4-DG) and parahippocampal (ERC and PRC) sub-regions at MRI smaller in early PD than in normal controls?
4. Are the levels of the CSF biomarkers associated with volume or thickness of the hippocampal and parahippocampal sub-regions in early PD?
5. Are the volume or thickness of the hippocampal and parahippocampal sub-regions associated with neuropsychological tests in early PD?

Paper 3: Impaired synaptic function is linked to cognition in Parkinson`s disease

6. Are the levels of CSF A β species, α -syn and neurogranin associated with ^{18}F -fludeoxyglucose positron emission tomography (FDG-PET) uptake in relevant regions, and are these regions correlated with cognition in early PD?
7. Are the levels of CSF neurogranin significantly different in early PD compared to NC, and are there correlations between neurogranin and A β , α -syn and PD motor stage?

4 Methods

4.1 Patient selection

4.1.1 Recruitment process

This project is based on cross-sectional baseline data from the ongoing longitudinal, prospective cohort study *Mechanisms and biomarkers of cognitive decline in Parkinson's disease and dementia with Lewy bodies* (the PD study) at the Department of Neurology, Akershus University Hospital. Patients with possible early PD were consecutively recruited to the study from the neurological outpatient clinic in the period of September 2011 to January 2014. Patients were recruited through courses held for early PD patients, from diagnosis lists of PD patients and from outpatient consultations.

Two control groups were used for comparison: Patients with SCI or MCI were recruited consecutively to the MCI-GO (Gothenburg and Oslo collaboration) study from referrals to the memory clinic at the same department in the period 2005-2013. Normal controls (NC) were recruited from spouses and relatives of the patients in the PD and MCI-GO studies and from volunteers and orthopedic patients undergoing spinal anesthesia in conjunction with orthopedic surgery at Akershus University Hospital in the period September 2012 to January 2014 as a part of the national Dementia Disease Initiation (DDI) study.

4.1.2 Inclusion and exclusion criteria in the studies

PD patients

Inclusion criteria

Inclusion criteria were diagnosis of PD according to the QSBB clinical diagnostic criteria (51) with the exception that more than one affected relative was not an exclusion criteria, in addition to Hoehn & Yahr (204) stage < 3 , disease duration ≤ 6 years and age 40-79.

Exclusion criteria

Exclusion criteria were dementia (criteria described under *Cognitive screening* on page 64) and other somatic or psychiatric diseases that might contribute to cognitive impairment, including drug abuse, moderate or severe depression, solvent exposure, ischemic stroke, cancer treatment with chemotherapy or radiotherapy, or contra-indications to MRI or lumbar puncture.

Inclusion

A total of 70 patients who had been examined and diagnosed by a neurologist at the neurological outpatient clinic were considered for the PD study. 15 patients were excluded before they were asked to participate due to (number in brackets) dementia [6], atypical parkinsonism [3], concomitant psychiatric disease [2], pacemaker [1], warfarin treatment [1], uncertain diagnosis [1] and concomitant multiple sclerosis [1]. 12 patients did not want to participate for different reasons (7 thought it was too demanding investigations, 1 was about to move, 1 had claustrophobia, 1 thought it would be too much focus on the disease and 2 did not want to participate of unknown reason). 2 patients withdrew from the study shortly after inclusion at the clinical evaluation. 1 patient turned out to have symptoms for more than 6 years. 5 patients did not fulfill the QSBB-criteria (due to (number in brackets) possible multiple system atrophy [2], dementia with Lewy bodies [1], progressive supranuclear palsy [1] and PDD [1]). Finally, 35 early PD patients were considered for the three papers.

SCI and MCI patients

Inclusion and exclusion criteria

Inclusion criteria in the MCI-GO study were SCI or MCI for at least 6 months according to the cognitive screening criteria as described on page 64 and age 40-79. Exclusion criteria were as for PD.

Inclusion

122 patients were included in the MCI-GO study; 85 had MCI, 33 had SCI, but 2 proved to have normal cognition and 2 actually had dementia.

Normal controls (NC)

Inclusion and exclusion criteria

Inclusion criteria were no known cognitive impairment and age 40-80 years. Exclusion criteria were as for PD in addition to PD or AD.

Inclusion

In the PD study, 13 NC were included. In the MCI-GO study, 49 NC were recruited, but 3 were excluded due to SCI. In the DDI study, 92 controls were included (6 volunteers and 86 orthopedic control patients with CSF biomarkers). This gives a total of 151 NC.

4.1.3 Representativeness and generalizability of the studies

PD patients

This project is not population-based, but the patients were recruited from the only public neurological outpatient clinic in the area, and thus should be representative for this population. Potential PD candidates were not routinely asked to participate, but all PD patients considered appropriate for this study according to the inclusion and exclusion criteria were asked to participate. 14 of 55 (25.5 %) patients refrained from participating for different reasons as mentioned under *Inclusion and exclusion criteria in the studies* on page 52, but it is possible that it was the patients with more advanced illness who abstained. A limitation is that the diagnosis of early PD is always somewhat uncertain. We did not have longitudinal follow-up in all the patients or pathological confirmation of the diagnosis, but all the patients fulfilled the QSBB-criteria and had a positive DaTSCAN. Thus, we believe that the study population is representative for the actual population, which are early PD patients in the catchment area of Akershus University Hospital and also for the target population, which are early PD patients in Norway.

SCI and MCI patients

The SCI and MCI patients were routinely asked to participate at the memory clinic, and thus should be representative for the actual and target populations.

Normal controls (NC)

The NC were recruited mainly from spouses or elective orthopedic patients. The orthopedic patients may have more comorbidity as they were selected based on a disease. In addition, it is possible that more individuals who have close relatives with neurodegenerative diseases and thus a higher risk of developing neurodegenerative disease themselves, agree to participate as controls. Anyway, we excluded NC not fulfilling the criteria, and thus we believe that the NC are representative for the middle aged and elderly general population in Norway.

4.1.4 Patient selection in paper 1: Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

PD patients

Inclusion

31 PD patients were included in the first paper according to the PD inclusion and exclusion criteria mentioned in *Inclusion and exclusion criteria in the studies* on page 52 and available CSF biomarkers. 4 of the 35 patients in the PD study were excluded due to (number in brackets) not proper CSF test tube [1], refrained from lumbar puncture [2] and uncertain PD diagnosis at time of inclusion [1].

Representativeness and generalizability

Compared to the population-based incident Norwegian ParkWest cohort study assessing CSF biomarkers and cognition (144), our study had numerically slightly higher proportion of women (38.7 vs. 34.9 %), younger age (64.7 vs. 67.2 years old), longer education (13.1 vs 11.3 years) and disease duration (2.5 vs. 2.2 years), lower UPDRS III (The unified Parkinson's disease rating scales of motor function part III motor examination (205)) (14.9 vs. 21.2) and Hoehn & Yahr (1.5 vs. 1.8) and higher MMSE (28.6 vs. 27.8) and Levodopa Equivalent Dose (LED) (385.2 vs. unmedicated). Thus, our population was younger, better educated and possibly more cognitively normal than in the population-based study, and this may lead to attenuated findings.

Compared to other similar studies in early PD with mean/ median disease duration < 5 years (see *table 1* on page 38), our PD population was mid-range with regard to gender distribution (30 to 50 % females), age (60-70 years old), education (11-16 years), disease duration (4 months to 5 years), UPDRS III (9-30), Hoehn & Yahr (1.5-2.3), MMSE (27-29) and LED (varied from unmedicated to 810.9). Thus, we believe that the included PD patients should be representative for the target population.

MCI non-PD patients

Inclusion criteria (number in brackets)

1. MCI [25] according to the National Institute on Aging - Alzheimer's Association (NIA-AA) core clinical criteria for AD-MCI (136)
2. or SCI with amnesic MCI [2] or Alzheimer's dementia [1] at follow-up
3. and an abnormal score on at least one CSF AD biomarker (A β 42, T-tau or P-tau)

Exclusion criteria

Exclusion criteria were as for PD and in addition cognitive improvement during follow-up or PD.

Inclusion

Of the 118 participants with SCI or MCI in the MCI-GO study, 67 were excluded due to normal CSF. 23 were excluded due to (numbers in brackets) borderline T-tau or P-tau levels [5], cognitive improvement during follow-up [6], SCI at inclusion and follow-up [4] or no follow-up data [2], cancer [2], ischemic stroke [2] and Lewy body disease [2].

Thus, 28 non-PD patients with MCI (MCI non-PD) with increased risk of developing AD were included. 22 had amnesic MCI and 3 had non-amnesic MCI with subjective memory problems at inclusion.

Representativeness and generalizability

Only 15 of the included patients had both pathological CSF A β 42 and tau and thus fulfilled the criteria for AD-MCI high likelihood (136) at inclusion. This could lead to smaller

differences in the CSF biomarker levels between the PD and MCI non-PD groups compared to a homogenous AD-MCI group.

Normal controls

Inclusion criteria

Inclusion criteria were available CSF biomarkers and normal cognition based on MMSE ≥ 28 , and a T-score above 35 on the Rey Auditory Verbal Learning Test (RAVLT) delayed recall, Trail Making Test-B (TMT-B) and Controlled Oral Word Association Test (COWAT) with respect to the NIA-AA criteria (136) (see under *Neuropsychological tests* on page 66).

Inclusion

Only 5 of the 59 controls in the MCI-GO and PD studies had lumbar puncture as this was not a part of the routine, and one control was excluded due to abnormal neuropsychological test results. 64 of the 86 orthopedic controls had neuropsychological examination, but only 36 controls fulfilled the neuropsychological criteria, and 6 of these controls were excluded for other reasons (SCI, vitamin B12 polyneuropathy, kidney transplantation and immunosuppressive therapy, extensive white matter lesions and dilated ventricles at MRI, depression and MMSE score 23).

Thus, 34 NC with CSF biomarkers and normal neuropsychological tests were included. 4 were spouses or relatives to the MCI-GO or PD patients, and 30 were recruited from the orthopedic patients in the DDI-study.

Representativeness and generalizability

54 of the NC were excluded due to missing lumbar puncture and 22 refrained from neuropsychological examination, and this may lead to selection bias. In addition, we excluded 29 patients with abnormal neuropsychological tests to be sure that none of the NC actually had cognitive impairment. Admittedly, this could have lead to a control group which performs cognitively better than the general population. Anyway, the focus was not to compare the neuropsychological function, but CSF biomarkers in a cognitively normal control group and early PD. It is possible that differences in the CSF biomarker levels between the NC and PD groups would have been smaller if we had used a more cognitively heterogeneous NC group.

Comparison of the groups

The three groups were not matched for gender, age or education, and there was numerically a higher percentage of males in the PD than in the NC and MCI non-PD groups (61.3 vs. 58.8 vs. 42.9 %), the age in the PD group was between the NC and MCI non-PD (64.7 vs. 62.6 vs. 66.9 years old) and the education was shorter (13.1 vs. 14.0 vs. 13.7 years). Anyway, there were no significant differences in age, gender or education, so this has probably not affected the results.

The sample sizes in the three groups were quite small, and larger sample sizes could have strengthened and added to the significant results.

4.1.5 Patient selection in paper 2: Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

PD patients

Inclusion

33 PD patients were included in the second paper in accordance with the criteria in paper 1 and available Achieva 3 T MRI data instead of CSF biomarkers. 2 of the 35 patients in the PD study were excluded due to missing MRI data. 3 patients included in this paper were not included in the first paper due to missing CSF biomarkers and uncertain diagnosis at that time, and 1 patient included in the first paper was not included in this paper due to missing MRI.

Representativeness and generalizability

Almost the same PD patients were included in this paper as in the first, and thus should be representative for the early PD population in Norway. Compared to the population-based incident Norwegian ParkWest cohort study assessing hippocampal subfields and cognition in PD and PD-MCI (197), our study had numerically slightly lower proportion of women (36 % vs. 40.4 % in PD and 37.9 % in PD-MCI), younger age (64.9 vs 65.8 and 70.6 years old), longer education (13.1 vs. 11.3 and 11.4 years), lower UPDRS (14.4 vs. 21 and 24.2) and Hoehn & Yahr (1.5 vs. 1.8 and 2) and higher MMSE (28.6 vs. 28.3 and 26.9) and LED (392.2

vs. unmedicated). Thus, our population was possibly more cognitively normal than in this population-based study, and this may lead to less findings.

Normal controls

Inclusion

The inclusion and exclusion criteria were as in paper 1 with the exception that all had to have available Achieva 3 T MRI data instead of CSF biomarkers.

Of the 59 controls in the MCI-GO and PD studies, 48 were excluded due to (number in brackets) missing MRI data [37], missing [5] or not normal [4] neuropsychological data, and 2 females were excluded to balance gender with the PD group. Of the 92 controls in the DDI-study, 71 were excluded due to (number in brackets) missing MRI data [55], missing [1] or not normal [9] neuropsychological data, for different reasons [3] (SCI, vitamin B12 polyneuropathy, kidney transplantation and immunosuppressive therapy) and 3 were excluded to balance gender with the PD group.

Thus, 32 NC were included. 11 were controls in the MCI-GO or PD studies (in 3 of the controls we had to use the MRI data at follow-up) and 21 (5 volunteers and 16 orthopedic patients) were controls in the DDI-study.

Representativeness and generalizability

As many as 98 NC were excluded due to missing MRI or neuropsychological data, and this may have led to selection bias. As previously mentioned, the exclusion of 13 controls with abnormal neuropsychology may lead to a control group which performs cognitively better than the general population. Thus, it is possible that differences in hippocampal subfields between the PD and NC group would have been smaller if we had not excluded NC based on neuropsychological tests.

Comparison of the groups

As for the first paper, the two groups were not matched. There was numerically a higher percentage of males (64 vs. 44 %), higher age (64.9 vs. 62.9 years old) and shorter education

(13.1 vs. 13.5 years) in the PD group compared to NC, but as there were no statistically significant differences, this has probably not affected the results.

The sample sizes in both groups were quite small. We performed power size calculations, which indicated that the sample sizes were adequate to compare the groups with regard to hippocampal subfields with the exception for subiculum and presubiculum.

4.1.6 Patient selection in paper 3: Impaired synaptic function is linked to cognition in Parkinson`s disease

PD patients

Inclusion

30 PD patients were included in the third paper in accordance with the PD inclusion and exclusion criteria and available Siemens Biograph 64 FDG-PET, MRI and CSF biomarkers. In one of the patients with normal cognition we used FDG-PET data at follow-up after 2 years as this was missing at baseline.

5 of the 35 PD patients were excluded due to (number in brackets) missing FDG-PET data [1], refrained from lumbar puncture [2], not proper CSF test tube [1] and uncertain PD diagnosis at time of inclusion [1]. One patient not included in paper 2 due to missing MRI, was not included in this paper either due to missing FDG-PET, otherwise these are the same patients as in the first paper.

Representativeness and generalizability

As this study included approximately the same PD patients as the first paper, our population was possibly slightly less cognitively impaired than in the population-based study (144), and this may lead to attenuated findings, but should be representative for an early PD population in Norway.

Normal controls

Inclusion

Inclusion criteria were available Siemens Biograph 16 FDG-PET, MRI and CSF biomarkers.

Of the 92 controls in the DDI study, 62 were excluded due to missing FDG-PET and 2 due to missing CSF biomarkers. 26 NC were included; 3 were volunteers and 23 were recruited from the orthopedic patients in the DDI-study. None of the 59 NC in the PD or MCI-GO study had 16 FDG-PET.

Representativeness and generalizability

In the NC group, 123 controls were excluded due to missing FDG-PET or CSF data, which may lead to selection bias. In contrast to the two previous papers, the NC were not excluded based on neuropsychological tests, and as such they represent a more cognitively heterogeneous control group. Some of them actually had cognitive impairment according to the neuropsychological criteria, as one also would find in a non-selected general population. This may lead to smaller differences in the associations between CSF A β and cortical glucose metabolism between the PD and NC groups.

MCI non-PD patients

Inclusion

In addition to the PD and NC groups described in the paper, we also assessed a group of MCI non-PD patients (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) for comparison. Inclusion criteria were MCI according to the criteria in the MCI-GO study and available Siemens Biograph 16 FDG-PET, MRI and CSF biomarkers.

Of the 85 MCI patients, 26 were excluded due to (number in brackets) missing FDG-PET [21], missing MRI [3], ischemic stroke [1] and psychiatric comorbidity [1]. Thus, 59 patients with non-PD MCI were included from the MCI-GO study. From this non-PD MCI group, a subgroup of 30 A β negative MCI patients with CSF A β levels above pathological cutoff (700 ng/ L) was selected.

Representativeness and generalizability

In the non-PD MCI group, 24 subjects were excluded due to missing MRI or FDG-PET data, which may lead to selection bias. This group was not selected based on pathological AD CSF biomarkers, but represented a heterogeneous MCI group, and a pure AD-MCI group would probably show stronger associations between CSF A β and cortical glucose metabolism.

Comparison of the groups

As for the previous papers, the groups were not matched. There was numerically a higher percentage of males in the PD group compared to NC and non-PD MCI (63.3 vs. 50 vs. 52.5 %), higher age compared to the non-PD MCI group (65 vs. 65 vs. 62.2 years old) and longer education (13 vs. 12.6 vs. 12.6 years). The only statistically significant difference was younger age in the A β negative non-PD MCI group, which could contribute to less findings in this group. Otherwise the insignificant differences have probably not affected the comparisons between the groups.

The sample sizes in the groups were quite small, and we have not performed power size calculations, so the findings need to be reproduced in larger populations.

4.2 Study design

The three publications in this project are based on cross-sectional baseline data from the ongoing longitudinal, prospective cohort PD study, MCI-GO study and DDI study. The cross-sectional design limits the opportunities to investigate possible predictors of cognitive decline and dementia.

All the examinations were conducted within approximately three months in the PD study and over some longer time periods up to ½-1 year in the MCI-GO study. For the orthopedic controls in the DDI study, the examinations were conducted over longer time periods up to 1-2 years. For the MCI-GO patients, it is possible that the disease progressed during the assessment period, and thus that different examinations correspond to different time periods in the disease development. This is most relevant for the FDG-PET analysis (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) as the lumbar puncture was usually performed before FDG-PET. It is possible that this may have led to stronger associations between low CSF A β levels and decreased cortical glucose metabolism as the disease has progressed. For the NC, this prolonged collection of data should not have any major consequences as this group per definition is normal, but we cannot exclude that a few of the controls have declined cognitively from the time of inclusion and lumbar puncture to the neuropsychological or imaging assessments.

There were partially different research physicians, neuropsychologists, study nurses and occupational therapists performing the examinations, and the participants were included in partially different time periods in the different studies. Although we have tried to coordinate the practice, we cannot exclude that this may have affected the clinical evaluations.

4.3 Examinations

4.3.1 Clinical examination

The PD patients were examined and diagnosed by neurologists at the outpatient clinic. All the included participants in the PD and MCI-GO studies and most of the orthopedic participants in the DDI study were examined by trained research physicians. This included thorough anamnesis and physical examination of the nervous system. Depressive symptoms were assessed by the physicians or study nurses with the 15-item Geriatric Depression Scale (GDS) (206) with cutoff score < 7. In addition, a battery of blood tests to examine for comorbidity was analyzed.

Specific examinations in the PD study

In the PD group, standardized rating scales of motor function including The unified Parkinson's disease rating scales of motor function (UPDRS) part III motor examination (205) and Hoehn & Yahr (204) were performed by research physicians. All the patients had their medication as usual when assessed, but were unfortunately not tested at a standardized time in relation to medication. Total daily levodopa equivalent doses (LED) were calculated based on conversion formulas giving standardized LED for each antiparkinsonian drug (207). The PD patients, and relatives when available, were asked questions about the patients cognitive functioning, psychiatric symptoms and activities of daily living (ADL).

4.3.2 Dopamine transporter scan (DaTSCAN)

Dopamine transporter scan (DaTSCAN) was performed in all included PD patients by intravenous injection of 185 MBq (^{123}I) FP-CIT (supplied by GE Healthcare), and after 3 hours, single photon emission computed tomography (SPECT) images were acquired with 2-headed gamma camera (Infinia Hawkeye 4) in a procedure that lasted about 30 minutes. 10-30

minutes before the intravenous injection of 185 MBq (^{123}I) FP-CIT, all the patients got a thyroid-blocking preparation (200 mg intravenous sodium perchlorate) to stop local uptake of the ligand.

The raw image data were reconstructed on a VISION workstation (Sopha medical/ GE) using filtered back projection (FBP) reconstruction, Butterworth filter, order 6, cutoff 0.30 and no attenuation correction. Reconstructed images were initially analyzed visually by a nuclear medicine physician. In addition, volume-of-interest based semi-quantification of regional DAT striatal binding was undertaken calculating the Total Striatal Binding Potential Index (BP) and the Asymmetry Index (AI) using Dr. Durval Costa's method (reference area for BP $\% \geq 60 \%$ and for AI $> 10 \%$). The relationship between the uptake in striatum compared to the occipital cortex (ratio 1) and the relationship between the uptake in putamen and nucleus caudatus (ratio 2) were calculated using the Dr. Freiberg Bispebjerg Hospital method (reference area for ratio 1 ≥ 2 and for ratio 2 ≥ 0.8). (See European Association of Nuclear Medicine (EANM) procedure guidelines for Brain neurotransmission SPECT using ^{123}I -labelled dopamine transporter ligands (208). www.eanm.org) (209).

Limitations

All the PD patients had pathological DaTSCAN supporting the clinical diagnosis of PD, but this examination discriminates from essential tremor and secondary parkinsonism and not from atypical parkinsonism (51).

4.3.3 Cognitive screening

Cognitive screening tests

All the patients in the PD and MCI-GO studies underwent three cognitive screening tests performed by research physicians, study nurses or occupational therapists at the memory clinic: MMSE (210) is a global cognitive screening test. I-Flex (interference, word fluency and number-letter items) is a short form of the executive interview (EXIT) (211). The Neurobehavioral Cognitive Status Examination (Cognistat) assesses orientation, attention, language, visuoconstruction, memory, calculation and logical ability (212).

Based on these screening tests and the clinical examination and interview with the patients and relatives, the Stepwise comparative status analysis (STEP) parameters 13-20 (assessing memory disturbance, disorientation, impairment of abstract thinking, visuospatial impairments, poverty of language, sensory aphasia, visual agnosia, and apraxia) (213) and the Clinical Dementia Rating (CDR) which is a six-item (memory, orientation, judgment and problem solving, community affairs, home and hobbies and personal care) global measure of cognitive functioning (214) were completed by the research physicians.

Global Deterioration Scale

The screening tests, STEP and CDR were in addition to the clinical interview used for cognitive staging in consensus with at least two research physicians according to the Global Deterioration Scale (GIDS) (215, 216).

Patients were classified as no CI (GIDS 1) or SCI (GIDS 2) if they scored better than published cutoffs on all the screening tests (≥ 28 for MMSE, ≤ 1 point at I-flex, average level at Cognistat adjusted for age). If they reported subjective cognitive symptoms, they were classified as SCI in accordance with SCI-criteria (217), otherwise they were classified as cognitively normal.

Patients reporting subjective cognitive symptoms, scored poorer than the cutoffs on at least one of the cognitive screening tests and had no or very mild deficits in activities of daily living (ADL) were classified as MCI (GIDS 3) in accordance with MCI-criteria (218).

Patients with cognitive impairment to such an extent that it affects ADL scoring GIDS ≥ 4 , CDR ≥ 1 or the sum of STEP variables 13-20 ≥ 2 , were considered to have dementia and excluded. This method of determining GIDS stage is in agreement with previously published methods and has been applied in several papers (219-221).

Limitations

A limitation with this method is that, except that Cognistat is age-adjusted, the screening tests are not adjusted for age or education, and thus may patients with higher pre-morbid cognitive capacity and younger age apparently score normal despite cognitive decline.

Unfortunately, we did not use the latest published PD-MCI criteria (55) as these were not published at the time of the start of this project and as we already had used the described screening criteria in the parallel ongoing MCI-GO study. These criteria require impairment on a scale of global cognitive abilities validated for use in PD such as MoCA or at least two neuropsychological tests. Anyway, due to the low number of PD patients, we could not perform and compare the different analyses in subgroups with and without PD-MCI, so this has not affected the results.

4.3.4 Neuropsychological tests

A well-validated neuropsychological test battery assessing five domains (memory, visuospatial, attention, executive and language functions) was administered by clinical neuropsychologists and for the orthopedic NC by a trained study nurse. From this battery we chose to use tests of verbal learning and verbal and visual memory, visuospatial construction, attentional and executive (response inhibition and verbal fluency) functions, but not language tests as this is less affected in PD. The battery was repeated for the PD patients after two years.

Rey Auditory Verbal Learning Test (RAVLT)

Verbal learning and delayed memory were measured by RAVLT (222). The patient is supposed to learn a list of 15 words over 5 trials and then recall the words after 30 minutes. The numbers of learned and recalled words are counted, and high scores represent good performances.

Rey Complex Figure Test (RCFT)

Visuospatial copying and delayed visual reproduction after 30 minutes were measured by RCFT (223-225). The patient is supposed to copy a complex figure on a piece of paper and recall the figure after 30 minutes. High scores represent good performances.

Trail Making Test A (TMT-A)

TMT-A (226) was used to measure psychomotor speed in the third paper. The patient is supposed to draw a line between numbers on a paper while the time is recorded. The score corresponds to the time spent, and a high score represents poor performance.

Trail Making Test B (TMT-B)

TMT-B (226) was used to measure divided attention which involves complex visual scanning, processing speed and mental flexibility. The patient is supposed to draw a line between numbers and letters (1-A-2-B-3-C etc.) on a paper while the time is recorded. The score corresponds to the time spent, and a high score represents poor performance.

Stroop Color Word (SCW)

The SCW subtest from Delis-Kaplan Executive Function System (D-KEFS) (223, 227) was used to assess response inhibition. The patient is supposed to specify the color of the ink when the name of a color is printed in another color (e.g. the word “blue” is printed in black ink, then the patient is supposed to say “black”). A high score represents poor performance.

Controlled Oral Word Association Test (COWAT)

COWAT (226) was used to evaluate letter fluency. The patient is supposed to say as many words as possible within one minute starting with the letters “F”, “A” and “S”. The score corresponds to the total number of words, and a high score represents good performance.

Use of neuropsychological tests in the papers

The neuropsychological raw scores were used to explore the associations with CSF biomarkers in the first paper and hippocampal and parahippocampal sub-regions in the second paper.

The raw scores were converted to T-scores, a normally distributed scale with a mean score of 50 and a SD of 10. A T-score of 35 correspond to 1.5 SD below the normative mean. T-scores for RAVLT delayed recall, TMT-B and COWAT were used for inclusion and exclusion of NC in the first and second paper. In the third paper, T-scores were used to compare the

neuropsychological tests between the PD and NC groups and to correlate with the CSF-biomarker based regional glucose metabolism clusters at baseline and follow-up.

Limitations

We lacked a test for visual recognition memory. A limitation with TMT-B is that it has a motor component, and although our PD patients were in mild to moderate motor stages, we cannot exclude that this may contribute to poorer results on this test.

4.3.5 CSF analysis

Collection and handling

CSF samples were collected in 4.5 mL sterile polypropylene tubes by lumbar puncture in the L3/4 or L4/5 intervertebral spaces. The samples were handled under standard condition with centrifugation at 2000 g for 10 minutes to eliminate cells and other insoluble material and stored at -80 °C until biochemical analysis. All the CSF samples were analyzed at the laboratory at Akershus University Hospital except for neurogranin.

CSF A β 42, T-tau and P-tau analyses

ELISA Innotest

CSF A β 42, T-tau and P-tau were consecutively analyzed with commercially available ELISA (enzyme-linked immunosorbent assay) kits (Innotest β -amyloid, Innotest hTAU-Ag and Innotest Phospho-Tau (181P), Fujirebio Europe, previously Innogenetics, Gent, Belgium) adapted to a Tecan Robotic Microplate 150 Processor (Tecan AG, Switzerland) as described in the laboratory handbook at Akershus University Hospital: After defrosting and a short centrifugation, the CSF and conjugate 1 (IgG monoclonal anti-amyloid/ anti-Tau/ anti-Ph-Tau detection antibodies marked with biotin) are added to the wells coated with monoclonal anti-amyloid (1-42)/ anti-Tau/ anti-Ph-Tau capture antibodies from mice, and an antibody-antigen-antibody marked complex is formed. After incubation and washing, conjugate 2 (peroxidase-marked streptavidin) is added. Streptavidin and biotin form a complex. After incubation and washing, adding substrate TMB evokes a blue color when reacting with peroxidase. After incubation, a stop solution (acid) is added and the color change to yellow. The color is

measured photometric and is proportional with A β 42/ T-tau/ P-tau in the CSF. Intern sample controls are analyzed each time and extern sample controls are analyzed three times a year, but sampling and handling may lead to pre-analytical variation.

Cutoff values

CSF A β 42 was considered pathological if ≤ 600 ng/ L, P-tau if ≥ 80 ng/ L and T-tau if > 450 ng/ L in the first paper, according to local cutoffs. In the non-PD MCI group related to the third paper (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*), a subgroup of A β negative MCI patients with CSF A β levels above 700 ng/ L was selected.

Limitations, validity and reliability

There are limitations related to the assays for CSF A β 42, T-tau and P-tau detections. Both interassay and interlaboratory variabilities are high, making it difficult to compare results from different laboratories and to perform longitudinal studies (60, 228). However, interlaboratory variability may be higher than intralaboratory variability (228), and we analyzed all the samples at the same laboratory. We have used the laboratory's own cutoff values using locally samples from well-characterized individuals, as recommended (60).

Biases may be preanalytical due to patient selection, lumbar puncture procedures, sample handling and storage, analytical due to difference in assay kit and sample processing and postanalytical due to data-handling including curve fitting for the standard curve or include assay related factors such as method optimization and batch variation in analytical kits.

Therefore, interlaboratory quality control is important to discover whether the biomarker level is outside an acceptable range and sudden or longitudinal changes, giving information on the measured biomarker levels, the mean and variation in biomarker levels across laboratories for comparison and the longitudinal stability in CSF biomarker levels (60). We have used standard operating procedures based on international cooperation studies (BIOMARKAPD) regarding standardization of sample processing, laboratory techniques and assay implementation, as proposed (60).

CSF A β 38 and 40, t- α -syn and hemoglobin analyses

Electrochemiluminescence (ECL)

The CSF A β 38 and 40 and t- α -syn were analyzed at the same time in all the samples with ECL using immunoassay kits from Meso Scale Discovery (Rockville, Maryland, USA): Electrochemiluminescent SULFO-TAG labels are tagged to detection antibodies. For A β 38/40, anti-beta amyloid (1-16) 6E10 capture antibody and SULFO-TAG Anti-human A β 38/40 detection antibodies were used. For t- α -syn, anti- α -syn capture antibody and SULFO-TAG anti- α -syn detection antibodies were used (229). When electricity is applied to the plate electrodes, this leads to a light signal by the SULFO-TAG labels, and the light intensity is measured by an instrument to quantify the levels of the analytes in the sample.

Hemoglobin ELISA

Since red blood cells contain α -syn, we measured the level of hemoglobin to control for blood contamination (230) in the CSF sample with ELISA kit (Bethyl Laboratories, Inc., Montgomery, Texas, USA) after the same principles as for the InnoteSts: The hemoglobin in the sample is captured by anti-human hemoglobin capture antibody in the wells, and a biotinylated anti-hemoglobin detection antibody is added to bind to the captured hemoglobin. A streptavidin-conjugated peroxidase is added to catalyze a colorimetric reaction with the chromogenic substrate TMB, which produces a blue product, which turns yellow when reaction is terminated by sulfuric acid. The absorbance of the yellow product is proportional to the amount of hemoglobin in the sample.

One study found that CSF t- α -syn level (ELISA kit, Covance) was modestly increased by hemoglobin concentrations (ELISA kit, Bethyl Laboratories) higher than 1000 ng/mL (175), while another study found that CSF t- α -syn (measured by Luminex assay) increased substantially when hemoglobin level was (ELISA kit from Bethyl Laboratories) > 200 ng/mL (230). Two other studies did not find any correlation between CSF t- α -syn and hemoglobin (184) after removing a few outliers (187). Thus, we chose a cutoff level in the lower range between these values of 350 ng/mL for hemoglobin, and samples with higher hemoglobin concentrations were excluded from the further analysis as previously recommended (175).

Limitations

The analyses of CSF A β 38 and 40 and t- α -syn were performed in the PD patients, but only in a subgroup of the control groups considered for the first paper at that time where all the NC had normal CSF AD biomarkers. As we changed the inclusion and exclusion criteria for the NC and MCI non-PD groups after these analyzes were performed, this may have led to selection bias for these analyzes in the first paper. T- α -syn values in participants with too high hemoglobin concentrations in the CSF were excluded, reducing the number of participants for this part of the analysis. We did not check whether there was any correlation between t- α -syn and hemoglobin, and thus can not rule out that blood contamination has affected the t- α -syn level. There were no reference values available.

Neurogranin analysis

CSF neurogranin concentration was measured at the same time in all the samples using a previously described in-house ELISA (75). Briefly, an assay based on the monoclonal antibody Ng7 (epitope including amino acids 52–65 on neurogranin) was used for capture, a polyclonal neurogranin anti-rabbit antibody (ab23570; Upstate Biotechnology, Lake Placid, NY, USA) for detection, and full-length neurogranin protein as calibrator. These analyses were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

Limitations

We were able to measure neurogranin concentrations in all included PD patients, but one outlier in the PD group with values 10-20 times higher than the mean in the group, was removed (this outlier was included in some analyses for reference). For 19 of the 26 included controls in the third paper there were sufficient CSF available to assess neurogranin concentrations, and we added an additional 11 controls for which there was no available PET data. The controls in this control material (N = 30) were younger (mean age 6.15 years) and there were more females (N = 18). No reference values were available for CSF neurogranin.

Use of CSF biomarkers in the papers

CSF A β species, T-tau, P-tau and t- α -syn were used in the first and second paper to compare the levels between PD, non-PD MCI and NC groups and to assess the associations with cognition and hippocampal and parahippocampal sub-regions in the PD patients. In the third paper, A β species, t- α -syn and neurogranin were used to evaluate the association with cortical glucose metabolism and cognition in the PD group. For comparison A β 42 was used to evaluate the association with cortical glucose metabolism in NC and non-PD MCI groups (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*). CSF neurogranin was used to compare the levels between PD and NC groups and correlate with A β species, t- α -syn and UPDRS motor score.

4.3.6 MRI

MRI scanners

The PD and NC groups in the second and third paper underwent 3 T Philips Achieva MRI scanning (Philips Medical Systems, Best, The Netherlands) as previously described (231). A single 3D turbo field echo sequence was acquired for morphometric analysis with the following sequence parameters: repetition time/ echo time/ inversion time (TI)/ flip angle (FA) = 4.5 ms/ 2.2 ms/ 853 ms/8°, matrix = 256 x 213, 170 slices, thickness 1.2 mm, in-plane resolution of 1 mm x 1.2 mm.

The non-PD MCI group in the FDG-PET analysis (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) underwent 1.5 T Siemens Espree MRI scans (Siemens Medical Solutions, Erlangen, Germany). One magnetization-prepared rapid acquisition gradient echo (MPRAGE) 3D sequence was acquired (TR/TE/TI/FA = 2400/3.65/1000/8°, matrix = 240 × 192), 160 sagittal slices, thickness = 1.2 mm, in-plane resolution of 1 mm × 1.2 mm.

Limitations

A limitation is that there was used a different MRI scanner in the non-PD MCI group in the third paper (unpublished data, see *Supplementary material in paper 3* on page 140 under

Attachments). Anyway, the MRI data were not compared between the groups and thus should not have affected the results.

MRI segmentations and analyses

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.3.0 (<http://surfer.nmr.mgh.harvard.edu/>) as previously described (231). This includes segmentation of the subcortical white matter (WM) and deep grey matter volumetric structures (232) and parcellation of the cortical surface according to a previously published parcellation scheme (233). This labels cortical sulci and gyri, and thickness values are calculated in the regions of interest (ROIs).

Hippocampal and parahippocampal sub-regions

In the second paper, the thickness values of the ERC and PRC were calculated using methods based on ultra-high resolution ex vivo applied to in vivo MRI, as implemented in FreeSurfer (234, 235). Further, an automated procedure for segmentation of the subfields of the hippocampus, as implemented in FreeSurfer, was employed (199). From the hippocampal segmentation we chose to analyse volume of the following sub-regions: subiculum, presubiculum, cornu ammonis sector 1 (CA1) separately, sector 2 and 3 combined (CA2-3), sector 4 combined with the dentate gyrus (DG) and the hippocampal tail, the posterior part of hippocampus which cannot be segmented into the different subfields (199).

We compared the volume or thickness of the sub-regions in PD and NC, and in the PD group we also assessed the associations with CSF biomarkers and cognition.

Cortical thickness region of interests (ROIs)

In addition, we analysed the 34 cortical thickness ROIs (233) to differentiate this to hippocampal changes in the PD group in the second paper and to assess correlations between A β and cortical thickness in the PD and NC (and non-PD MCI, unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) groups in the third paper.

Limitations

Manual segmentation is assumed to be more accurate than automated segmentation and is thus considered as the gold standard (236).

FreeSurfer is applied on lower resolution (1 mm^3 , T1-weighted, 1.5-3 T) than many other manual or automated segmentation techniques ($0.2\text{-}0.7\text{ mm}^2$, T2-weighted). The resolution of the atlas in FreeSurfer is only sufficient to provide a coarse segmentation of the sub-regions (237), and it has only been validated at high-resolution 3T MRI (T1-weighted) (199, 238). Due to limited MRI resolution in our study, it is difficult to accurately map histoanatomic areas, but we used 3 T high field strength which allows more detailed imaging than 1.5 T.

Neuroanatomical boundary selections vary from different segmentation techniques. The parcellation scheme used for the FreeSurfer segmentation is based on that one coronal section in the body of hippocampus is used to segment the subfields along the axis of the hippocampus (199), but the position of the subfields anatomically actually differ along the axis (238). The consequence is that parts of subfields are allocated to neighbouring subfields. For instance a part of the CA1 is with FreeSurfer segmented as a part of subiculum and CA2-3, while in other techniques and anatomical studies it is defined as a part of CA1 (238-240). This may lead to that associations found to be linked to subiculum or CA2-3 actually are related to CA1 (238). It has also been suggested that the subfield defined as presubiculum with FreeSurfer actually is a part of subiculum (241). This makes comparisons between studies difficult, and therefore a unified segmentation protocol is needed (242).

In a validation study, automated volume measurements of the larger subfields CA2-3 and CA4-DG and to a lesser degree subiculum using FreeSurfer on high resolution 3T MRI correlated well with manual volume estimates (199). In another study comparing manual with automated segmentations of hippocampus, the segmentations were more often incorrect with FreeSurfer than with manual methods (243).

One study compared automated hippocampal subfield segmentation using FreeSurfer on lower resolution 3 T MRI with manual segmentation on high-resolution 3 T MRI (244). They found moderate correlations for subiculum combined with presubiculum, CA2-3 together with CA4-DG, and the whole hippocampus, but no correlations for CA1. The subfields were larger with the automated compared to the manual method with exception for CA1, which was smaller. For CA1 larger volumes were more underestimated than smaller volumes, introducing a bias which makes it harder to detect atrophy. When they considered CA1, subiculum and presubiculum defined by FreeSurfer as a single region compared to the manually defined CA1 region, the correlation was much higher and the bias was lower. Thus,

we could have merged CA1 together with subiculum and presubiculum to better correlate with the CA1 region. This underestimation of CA1 with FreeSurfer may be due to lower resolution, but may also reflect anatomical differences in the definition of the subfields as mentioned above. Thus, the lower resolution FreeSurfer and high-resolution manual subfield segmentations only partly overlap (244), and this has to be taken into account when interpreting our results.

Another study found sufficiently high correlations between manual and automatic hippocampal segmentations on high-resolution 3 T MRI, but the volumes were overestimated in FreeSurfer, more so in the younger (20-30 years) than the older (60-70 years) age group (245). In the older age group the overestimation was higher for smaller than larger volumes, leading to an age bias and a reduced sensitivity to detect decreases in hippocampal volume in the older. This may be due to that the segmentation atlas used in FreeSurfer is based on middle-aged brains (mean age 38 years) and not optimized for older brains (245).

However, we used a newer version of FreeSurfer, all segmentations were manually inspected, and the image and segmentation quality were considered good. We only included the larger hippocampal structures with the best Dice spatial overlap coefficients with manual segmentation (199). The automated technique has a higher reproducibility compared to manual segmentation (199, 243), and CA2-3, CA4-DG, and subiculum, presubiculum and CA1 together have reproducibility similar to the whole hippocampus (246). Manual segmentation is dependent on operator skills with intra- and interrater variability, the operator may repeatedly make the same mistakes, the segmentations are more consistent in the plane they were drawn than in other directions, and it is much more time-demanding compared to automated methods (199). A new version of FreeSurfer (v6.0) based on an atlas of the hippocampal sub-regions with a much higher resolution better matching histological studies and a larger number of structures has become available and should be used to replicate our findings (237).

4.3.7 FDG-PET

FDG-PET scanners

In the third paper, FDG-PET/CT imaging was performed with a Siemens Biograph 64 PET/CT scanner in the PD group, while in the NC group (and non-PD MCI group, unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) it was performed with a Siemens Biograph 16 PET/CT scanner.

Procedure

All subjects fasted at least 4 hours before the image acquisition, and had an intravenous bolus of 218 MBq (± 23 MBq) injected and rested for 45 minutes after the injection before being positioned head-first supine in the scanner. Prior to the PET acquisition, a low-dose CT scan for attenuation correction was acquired. Patients were scanned for 15 minutes in 3D mode using one bed position (axial range = 16 cm). Acquired data were corrected for random events, dead time, attenuation (CT-derived μ -map), scatter (model based), and decay. PET volumes were reconstructed using an iterative algorithm (OSEM 2D, 4 iterations, 8 subsets), and a post-reconstruction 3D Gaussian filter of width 3.5 mm full-width at half maximum was applied. The axial image format was 256×256 (pixel size: 2.67 mm $\times$ 2.67 mm), with a slice thickness of 2.00 mm. Blood glucose at FDG injection time was measured routinely, and the subject was excluded if blood glucose exceeded 8.0 mmol/l.

Whole head PET volumes were co-registered to the anatomical volume using a 6-parameter rigid body spatial registration as implemented in the Spatial Parametrical Mapping (SPM 8, Wellcome Trust Centre for Neuroimaging, UCL, UK) coregistration tool. PET volumes were resliced into 1 mm MRI space using a spline interpolation. For each subject, a voxel-based intensity normalization to the mean uptake in the cerebellar white matter was performed. Prior to intensity normalization, the cerebellar white matter mask was eroded to avoid influence due to partial volume effects, inaccurate segmentation or co-registration. Intensity normalized volumes were used for all further analyses. Because there is both global cerebral, brainstem and cerebellar cortical hypometabolism in PD, neither global mean nor cerebellar normalization is suitable. We therefore chose to normalize the FDG-PET uptakes against mean cerebellar white matter (22).

Use of FDG-PET data

In the third paper, the FDG-PET imaging was used to assess the correlation between CSF A β species, t- α -syn and neurogranin and regional glucose metabolism in the PD and NC groups (and non-PD MCI groups, unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*). In addition, correlations between the CSF-biomarker based regional glucose metabolism clusters at baseline and neuropsychological tests at baseline and follow-up were considered.

Limitations

A limitation is that we applied two similar, but not identical PET/ CT scanners in the PD and control groups. Anyway, the scan and image reconstruction parameters and the post-processing procedure were identical, and all the analyses were conducted within the groups, but we could not directly compare the results between the groups.

4.4 Statistical analysis

IBM® SPSS® Statistics for Windows (Armonk, NY, IBM Corp, USA) was used for statistical analyses; version 20 in the two first papers and version 22 in the third paper.

4.4.1 Paper 1: Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

Comparison of demographical and clinical data and CSF biomarkers

Comparisons between PD, MCI non-PD and NC with regard to continuous variables of demographic and clinical data and CSF biomarkers were made using non-parametric Kruskal-Wallis test as not all of the variables were normally distributed. Post-hoc Mann-Whitney U test was performed when significant differences emerged. When comparing the CSF biomarkers, we did not correct for multiple comparisons as the CSF biomarkers were compared in an exploratory setting. For gender, which is a categorical variable, Pearson Chi Square test was used. Correlations between the CSF biomarkers in the different groups were assessed using Spearman Correlation.

Associations between CSF biomarkers and neuropsychological tests

Correlations between the CSF biomarkers and raw scores of the neuropsychological tests in the PD group were assessed with Spearman Correlation. Significant correlations were further assessed with linear regression with the neuropsychological tests as dependent variables. MMSE, LED, UPDRS part III, GDS and disease duration were used as independent variables in single linear regressions for each neuropsychological test, and if significant at a 10 % level, included in the multiple linear regression analyses with each CSF biomarker as predictor adjusting for gender, age and education (independent variables), which are potential confounders. There were no relevant interaction effects. The residuals were normally distributed. $P \leq 0.05$ was considered statistically significant.

4.4.2 Paper 2: Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

Comparison of demographical and clinical data

Continuous variables of demographical and clinical data in the PD and NC groups were compared by Mann-Whitney U tests, and for categorical variables (gender) Pearson Chi-square test was used.

Comparison of hippocampal and parahippocampal sub-regions and associations with CSF biomarkers and neuropsychological tests

The sum of the left and right side MRI subfield volume variables were calculated and normalized to intracranial volume (ICV) by dividing the volume variables by ICV, and multiplied with 1000 to get promille of ICV. The average of the left and right side PRC, ERC and 34 cortical thickness variables were calculated.

The normalized subfield volume variables and the average PRC, ERC and 34 cortical thickness variables were used as dependent variables in multiple linear regression analyses with group (PD or NC) as predictor, adjusting for gender and age (independent variables). The multiple comparisons of the 9 hippocampal and parahippocampal sub-regions were corrected for by dividing the p-value 0.05 with the number of dependent variables according to Bonferroni, giving a threshold of significance of $p \leq 0.0056$ for these analyses.

The normalized subfield volume variables and the average PRC and ERC were further assessed as dependent variables in multiple regression analyses with each CSF biomarker as predictor adjusting for age and gender (independent variables) in the PD group.

Finally, we performed multiple regression analyses with raw scores of the neuropsychological tests as dependent variables and MRI variables as predictors adjusting for age, gender and education. CSF biomarkers, UPDRS part III and LED if proved to be significant predictors of a neuropsychological test at a 10 % level, were included in the multiple regression analysis together with the MRI variables. The residuals in the multiple regression analyses were normally distributed. P-values ≤ 0.05 were considered significant.

4.4.3 Paper 3: Impaired synaptic function is linked to cognition in Parkinson`s disease

Comparison of demographical and clinical data, CSF biomarkers and neuropsychological tests

Continuous variables of demographical, clinical data, CSF biomarkers and neuropsychological tests in the PD and NC groups were compared by T-test for age and Mann-Whitney U tests. For categorical variables (gender) Pearson Chi-square test was used.

According to examination of histograms and as assessed by the Kolomogorov-Smirnov test of normality, CSF neurogranin concentrations were not normally distributed in neither the PD nor the control group. We therefore assessed group differences using Mann-Whitney U. There were no significant correlations (Spearman`s rho) between CSF neurogranin concentrations and age or sex (neither within the PD group nor within the control group). We did therefore not correct for age or sex when comparing the groups. One outlier in the PD group with values 10-20 times the mean in the group, was removed. (The outlier was included in additional analyses for reference.)

Within the PD group, the distribution of CSF neurogranin, A β 38, 40 and 42 concentrations as well as UPDRS scores were borderline normally distributed according to examination of histograms and as assessed by the Kolmogorov-Smirnov test of normality. We therefore chose to assess the correlation between these variables with Spearman correlations. (The

estimations of these correlations were repeated with parametric Pearson correlations with very similar results, data not shown.)

Associations between FDG-PET and CSF biomarkers and cognition

Statistical maps were generated using the general linear model as implemented in FreeSurfer. Auxiliary statistical analyses were performed using IBM SPSS version 22. Statistical maps of correlations (while correcting for the effects of age and gender) between FDG-PET uptake (or cortical thickness) and each CSF biomarker concentration were created. This operation was performed separately in the PD and control groups. Also, similar statistical maps of correlations between cortical thickness and CSF biomarker concentrations were created. These statistical operations on global data and surface maps were generated by computing a general linear model of the effects of CSF biomarker concentrations on FDG uptake (or cortical thickness) at each vertex, while controlling for the effects of age and gender. A cluster-wise correction for multiple comparisons was employed, based on Hagler et al. (247). A cluster forming threshold of $P < 0.01$ was chosen, and only clusters with a cluster-wise $P < 0.01$ (Bonferroni corrected for examining both hemispheres) were kept.

The coefficient of determination, R^2 , was determined for the relation between CSF biomarkers (separately) and FDG uptake or cortical thickness (in pertinent areas as determined above). The effects of age and gender were regressed out of FDG uptake or cortical thickness, and the resulting residual was entered in a simple regression model as dependent variables, with the CSF biomarker concentration as explanatory variable.

To assess the relationship with cognitive function, mean FDG uptake (corrected for age and gender) in the pertinent areas as determined above for each CSF biomarker were also correlated (Spearman) with T-scores of neuropsychologic tests.

Two-year follow-up cognitive data were available for the PD subjects. To assess the association between cognition, time and FDG-PET clusters as determined above, a mixed models approach was used: each of the examined neuropsychological variables were entered as dependent variables accounting for repeated measures (in separate models) with age, the FDG-PET cluster corresponding to CSF A β 42, and also an interaction between time and the FDG-PET cluster (to model the association between FDG-PET and change over time) as independent variables. *A priori*, the FDG-PET cluster corresponding to CSF A β 42 was chosen

for this analysis because it is one of the larger, representative clusters and the well-documented role of A β 42 in synaptic pathology.

4.5 Ethics

The World Medical Association developed the Declaration of Helsinki in 1964 (revised in 2013 (248)) as a statement of ethical principles for medical research involving human subjects to ensure that the health and rights of the participants are safeguarded.

In accordance with the Helsinki Declaration and the Norwegian Health and Research Act, the study protocol was approved by the South-Eastern Norway ethical committee for medical research 24.02.11, last approved change 12.05.14, approval number 2011/99.

All participants were given oral and written information, and written informed consent was obtained before any study specific procedures. The participants are assumed to be competent to give consent as dementia was an exclusion criterion. The participants could withdraw from the study without explanation at any time. The collected data were anonymised by using a code key and stored in an electronic research database available for the researchers only.

The examinations in the project were time-consuming for the participants and demanded attendance for a few hours on 4-5 different days at Akershus University Hospital and Oslo University Hospital. All the examinations were for free, and travel expenses were refunded if desired. Some of the examinations were challenging for the participants, for instance the MRI examination lasted for as long as about 45 minutes and the neuropsychological examination for up to 3-4 hours. All patients were encouraged to perform a lumbar puncture if there were no contraindications. This procedure involves some degree of pain, and the patients were informed about the procedure and potential complications such as post puncture headache. The patients were informed about the examination results, which were followed up clinically if necessary.

The participants received a more thorough assessment than what is normal in the clinic, as the study examinations are not routinely performed in PD patients and of course not in the healthy controls. There is a known increased risk of developing dementia in PD on group level. An ethical dilemma is that when examining the participants, we are evaluating potential risk factors for development of dementia on an individual basis even before any cognitive

symptoms have arisen, and there are limited opportunities to slow the progression. Thus, the participants must eventually live with the knowledge of an increased risk of dementia in their mind. This potential burden can be justified on the basis of the aim of the research which is of benefit for the patient group.

5 List of publications

5.1 Publications included in the thesis

1. Stav AL, Aarsland D, Johansen KK, Hessen E, Auning E, Fladby T. Amyloid-beta and alpha-synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease. *Parkinsonism & related disorders*. 2015;21(7):758-64. (249)
2. Stav AL, Johansen KK, Auning E, Kalheim LF, Selnes P, Bjornerud A, et al. Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study. *NPJ Parkinson's disease*. 2016;2:15030. (250)
3. Selnes P, Stav AL, Johansen KK, Bjornerud A, Coello C, Auning E, et al. Impaired synaptic function is linked to cognition in Parkinson's disease. *Ann Clin Transl Neurol*. 2017;4(10):700-13. (53)

5.2 Other publications

- Haram A HE, Auning E, Stav AL, Boeve B et al. Clinical Correlates of RBD in Early Parkinson Disease. *J Alzheimers Dis Parkinsonism*. 2014;4:174. (251)
- Auning E, Selnes P, Grambaite R, Saltyte Benth J, Haram A, Lovli Stav A, et al. Neurobiological correlates of depressive symptoms in people with subjective and mild cognitive impairment. *Acta psychiatrica Scandinavica*. 2015;131(2):139-47. (252)
- Hessen E, Stav AL, Auning E, Selnes P, Blomso L, Holmeide CE, et al. Neuropsychological Profiles in Mild Cognitive Impairment due to Alzheimer's and Parkinson's Diseases. *J Parkinsons Dis*. 2016;6(2):413-21. (253)
- Fladby T, Palhaugen L, Selnes P, Waterloo K, Brathen G, Hessen E, et al. Detecting At-Risk Alzheimer's Disease Cases. *Journal of Alzheimer's disease : JAD*. 2017;60(1):97-105. (254)

6 Summary of publications

6.1 Paper 1: Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

Introduction

Cognitive impairment in early Parkinson's disease (PD) is common and distinct from early Alzheimer's disease. Predictors and mechanisms are only partially known, but α -synuclein, amyloid- β and tau dysmetabolism may be involved. Our aim was to study associations between cerebrospinal fluid biomarkers (CSF) and cognition in non-dementia PD compared to normal controls (NC) and non-PD patients with mild cognitive impairment (MCI non-PD).

Methods

Patients were classified as having normal, subjective or mild cognitive impairment after cognitive screening. Cerebrospinal fluid biomarker levels of total α -synuclein (t- α -syn), amyloid- β (A β) 38, 40 and 42, total tau (T-tau) and phosphorylated tau (P-tau) were measured in 34 NC, 31 early, non-dementia PD and 28 MCI non-PD patients. A well-validated neuropsychological test battery was administered.

Results

In the PD group, 13 had normal cognition, 4 had subjective and 14 mild cognitive impairment. The PD and MCI non-PD groups had lower MMSE scores compared to the NC group.

All PD patients had A β 42 levels above in-house diagnostic cutoff for AD. The PD group had significantly lower, but overlapping CSF biomarker levels of t- α -syn, A β 38, 40 and 42, T-tau and P-tau compared to NC. Compared to MCI non-PD, t- α -syn, A β 38 and 40, T-tau and P-tau were also lower, while A β 42 was significantly higher in the PD group. A β 38 and 40, but not A β 42, correlated strongly with t- α -syn levels in PD, but not in the MCI non-PD group.

Lower A β 42 was associated with decreased verbal learning, delayed verbal recall and response inhibition in PD. Also lower education was associated with poorer verbal learning and delayed verbal recall. Higher UPDRS part III motor score was associated with decreased response inhibition. Gender and age did not have any significant effect on the neuropsychological tests. There were no significant correlations between A β 38 or 40, t- α -syn, T-tau or P-tau and any of the neuropsychological tests.

Conclusion

The CSF biomarkers in PD were significantly different from both NC and MCI non-PD patients with increased risk of developing AD. While A β 38, 40 and t- α -syn levels are strongly correlated, only lower A β 42 was associated with reduced cognitive functions in early PD, mainly connected to medial temporal lobe-based cognitive functions.

6.2 Paper 2: Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

Introduction

Cognition is often affected early in Parkinson's disease (PD). Lewy body and Amyloid β (A β) pathology and cortical atrophy may be involved. The aim of this study was to examine whether medial temporal lobe structural changes may be linked to cerebrospinal fluid (CSF) biomarker levels and cognition in early PD.

Methods

33 early PD patients (13 without, 5 with subjective and 15 with mild cognitive impairment) and 32 normal controls (NC) had 3 Tesla magnetic resonance imaging (MRI) scans. The MRI scans were post-processed for volumes of hippocampal subfields and entorhinal and perirhinal cortical thickness. Lumbar puncture for CSF biomarkers A β 38, 40, 42, total tau, phosphorylated tau (Innogenetics) and total α -synuclein (Meso Scale Diagnostics) were performed. Multiple regression analyses were used for between-group comparisons of the

MRI measurements in the NC and PD groups and for assessment of CSF biomarkers and neuropsychological tests in relation to morphometry in the PD group.

Results

PD patients had smaller volumes of total hippocampus, presubiculum, subiculum, CA2-3, CA4-DG and hippocampal tail compared to NC, but after adjusting for multiple comparisons only the subiculum and hippocampal tail remained significantly smaller. None of the 34 cortical thickness variables were significantly thinner in the PD patients compared to the NC group.

Lower CSF A β 38 and 42 were significant predictors for thinner perirhinal cortex in the PD group, but not in the NC group. There were no other significant relationships between CSF biomarkers and MRI subfields.

In the PD group, lower A β 42, and smaller presubiculum and subiculum in combination with A β 42 predicted poorer verbal learning and delayed verbal recall. The combination of CSF and MRI biomarkers were better predictors of cognition than considered separately. Smaller total hippocampus, presubiculum and subiculum predicted poorer visuospatial copying. Lower A β 38 and 40 and thinner perirhinal cortex predicted poorer delayed visual reproduction separately, but this was not longer significant when combining perirhinal cortex with A β 38 or A β 40. Lower A β 42 only predicted poorer response inhibition, and no MRI variables predicted executive functions.

Conclusion

In conclusion, smaller volumes of hippocampal subfields and sub-hippocampal cortex thickness linked to lower CSF A β levels may contribute to cognitive impairment in early PD.

6.3 Paper 3: Impaired synaptic function is linked to cognition in Parkinson's disease

Objective

Cognitive impairment is frequent in Parkinson's disease, but the underlying mechanisms are insufficiently understood. Because 1) cortical metabolism is reduced in Parkinson's disease and closely associated with cognitive impairment, and 2) CSF amyloid- β species are reduced and correlate with neuropsychological performance in Parkinson's disease, and amyloid- β release to interstitial fluid may be related to synaptic activity; we hypothesize that synapse dysfunction links cortical hypometabolism, reduced CSF amyloid- β and presynaptic deposits of α -synuclein. We expect a correlation between hypometabolism, CSF amyloid- β , and the synapse related markers CSF neurogranin and α -synuclein.

Methods

30 patients with mild-to-moderate Parkinson's disease and 26 healthy controls underwent a clinical assessment, lumbar puncture, MRI, ^{18}F -fludeoxyglucose-PET and a neuropsychological test battery (repeated for the patients after two years).

Results

All subjects had CSF amyloid- β 1-42 within normal range. In Parkinson's disease, we found strong significant correlations between cortical glucose metabolism, CSF A β , α -synuclein and neurogranin, the latter also correlated with cortical thickness. All PET CSF biomarker-based cortical clusters correlated strongly with cognitive parameters. CSF neurogranin levels were significantly lower in mild-to-moderate Parkinson's disease compared to controls and correlated positively with amyloid- β and α -synuclein and inversely with motor stage. There was little change in cognition after two years, but the cognitive tests that were significantly different, were also significantly associated with cortical metabolism. No correlations between CSF A β 42 and cortical glucose metabolism or thickness were found in the control group.

Interpretation

CSF A β , α -synuclein and neurogranin concentrations are related to cortical metabolism and cognitive decline. Synaptic dysfunction due to A β and α -synuclein dysmetabolism may be central in the evolution of cognitive impairment in Parkinson's disease.

7 Discussion of the scientific results

7.1 Method limitations

As previously discussed under *Representativeness and generalizability of the studies* on page 54, we believe that the early PD, non-PD MCI and NC groups are representative for the actual population in the catchment area of Akershus University Hospital and also for the target population in Norway.

Nevertheless, a substantial proportion of the participants especially in the NC group were excluded due to missing data (either CSF biomarkers, neuropsychological, MRI or PET data) or weak neuropsychological tests in the two first papers, and this may have led to selection bias. Our PD population was possibly more cognitively normal than in the population-based incident Norwegian ParkWest cohort study (144), and this may have led to less findings. There were no significant differences in age, gender or education which potentially could have affected the results when comparing the groups.

The sample sizes were quite small, and therefore our results should be replicated in larger population-based studies. The cross-sectional design limits the opportunities to investigate possible predictors of cognitive decline and dementia, and this should be assessed in the follow-up data.

Other limitations which potentially affect all three papers are the interassay and interlaboratory variabilities related to the assays for CSF A β 42, T-tau and P-tau (60) as discussed in *CSF A β 42, T-tau and P-tau analyses* on page 68. All the CSF samples were analyzed for a specific biomarker at the same laboratory, but were collected over several years, and thus we cannot exclude interassay and intralaboratory variability.

7.2 Paper 1: Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

7.2.1 Research question 1: Are the levels of CSF A β 38, 40 and 42, T-tau, P-tau and t- α -syn biomarkers significantly different in early PD patients and non-PD MCI patients with increased risk of developing AD?

Results

CSF biomarkers in PD compared to NC

We found that even though all the PD patients had CSF A β 42 levels above in-house diagnostic cutoff for AD, the PD group had significantly lower, but overlapping CSF biomarker levels of t- α -syn, A β 38, 40 and 42, T-tau and P-tau compared to NC.

CSF biomarkers in PD compared to MCI non-PD with increased risk of developing AD

We found that compared to MCI non-PD, t- α -syn, A β 38 and 40, T-tau and P-tau were lower, while A β 42 was significantly higher in the PD group, although the levels were overlapping. A β 38 and 40, but not A β 42, correlated strongly with t- α -syn levels in PD, but not in the MCI non-PD group. We did not correct for multiple comparisons due to the exploratory setting.

Discussion

CSF biomarkers in PD compared to NC

Our findings are in line with most other studies, which have shown lower levels of t- α -syn and A β 38, 40 and 42, although some studies have shown similar levels in PD without dementia compared to controls (18, 29, 30, 137, 138, 144). For T-tau and P-tau, most studies have reported no changes in PD without dementia compared to controls, although others have found both decreased levels, as we did, and increased levels (18, 29, 138).

The inconsistent findings may be due to heterogeneity of patients and controls with different inclusion criteria, methodological differences in CSF biomarker analysis, different study designs, statistical analysis and for t- α -syn blood contamination (29) as not all studies have controlled for this.

CSF biomarkers in PD compared to MCI non-PD with increased risk of developing AD

In line with our findings, most other studies have shown a lower level of A β 42 and a higher level of t- α -syn, T-tau and P-tau in AD dementia compared to PD and NC (18, 29, 137, 138). Whether it is possible to distinguish a CSF biomarker profile of early PD from non-PD MCI with increased risk of developing AD has never been described before. Only one other study has assessed the levels of A β 42, T-tau and P-tau in both a PD (longer lasting) and a MCI (amnesic) group (255). They found that the PD-MCI group only had significantly lower levels of A β 42 and P-tau, while the amnesic MCI group had higher levels of T-tau and P-tau compared to controls. They did not compare the amnesic MCI and PD-MCI groups directly.

The patients in our MCI non-PD group were included based on the AD-MCI core clinical criteria in addition to at least one pathological CSF AD biomarker, and thus our findings were more consistent with the biomarker criteria for AD-MCI (136) with low A β 42 in the MCI non-PD group with increased risk of developing AD.

Correlations between CSF biomarkers

We found strong correlations between t- α -syn and A β 38 and 40 levels in PD, but in line with another study in early PD (175), we did not find correlations with A β 42. This may be due to that especially A β 42 is transformed into oligomers, which we did not measure. However, other studies have found correlations between t- α -syn and A β 42 in longer-lasting PD suggesting that this correlation first becomes significant later (161, 184, 188, 190), but they did not control for hemoglobin (161). As discussed in *Mechanisms: Lewy and AD pathology and synaptic dysfunction* on page 15, our findings are in line with studies showing associations between cortical A β plaques and Lewy pathology, even in PD-MCI (66, 67), and with studies pointing towards early synaptic dysfunction due to presynaptic accumulation of and interaction between A β and α -syn (6, 13-17, 41, 69, 71, 82, 86-92) as the cause of neurotransmitter deficiency and neurodegeneration in PD (17).

Limitations

CSF biomarkers in PD compared to NC

A limitation in our study is that the analyses of CSF A β 38 and 40 and t- α -syn were performed only in a subgroup of NC with normal CSF AD biomarkers, and this may have led to a selection bias with an apparently larger difference in the levels of CSF A β 38 and 40 and t- α -syn between the NC and PD groups.

In the third paper, we also compared the levels of CSF A β 42 (and T-tau and P-tau, unpublished data), and although all the levels were numerically lower, only T-tau was significantly lower in the PD group compared to NC. The discrepancies between the two papers may be due to different inclusion and exclusion criteria as the controls were excluded if they scored below normal on neuropsychological tests in the first paper, while this was not an exclusion criteria in the third paper. This shows that the results are affected by the definition of controls. In the first paper, we wanted to compare the CSF biomarkers with a cognitively normal control group to ensure that none of the controls actually had cognitive impairment due to a neurodegenerative disease, which potentially could have inappropriately influenced the results.

Another limitation is that we did not assess o- α -syn and p- α -syn. Others have found that soluble o- α -syn and p- α -syn are elevated in PD compared with controls, but the results are inconsistent (18, 29, 30, 137).

CSF biomarkers in PD compared to MCI non-PD with increased risk of developing AD

We compared the levels of CSF biomarkers in a MCI non-PD group selected based on pathological levels of at least one of the CSF AD biomarkers, but this is difficult to avoid due to the criteria for AD-MCI. We also compared the non-PD MCI group with a more heterogeneous PD group with both normal cognition, SCI and MCI. Thus, it is possible that the differences in the CSF biomarkers would have been larger if we had had an AD-MCI group according to the biomarker criteria and smaller if we had had a PD-MCI group.

Conclusion

Our study is to our knowledge the first to show that t- α -syn, A β 38 and 40, T-tau and P-tau are significantly lower, while A β 42 is higher in an early PD group compared to a MCI non-PD group with increased risk of developing AD, but lower than in NC.

Our results propose that the CSF biomarkers, even though partly overlapping, may be useful in the distinction between early PD and AD-MCI; α -syn and amyloid dysmetabolism with decreased CSF t- α -syn and A β 42 (to a lesser degree than in AD-MCI) are seen in early PD, but not neuronal damage with increased CSF T- and P-tau and t- α -syn (256) as in AD (257).

We also found strong correlations between CSF A β species and t- α -syn. This may be due to coincident A β and Lewy pathology or common pathologies affecting the presynaptic terminal and accumulation of and interacting mechanisms between A β and α -syn at the presynapses leading to synaptic dysfunction.

7.2.2 Research question 2: Are the levels of the CSF biomarkers associated with neuropsychological tests in early PD?

Results

Although all PD patients had CSF A β 42 levels above pathological cutoff, we found that lower CSF A β 42, but not A β 38 or 40, was associated with decreased verbal learning, delayed verbal recall and response inhibition in early PD. We did not find significant correlations between T-tau, P-tau or t- α -syn and any of the neuropsychological tests.

Discussion

CSF A β species and cognition

Our findings are partially in line with other cross-sectional studies (see *table 1* on page 38) showing that lower A β 42 is associated to poorer cognition in PD; lower A β 42 has been associated to poorer verbal (144) and visual memory, MoCA (181, 189) and MMSE (184) in early PD and recall (190) and phonetic fluency (160, 171) in longer lasting PD. We did not assess the associations with MoCA or MMSE. We did not find any association with visual

memory or phonetic fluency, and this may be due to different neuropsychological tests and shorter disease duration in our study, respectively. What we did find was an association with another executive function, response inhibition, measuring cognitive flexibility, but this has not been assessed in any of the other studies and thus must be replicated in larger studies. Anyway, not all cross-sectional studies have shown associations between A β 42 and cognition (172, 173, 175, 176, 178).

We did not have longitudinal neuropsychological data available, and thus we could not predict cognitive decline. In longitudinal studies in early PD, lower baseline A β 42 has been associated with a higher rate of decline in MoCA and MMSE (179) and predicted increased risk of cognitive impairment and dementia (64, 152, 185, 186, 189), with the exception of two studies (182, 187). In longer lasting PD, lower baseline A β 42 has been associated to decline in attention, memory, conceptualization (172) and delayed memory (183), poorer verbal recall and recognition and object-decision, and predicted PDD (160).

A β 38 and 40 have also been associated to poorer verbal memory in one study in early PD (144). We did not find this, and the discrepancy may be due to different neuropsychological tests. Other studies have also not found any correlation between A β 40 and visual memory or MoCA (181) or prediction of PDD in early PD (64).

CSF T-tau and P-tau and cognition

A study that assessed early PD patients with cognitive impairment and normal cognition, found cross-sectional correlations between higher T-tau and P-tau-S396 and poorer MoCA score in the group with cognitive impairment (178). Other studies, like ours, did not find any association with MoCA (175, 181, 189) or other cognitive tests (144, 160, 171-173, 175, 181-184, 187).

A large longitudinal study in early PD found that higher P-tau predicted cognitive decline in verbal learning and visuospatial working memory/ processing speed (182). Another study in early and longer-lasting PD found that an increase in P-tau, but not T-tau, over 2 years correlated with worsening letter fluency (188). Other longitudinal studies did not find any association with cognition (64, 160, 172, 179, 183, 185, 186, 189).

Thus, a few studies suggest that there is a relationship between higher tau and cognitive impairment in PD, but most studies have not found this.

CSF t- α -syn and cognition

We did not find any association between t- α -syn and cognition, which is in line with other cross-sectional studies (175, 177, 181, 184, 190), but the results have been conflicting: Lower t- α -syn has been associated with poorer verbal memory (180) and executive-attentional and global cognition in early PD (187). Low t- α -syn has been correlated with poorer phonetic fluency and visuospatial function and frontal cortical thinning in longer-lasting PD, but they did not control for blood contamination (161). This association may be explained by the hypotheses that toxic α -syn is sequestered in Lewy bodies (although not all patients with PD-MCI have cortical Lewy pathology (6, 9, 41, 57)) or in the presynaptic terminals causing synaptic dysfunction (17) leading to lower CSF t- α -syn levels and impaired cognition. This is in accordance with the associations between FDG-PET t- α -syn correlated cortical clusters and memory and executive functions described under *Research question 6* in the third paper (see *CSF biomarkers, glucose metabolism and cognition* on page 110).

In contrast, one longitudinal study in early PD found that lower t- α -syn predicted better preservation of verbal learning, memory and visuospatial working memory (180), and a study in longer-lasting PD found that higher baseline t- α -syn was associated with decline in cognitive speed (183). This may be explained by the hypothesis that a mechanism compensating for the sequestering of toxic α -syn retains functional α -syn in the neurons, leading to lower CSF levels and preserved cognition, but higher CSF levels and poorer cognition when this compensation fails (180). Another hypothesis is that early intracellular accumulation of α -syn leads to lower CSF levels, but that greater synaptic degeneration and neuronal damage lead to an increased release of α -syn into the CSF and poorer cognition as the disease progresses (183, 188). Other longitudinal studies have not found associations with decline in cognition (179, 185, 186, 188).

To summarize, three cross-sectional studies found that lower t- α -syn is associated to poorer cognition (161, 180, 187), two longitudinal studies found that lower t- α -syn is associated to preservation of cognition (180, 183), while other studies, like us, did not find any association (175, 177, 179, 181, 184-186, 188).

Conflicting results

Several different reasons may account for the divergent results and limitations of the studies, some already mentioned: Different CSF biomarker analysis techniques, sample sizes (with lack of power size calculations), patients selections (age, gender, education, dopaminergic medication, disease duration and severity, proportion with MCI), criteria for inclusion of controls, neuropsychological tests, study design (cross-sectional or longitudinal, neurological department or population-based, with or without control group), lack of clinicopathological correlations, statistical analysis and adjustments for confounders probably explain the inconsistent results and complicates the comparisons of the results. Different methods were also used to assess blood contamination, which potentially increases the concentration of α -syn in the CSF, and not all of the studies adjusted for this confounder (161, 177, 181). Thus, there is a need for more standardized methods to assess the relationship between CSF biomarkers and cognition.

The link between CSF biomarkers and cognition

Our and others' results suggest that at least CSF A β 42, and possibly also CSF A β 38 and 40, T-tau, P-tau and t- α -syn, reflect the pathological processes in the brain and are associated to cognition and especially memory and executive functions in PD patients. The CSF biomarkers are thus potential early predicting biomarkers of dementia in PD. However, compared to AD, tau biomarkers have clearly less impact.

As previously described in *Mechanisms: Lewy and AD pathology and synaptic dysfunction* on page 15, A β plaques and neurofibrillary tangles are found in a lesser degree in PD without dementia (6, 41), including in the limbic system, hippocampus and parahippocampal gyrus, and more widely distributed in the neocortex in PDD (57). The accumulation of A β plaques and decrease in CSF A β are likely to precede the accumulation of neurofibrillary tangles with neuronal damage and increase of tau as in AD (257), and this may explain why CSF A β is a better predictor of cognitive impairment in PD (258). Widespread Lewy pathology in limbic and neocortical areas correlates with cortical A β plaques (66, 67) and neurofibrillary tangles and with the development of more advanced cognitive impairment and dementia in PD (6, 8, 30, 41, 57, 79-81).

However, it has been hypothesized that A β (13-16, 18, 60) and α -syn interactions and aggregation of toxic oligomers at the presynaptic terminals (6, 30, 34, 41, 82, 86-92), prior to A β plaque (74) and Lewy pathology (17), cause synaptic dysfunction leading to neurotransmitter deficiency and cognitive impairment in early PD (13-17). This is in line with our findings, suggesting an association between CSF A β and cognition including memory functions in PD patients with CSF A β 42 within the normal range, and thus without evidence for A β plaques.

Our findings suggest a link between A β dysmetabolism and temporal (memory) and frontal (executive) lobe affection. This is supported by a MRI study in PD patients with and without dementia showing correlations between low A β 42 and high T-tau and P-tau and reduced gray matter volumes mostly temporally and frontally, which correlated with memory, executive and visuospatial functioning (159). Another explanation in accordance with *The dual-syndrome hypothesis* described on page 21, is that early synaptic dysfunction or Lewy pathology leads to dopaminergic depletion in the frontal dopaminergic pathways (9, 41) and cholinergic depletion in the cholinergic network from NBM to neocortex, limbic regions and hippocampus, which are associated with executive functions, such as response inhibition, and memory respectively (9).

It is somewhat surprising that we did not find any association between t- α -syn and cognition, but this may be due to low statistical power. Another possible explanation is that most of the accumulated α -syn in Lewy bodies are phosphorylated (29) and that α -syn oligomers may be more neurotoxic (2), and thus may be better biomarkers of cognition. In two other studies o- α -syn in the CSF correlated negatively with MMSE, semantic fluency and visuoperceptive tests across the premotor-motor-dementia PD continuum (161), but did not predict decline in MMSE or MoCA in PD (179). Another explanation is that not all patients with PD-MCI have limbic or neocortical Lewy pathology, and not all with cortical Lewy pathology will develop dementia (6, 9, 41, 57). Anyway, the results in other studies have been conflicting (161, 175, 177, 179, 181, 184-186), and there is need for more research regarding the underlying mechanisms.

Limitations

A limitation with our study is that we did not assess the associations between the CSF biomarkers and cognition in the NC, but two of the other studies in PD evaluated the relationship between CSF biomarkers and cognition in controls as well, and did not find any relationship (171, 179). Anyway, a population-based study in elderly women without dementia found that lower levels of CSF A β 42 may predict cognitive decline in MMSE (259), and this is in line with studies showing that CSF A β 42 levels are reduced in decades before development of dementia in AD (60, 135, 136, 141, 142).

Another limitation is that we did not measure oligomeric or phosphorylated forms of α -syn or A β .

Conclusion

We found that lower CSF A β 42 (although above cutoff) only was associated with decreased verbal learning, delayed verbal recall and response inhibition in early PD. This is in line with most other studies showing associations between A β 42 and cognition in PD.

This suggests that CSF A β 42 reflects and possibly also drives the pathological processes in the brain associated to memory (temporal) and executive (frontal) functions and thus is a potential early predictive biomarker of dementia in PD.

As cortical A β plaques are scarce in PD without dementia and not all have limbic or neocortical Lewy pathology, it has been hypothesized that prior to this, there are interactions and aggregation of toxic A β and α -syn at the presynaptic terminals, leading to synaptic dysfunction and subsequently early cognitive impairment. The synaptic dysfunction may arise cortically or strategically in dopaminergic (frontal) and cholinergic (including hippocampus) pathways (9, 41). Anyway, we did not find any association between t- α -syn and cognition, and the results have been inconsistent in other studies, but this may be due to low statistical power or that α -syn oligomers are more neurotoxic, and thus better biomarkers of cognition.

7.3 Paper 2: Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

7.3.1 Limitations with the hippocampal segmentation method

As previously discussed in *MRI segmentations and analyses* on page 73 there are some limitations with the FreeSurfer automated segmentation method, which only partly overlap with manual segmentation (244), the gold standard (236). The use of lower resolution of the MRI and hippocampal atlas leads to difficulties in mapping accurately hippocampal subregions (237). The use of different neuroanatomical boundaries, where for instance a part of CA1 is segmented as a part of subiculum and C2-3, may lead to that associations found to be linked to subiculum or CA2-3 actually are related to CA1 (238). The subfield defined as presubiculum with FreeSurfer is actually a part of subiculum (241). FreeSurfer may also underestimate larger volumes of CA1 more than smaller volumes (244), and in the older age group the overestimation of hippocampal volumes was higher for smaller than larger volumes, introducing biases which makes it harder to detect atrophy (244, 245). The segmentation method does not discriminate CA2 from CA3 or CA4 from DG (238).

However, the segmentations were manually inspected, we only included the hippocampal structures with best overlap with manual segmentation and the automated technique has a higher reproducibility (199). A new version of FreeSurfer (v6.0) based on an atlas of hippocampus with higher resolution is available per March 2017 and should be used to replicate our findings (237).

7.3.2 Research question 3: Are the volume or thickness of total hippocampus, hippocampal (subiculum, presubiculum, CA1, CA2-3 and CA4-DG) and parahippocampal (ERC and PRC) sub-regions at MRI smaller in early PD than in normal controls?

Results

We found that the PD patients had smaller volumes of total hippocampus, presubiculum, subiculum, CA2-3, CA4-DG and hippocampal tail compared to normal controls (NC). The subiculum and hippocampal tail were significantly smaller also after adjusting for multiple comparisons. None of the 34 cortical thickness variables were significantly thinner in the PD group.

Discussion

Hippocampal subfields

Our study is the first to assess volumes of the hippocampal subfields in a group of early PD patients compared to controls, and the findings are partly in line with studies comparing longer-lasting PD with controls (see *table 2* on page 46); studies using different MRI and manual segmentation methods have found that the whole hippocampus and hippocampal head (which partially consists of CA1) (191) and tail (192) were smaller in PD patients with and without dementia. The reason why we did not find smaller CA1 may be due to the segmentation method in FreeSurfer, as a part of CA1 was segmented as subiculum and C2-3 (238) and that FreeSurfer may underestimate larger volumes of CA1 more than smaller volumes (244).

Another study in longer-lasting PD also used automated hippocampal segmentation volumetry with FreeSurfer. Compared to controls, they found smaller CA2-3 and CA4-DG in PD with and without MCI, while subiculum and the whole hippocampus were only reduced in those with hallucinations after controlling for multiple comparisons (198). They did not assess the hippocampal tail. These findings are partly in line with our results. The reason why we did not find significantly smaller CA2-3 and CA4-DG after adjusting for multiple comparisons may be due to shorter disease duration, younger age and longer education in our PD group. We found that subiculum was significantly smaller in an early PD group without hallucinations,

while they also found affection of subiculum, but limited to a group of longer-lasting PD with hallucinations. Together, this suggests that the subiculum is vulnerable in PD.

Thus, although the studies used different MRI methods and differed in patient selections, all found reductions in different hippocampal sub-regions (hippocampal head or tail, CA1-4, DG, subiculum or whole hippocampus). An explanation may be that Lewy pathology is distributed to the temporal mesocortex and CA1-3 of the hippocampus in Braak stage 4 and 5 (39), and according to this, Lewy pathology is found in CA2-3 in PD without dementia (108-110). Early stages of AD, even preclinical, are primarily associated with atrophy of especially CA1, but also subiculum (241, 260, 261), and are also the subfields of strongest atrophy in DLB and other forms of dementia (260).

Parahippocampal sub-regions

We did not find thinner ERC or PRC in the early PD patients compared to NC. This is in line with other studies not showing smaller ERC in early PD using manual volumetric segmentation (193) or in longer-lasting PD using automated thickness segmentation with FreeSurfer (201). Nevertheless, other studies have shown smaller ERC compared to controls using manual volumetric segmentation in PDD (193) and longer-lasting PD (196), automated thickness segmentation in PDD and PD-MCI (201) or volumetric segmentation with FreeSurfer and manual tracing of the left ERC volume in PD-MCI and longer-lasting PD (202). This suggests that ERC is reduced in PD-MCI and PDD, but not in early PD, and may partly be explained by that Lewy, A β and tau pathology are found in ERC in PDD (79, 103, 111-117). The PRC and parahippocampus, which are linked to visual functions, are found to be damaged in DLB, but not in AD in one study (261).

Conflicting results

Divergent findings and limitations may in addition to the *Limitations* mentioned under *Research question 2* on page 98, be due to different imaging analysis techniques (the other studies mainly used 1/ 1.5 T MRI and different MRI scanners) and neuroanatomical boundary selections (differences in segmentation protocols, some studies used manual segmentation, while others used automated segmentation techniques).

Limitations

We performed power size calculations for the hippocampal subfields, which showed adequate numbers of participants except for subiculum and presubiculum. Anyway, limitations must be taken into consideration due to the accuracy of the hippocampal subfield segmentation technique with FreeSurfer as discussed in *MRI segmentations and analyses* on page 73. The control group, which although not significantly different, was not gender-matched, but preselected based on normal neuropsychology, and this may have led to greater differences in the hippocampal and parahippocampal sub-region sizes between the PD and NC groups. Thus, our results should be replicated in early PD studies using the newest version of FreeSurfer (v6.0) (237) with higher resolution.

Conclusion

Our study shows that different hippocampal subfields, especially subiculum, are reduced in early PD as previously shown in longer-lasting PD, before cortical thinning occurs. This suggests that regional hippocampal atrophy precedes cortical gray matter changes, which is in line with other studies describing hippocampal atrophy at MRI as a biomarker of initial cognitive impairment in PD (150) and predicting MCI and dementia in PD (149). The findings may be partly explained by Lewy pathology in the CA1-3 of the hippocampus in PD without dementia (108-110).

7.3.3 Research question 4: Are the levels of the CSF biomarkers associated with volume or thickness of the hippocampal and parahippocampal sub-regions in early PD?

Results

Lower CSF A β 38 and 42 were significant predictors for thinner PRC in the PD group, but not in the NC group. There were no other significant relationships between the CSF biomarkers and hippocampus or hippocampal subfields.

Discussion

To date, no other study has assessed the relationship between the CSF biomarkers and hippocampal and parahippocampal sub-regions in PD. One study did not find any association between CSF biomarkers and hippocampal radial distance thickness as a measure of total hippocampal atrophy (163), which is in line with our findings.

Studies in early and mild AD have shown that lower levels of A β 42 are correlated with decreases in hippocampal volume and deformation of CA1 (262) and that higher levels of T-tau and P-tau, but not A β 42, predict rates of hippocampal, parahippocampal and ERC atrophy (263). None of these studies assessed PRC as a single structure. T-tau correlated with hippocampal head and A β 42 with hippocampal head, body and tail in NC, MCI and AD (264). Another study in elderly with normal cognition, SCI or MCI using FreeSurfer found that low A β 42, high P-tau and poorer delayed recall were associated with volume loss of posterior (pre)subiculum (241). A recent study in asymptomatic elderly with familial history of AD found that persons with both reduced A β 42 and tau pathology had lower subiculum volume (265). A study using A β deposition at ^{11}C -Pittsburgh compound B (PiB)-PET in cognitively normal elderly, found that A β -PET positive participants had smaller hippocampal tail, presubiculum, subiculum and total hippocampal volumes using hippocampal subfield segmentation with FreeSurfer compared to A β -PET negative participants, suggesting an association between hippocampal subfield atrophy and A β plaques in preclinical AD (266). In contrast, there was no association between CSF biomarkers and hippocampal subfields in our PD group, even though we found associations between memory and A β 42 which may be caused by early synaptic dysfunction and not A β plaques as discussed in *The link between CSF biomarkers and cognition* under *Research question 2* on page 93. This discrepancy can be explained by different pathophysiology and stages of the diseases.

Even though Lewy pathology is found in CA2-3 in PD without dementia (108-110), we did not find any association with t- α -syn. This may be due to that the subfields are too small to affect the level of t- α -syn in the CSF or that we did not measure the more toxic forms of α -syn.

CSF A β and PRC

PRC is a part of the input and output structures to the hippocampus. The transentorhinal cortex, the medial part of PRC (105), is one of the first areas affected by neurofibrillary tangles in pre-dementia AD (267) and is also initially affected by both neurofibrillary tangles and Lewy pathology in Braak stage 4 in PD (39, 117).

A β , P-tau and α -syn, especially oligomeric forms, may interact and disrupt synaptic plasticity in PD, leading to synaptic loss (13-17). In mouse models, hippocampal synaptic dysfunction has been related to increasing levels of soluble A β at A β levels too low for plaque deposition (74). Intracellular A β 42 and α -syn cooperate to produce toxic α -syn oligomers (71). A β may contribute to hyperphosphorylation of tau, the main component of neurofibrillary tangles (93). Accordingly, a study combining amyloid PET and fMRI in cognitively normal elderly found that cortical A β load was related to disrupted intrinsic functional connectivity of the PRC, suggesting that dysfunction in PRC due to tauopathy is a very early sign of cortical amyloid deposition and may predict dementia (268). Another study found that Brodmann area 35, corresponding to the transentorhinal cortex, was the only MTL region to discriminate cognitively normal adults with and without positive A β PET (269), supporting early affection of this area related to A β pathology.

Taken together, this may explain why we find an association between lower CSF A β species and thinner PRC in early PD, but not in NC; we hypothesize that A β dysmetabolism leads to thinning of PRC, possibly through development of neurofibrillary tangles or alternatively through Lewy pathology, toxic α -syn oligomers and synaptic dysfunction. The reason why there was no association between tau and PRC may be due to that the initial formation of neurofibrillary tangles is not severe enough to increase the level of tau in the CSF. The connection to neurofibrillary tangles is not supported by biomarker findings in PD. However, methodological causes for this lack of support can not be excluded. Neither was it any association between t- α -syn and PRC, but we did not measure the more toxic oligomeric forms of α -syn. Another possibility is that the A β , tau and α -syn pathologies are independent, but coinciding factors, for example resulting from the development of presynaptic pathology.

Although we did not find thinner PRC in the PD group compared to NC, our study shows that there is a relationship between lower CSF A β species and thinner PRC in the PD group only,

suggesting an initial involvement of PRC. However, the results should be replicated in larger studies with the newest FreeSurfer software with higher resolution.

Conclusion

This is the first study to show associations between lower CSF A β 38 and 42 and thinner PRC in PD. This may be due to that the transentorhinal cortex, the medial part of PRC (105), is initially affected by both neurofibrillary tangles and Lewy pathology in PD (39, 117) and that toxic forms of A β may interact with P-tau and α -syn and disrupt hippocampal, including PRC, synaptic functioning in early PD (13-17, 74, 268) leading to thinning of PRC.

7.3.4 Research question 5: Are the volume or thickness of the hippocampal and parahippocampal sub-regions associated with neuropsychological tests in early PD?

Results

In the PD group, smaller presubiculum and subiculum predicted poorer verbal learning and delayed verbal recall, and this association was even stronger when adjusting for A β 42. Smaller total hippocampus, presubiculum and subiculum predicted poorer visuospatial copying. Thinner PRC predicted poorer delayed visual reproduction, but this was not longer significant when adjusting for A β 38 or A β 40. No MRI variable predicted executive functions.

Discussion

Hippocampal subfields

Two studies in longer-lasting PD with or without dementia using different MRI and manual segmentation methods found that the hippocampal head (which partially consists of CA1) was smaller compared to controls and correlated with verbal and visual recall (191) and verbal learning impairment (192). This is partially in line with our findings, as with FreeSurfer a part of CA1 is segmented as a part of subiculum (238). These studies did not assess presubiculum or subiculum as single structures.

Only one previous study has been performed in newly diagnosed PD patients with and without MCI using automated hippocampal segmentation and radial distance mapping for

thickness measures. They found that atrophy in CA1, CA3 and subiculum was associated with verbal long delay free recall, even in those without MCI, and that CA1 and subiculum were associated with verbal short delay free recall and recognition (197). Again, this is mainly in accordance with our results, and the discrepancies may be due to that our population was possibly more cognitively normal than in this population-based study, and this may have led to more subtle findings in our study.

Another study in longer-lasting PD with and without MCI using the same FreeSurfer segmentation technique and the same verbal learning and recall tests (RAVLT) as us, found that CA2-3, CA4-DG and the whole hippocampus, which were smaller compared to controls, correlated with verbal learning in the PD patients (198).

The last study used automated volume segmentation of the hippocampus into 13 subfields with the newest version of FreeSurfer 6.0 (203). They found that PD-MCI patients had lower volumes in the left fimbria and right CA1 and hippocampus-amygdala-transition-area compared to PD, and that baseline right CA1 volumes correlated with baseline attention. Longitudinal follow-up showed that PD patients who converted to PD-MCI had greater atrophy in the right CA2-3. Decreasing baseline volumes of granule cell layer of DG, right CA4 and left parasubiculum and hippocampus-amygdala-transition-area predicted conversion to PD-MCI.

Thus, all the studies found reductions in different hippocampal sub-regions (hippocampal head or tail, CA1-4, DG, presubiculum, subiculum or whole hippocampus) in relation to poorer memory and visual functions (verbal recall, learning and recognition and visual copying and recall) or attention. This is consistent with studies in primates and patients with (amnesic) MCI and AD, which have shown associations between hippocampus, CA1-3, DG, subiculum and presubiculum and memory and visuospatial functions (104, 106, 200, 239, 241, 260, 270), especially associations between CA1 atrophy and memory deficits (260).

Parahippocampal sub-regions

Using manual volumetric segmentation, studies have found that ERC is associated with learning and verbal memory in PD with and without dementia (193, 196) and with logical memory, but not verbal memory composite score, in both PD and controls (202). One study conducting automated cortical segmentation with FreeSurfer did find that baseline decreased

ERC volume was associated with decline in MoCA over time (152), while another study, in line with our findings, did not find any correlations between ERC thickness and neuropsychological tests in PD with or without cognitive impairment (201).

To date, no other study has assessed the size of PRC in relation to cognition in PD. We found that thinner PRC predicted poorer delayed visual reproduction, but this was not longer significant when adjusting for A β 38 or A β 40, suggesting that they are confounders. PRC has been linked to visual recognition memory (104), which both are affected in amnesic MCI and early AD (271-273). A study in early PD found associations between low CSF A β 42 and poorer visual recognition memory (181). We found a relationship between lower CSF A β 42 and thinner PRC. All this points to a possible association between PRC and visual memory.

Other studies have found association between MoCa and ERC in AD (274) and elderly with cognitive decline (275), but did not find any association with PRC (274). A study in MCI patients found that atrophy in PRC was associated with poorer category fluency and paired associates learning tasks, and the atrophy and cognitive impairment were most pronounced in those who converted to dementia (276). Another study in amnesic MCI and early AD found that atrophy of medial PRC (transentorhinal cortex) was associated with difficulties in naming of living relative to non-living things (277). Studies in normal controls, amnesic MCI and early AD have found that poorer episodic and semantic memory are associated with reduced ERC and medial PRC sizes (278, 279).

All this findings suggest that parahippocampal sub-regions are associated with memory and visual memory in PD and early stages of AD.

Conflicting results

The divergent findings may be due to the same factors as mentioned under *Research question 3* on page 100.

Limitations

Unfortunately we did not include a test of visual recognition memory. Thus, the relationship between PRC, A β species and different aspects of visual memory has not been clarified, and this should be investigated in larger studies.

Another weakness is that we did not assess the relationship between MRI hippocampal subfields and cognition in NC, but two of the other studies also examined the relationship between cognition and hippocampal sub-regions in controls did not find any association (191, 198). A review summarize that some studies in controls have not found any association, while others have found associations between CA1-4 and DG and memory functions (260). A recently published study in cognitively normal elderly found that tau PET uptake particularly in the entorhinal cortex including the transentorhinal cortex (the medial part of PRC) was related to episodic memory (280).

Conclusion

Our results show that smaller presubiculum, subiculum, total hippocampus and PRC are associated to poorer verbal learning and memory and visuospatial copying and reproduction respectively. This is partly in line with other studies in PD, which have found reduction in different hippocampal sub-regions and ERC in relation to poorer verbal and visual memory functions. This suggests that different hippocampal and parahippocampal sub-regions are related to verbal memory and visuospatial functions in PD, probably even in early stages, which is in line with studies in patients with amnesic MCI and AD.

7.4 Paper 3: Impaired synaptic function is linked to cognition in Parkinson`s disease

7.4.1 Research question 6: Are the levels of CSF A β species, α -synuclein and neurogranin associated with ^{18}F -fludeoxyglucose positron emission tomography (FDG-PET) uptake in relevant regions, and are these regions correlated with cognition in early PD?

Results

CSF A β species and glucose metabolism

Even though all PD patients had A β 42 within normal range, we found correlations between lower CSF A β 42 levels and lower glucose metabolism in frontal, parietal, occipital and

smaller temporal areas and between lower A β 38 and 40 levels and lower glucose metabolism in mainly posterior lateral areas in the early PD patients. In the NC group, there was no correlation between CSF A β 42 levels and glucose metabolism. There were no correlations between CSF A β 42 levels and cortical thickness in neither of the groups (see *Supplementary figure 1* on page 144 and *Supplementary figure 2* on page 145 under *Attachments*).

There were no significant differences in any of the neuropsychological tests between the PD and NC groups except poorer divided attention, an executive function early affected by both impaired dopaminergic and cholinergic networks and frontoparietal hypometabolism (9), but this may also have been affected by a motor component in the PD group. In contrast to the two previous papers, cases included as NC were not excluded based on low neuropsychological test scores, and thus NC here represent a more cognitively heterogeneous control group. Nevertheless, all the neuropsychological tests were within the normal range for both groups and there were no associations between A β 42 and glucose metabolism in the NC, suggesting that the findings in the PD group are specific for PD.

In the non-PD MCI group (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) there were extensive correlations between CSF A β 42 concentrations and glucose metabolism and also distributed correlations with cortical thickness, as expected in a group including AD-MCI patients. In the younger A β negative non-PD MCI patients there were no such correlations. The estimated size of the effects of CSF A β 42 on glucose uptake was larger in PD than in non-PD MCI, but this was not directly comparable as we used two different PET scanners, and a pure AD-MCI group would probably have shown stronger associations between CSF A β and cortical glucose metabolism.

CSF t- α -syn and glucose metabolism

In the PD patients, there were significant correlations between lower CSF t- α -syn levels and lower glucose metabolism in several cortical clusters, especially frontally left and laterally right clusters. There were no correlations with cortical thickness.

CSF neurogranin and glucose metabolism

In the PD group, there were significant correlations between lower CSF neurogranin levels and lower glucose metabolism within the same cortical clusters as for CSF A β , both lateral

and medial left and a larger right cluster. There were also significant correlations between CSF neurogranin levels and cortical thickness in small frontal and parietal left clusters and frontal, medial, parietal and inferior temporal right clusters (see *Supplementary figure 6* on page 149 under *Attachments*).

CSF biomarkers, glucose metabolism and cognition

For the cortical areas in which glucose metabolism was correlated with either CSF A β species, α -syn or neurogranin in the PD group, there were strong correlations between mean FDG uptake and most tests in the neuropsychological test battery, including measures of executive function (word fluency, response inhibition and psychomotor speed) and memory (verbal and visual recall). There was no correlation to divided attention, but when using mixed models (as described in the *Statistical analysis* on page 80), also divided attention correlated with cortical areas in which glucose metabolism correlated with CSF A β 42.

Only psychomotor speed and verbal fluency improved significantly from baseline to two years follow-up, and for both higher FDG-PET cluster uptake corresponding to CSF A β 42 was associated with better performance at follow-up.

Discussion

CSF biomarkers, glucose metabolism and cognition

Widespread cortical hypometabolism, most prominently in frontoparietal and occipital cortices, has been shown even in early stages of PD before development of substantial atrophy, and with progressive hypometabolism with increasing disease duration and severity (22). It has been associated with cognitive impairment including memory, visuospatial and executive dysfunctions (166-168).

To our knowledge, this is the first study to explore and link regional cortical glucose metabolism to CSF A β species, t- α -syn and the postsynaptic marker neurogranin and cognitive impairment in PD. This suggests that synaptic dysfunction is related to both reduced cortical glucose metabolism and reduced levels of CSF A β species, t- α -syn and neurogranin, assumed to be related to presynaptic deposition of α -syn (17, 37). Studies in controls, MCI and AD have also found associations between lower A β 42 or higher T-tau and P-tau and reduced glucose metabolism in different cortical regions, mainly frontal, parietal, temporal

and posterior cingulate gyrus (265, 281-287) while one of the studies did not find any association in controls (281).

The strong associations to memory and executive functions and also to changes at follow-up in our study demonstrate the clinical relevance. These findings are partly in line with the first paper where we did find associations between memory and executive functions and A β 42, but not t- α -syn. The latter may be due to insufficient statistical power in the analyzes of t- α -syn, but the results in other studies have been conflicting as discussed in *Research question 2* on page 93. Anyway, we did find associations between FDG-PET/ t- α -syn correlated cortical clusters and memory and executive functions. This suggests that there is a relationship between lower α -syn and poorer cognition, possibly through synaptic dysfunction leading to reduced cortical glucose metabolism.

Two small studies combining FDG-PET and PiB-PET in longer-lasting PD patients found glucose hypometabolism in PD and even more widespread in PDD, but not significantly increased A β deposition compared to controls (288, 289). This suggests that hypometabolism precedes and is much more pronounced than A β deposition in PD and PDD. This is in line with most studies that not have found any significantly increased PiB-uptake in PD, PD-MCI and PDD compared to controls (290-294). These studies are also in agreement with our findings of widespread correlations between glucose metabolism and CSF A β 42 even though all had A β 42 levels within normal range, and therefore unlikely occurrence of amyloid plaques in the early PD group. Nevertheless, increased PIB-uptake has been associated to cognitive impairment (293) and predicted cognitive worsening and conversion to dementia in both longer-lasting PD (295) and healthy controls (292).

Synaptic dysfunction and interactions

As discussed in *Mechanisms: Lewy and AD pathology and synaptic dysfunction* on page 15 and *Research question 2* on page 93, there are indications of interacting mechanisms between A β species and α -syn which are linked to synaptic activity. Defective presynaptic proteolysis and lysosomal degradation could affect both A β and α -syn metabolism leading to increased levels of presynaptic A β and α -syn (15, 84, 85). The neurodegeneration and neurotransmitter deficiency in early PD may be caused by synaptic dysfunction due to presynaptic α -syn aggregates that slightly reduce A β in proportion to the degree of α -syn dependent neurodegeneration (17, 70). A β 42 may escalate the production of toxic α -syn oligomers,

directly disrupting SNARE-dependent synaptic vesicle function (70, 71) and impair neurotransmitter release leading to postsynaptic dendritic spine loss (17), synaptic dysfunction and loss of synaptic markers such as synaptophysin (37, 70) and neurogranin. This hypothesis could be tested by measuring pre- and postsynaptic markers, like CSF neurogranin as we did in *Research question 7* on page 115, synaptosomal-associated protein 25 (SNAP25, a component of the trans-SNARE complex) (296) or synaptotagmin, expected to be changed if vesicle fusion is impaired.

The alternative amyloid species A β 38 and A β 40 are considered less prone to aggregate, but the correlations between glucose metabolism and A β 38 were comparable to those with A β 42 (whereas the correlations with A β 40 were more sparse) in the PD group. As we have described in the first paper, A β 38 and 40 correlated strongly with t- α -syn levels strengthening the assumption that presynaptic α -synuclein and A β metabolism may interact to produce the current findings.

Another hypothesis is that there are increased concentrations of soluble synaptotoxic A β oligomers that are only beginning to deposit in plaques, or that these oligomers are less prone to aggregate in early PD. In AD, A β oligomers are responsible for causing synaptic dysfunction and neuronal death, more so than the plaques themselves (16). A β 42 fibrils or oligomers might have synaptotoxic effects in PD as in AD, even in the absence of positive amyloid-PET or pathological CSF A β 42 concentrations (13). Measuring A β oligomers in the CSF of patients with PD can test this hypothesis.

Cortical or subcortical pathology

Both reduced cortical glucose metabolism and cortical presynaptic accumulation of α -syn, interactions with A β and postsynaptic spine retractions point toward a cortical pathology. Our findings also supports a cortical etiology, describing strong correlations between cognition, cortical glucose metabolism and CSF A β species, t- α -syn and neurogranin.

Anyway, we cannot exclude a subcortical contribution; the correlations between CSF A β 42 (and A β 38) and the frontal area in early PD were mainly located in the prefrontal cortex, which is connected to the basal ganglia via the frontostriatal dopamine pathway and plays a role in executive functions (9). There were also correlations between CSF A β species and the more posterior parietal, temporal and occipital areas. The NBM (in the basal forebrain) has

widespread projecting cholinergic neurons to the entire neocortex, and is known to degenerate in PD, leading to cortical dysfunction and cognitive decline (9, 297). Reduced dopaminergic and cholinergic transmission in PD corresponding to frontal and posterior areas suggesting that our findings may be due to reduced dopaminergic (frontal cluster) and cholinergic (posterior cluster) networks (102). This is partly in accordance with *the dual syndrome hypothesis* described on page 21 and *Research question 2* on page 93 suggesting that dopamine deficiency in the frontostriatal pathway causes (mild) impairment in executive functions in early PD, while widespread Lewy pathology and dysfunction in cortical cholinergic networks lead to (more rapid) cognitive decline in memory and visuospatial functions, and development of dementia (100). The dual-syndrome hypothesis may also explain the improvement in two executive tests (psychomotor speed and verbal fluency) at follow-up.

Thus, the neurodegeneration could either affect the synapses diffusively across over the cortical mantle, or possibly the brain stem and basal ganglia synapses in relevant transmitter systems, making the present findings a diaschisis effect.

Limitations

The sample sizes in the groups were quite small, and we have not performed power size calculations, so the findings need to be reproduced in larger populations. We only included early-stage PD patients, they performed as well as NC on most neuropsychological tests, and the results may have been more informative if we included more cognitively impaired patients and if the time from baseline to follow-up was longer. The findings should also be replicated in drug-naïve populations.

In some of the MCI (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*) and NC patients, the examinations were conducted over longer time periods up to 1 (MCI) to 2 (NC) years. This should not have any consequences in the NC group, but in the MCI group this may have led to stronger associations between low CSF A β levels and decreased cortical glucose metabolism as the disease has progressed. Another limitation is that we applied two similar, but not identical PET/ CT scanners in the PD and control groups, and thus we could not directly compare the results between the groups.

Although FDG uptake reflects synaptic function, we have not used any direct measure of synaptic function.

Conclusion

We found strong correlations between lower CSF A β species, t- α -syn and neurogranin and regional cortical glucose hypometabolism, but not cortical thickness (except for neurogranin), and these regions correlated with cognition and cognitive change at 2 years follow-up in early PD. This was true even though all had A β 42 and neuropsychological tests within the normal range. In comparison, there were no correlations between A β 42 and glucose metabolism in NC and A β negative non-PD MCI (unpublished data, see *Supplementary material in paper 3* on page 140 under *Attachments*).

This suggests that A β and α -syn dysmetabolism in the likely absence of A β plaques, cortical Lewy bodies and cortical thinning, as in contrast to the non-PD MCI group, affects cortical synaptic function and metabolism related to cognitive functioning in early PD. Possible explanations of the findings include cortical presynaptic accumulation and interactions between α -syn and A β species and postsynaptic A β oligomers leading to synaptic dysfunction as reflected by neurogranin as a postsynaptic marker, early AD cross-pathology including synaptotoxic A β oligomers and reduced dopaminergic and cholinergic transmission. However, we cannot rule out other mechanisms acting as confounders affecting both cortical and A β metabolism.

If confirmed, the present results will have important implications for understanding the role of A β and α -syn in the evolution of PD and point towards synaptic pathology as a possible target for treatment. We hypothesize that baseline cortical glucose metabolism would predict future neuropsychological performance and possibly predict time from disease onset to dementia. The included PD subjects are being reexamined, and the hypothesis will be tested in future work.

7.4.2 Research question 7: Are the levels of CSF neurogranin significantly different in early PD compared to NC, and are there correlations between neurogranin and A β , α -synuclein and PD motor stage?

Results

CSF neurogranin in PD compared to NC

The CSF neurogranin levels were significantly lower in the PD group as compared to the NC group.

Correlations with CSF neurogranin

The CSF neurogranin levels were significantly correlated with concentrations of all CSF A β species and t- α -syn. Both CSF neurogranin and t- α -syn levels, but not CSF A β species, were significantly inversely correlated with UPDRS motor score.

Discussion

The lower level of CSF neurogranin in the PD group is consistent with cortical presynaptic pathology with accumulation of α -syn oligomers impairing neurotransmitter release leading to loss of postsynaptic dendritic spines, but no other postsynaptic injury (17). This is supported by the significant association between CSF neurogranin and t- α -syn in our study, but other factors such as altered degradation or transport mechanisms may also be implicated.

In contrast, in AD increased CSF level of neurogranin is thought to reflect synaptic degeneration with loss of integrity of postsynaptic spines (75, 145, 146). To our knowledge, only two other studies have assessed the level of CSF neurogranin in PD. The first study did not find any difference from controls (145). They included PD patients with longer mean disease duration (8 years), and later in the disease course more complex neurodegeneration is to be expected, including A β pathology, which may explain why they did not find reduced level of neurogranin compared to controls. The other study found increased neurogranin level in drug-naïve PD associated with reduced cognition (MoCA) and more advanced motor stage, which may reflect postsynaptic loss (148). The discrepancies between the studies may be due to different laboratory analysis methods.

The correlations between CSF neurogranin and A β species and t- α -syn levels and with UPDRS motor score in our study suggest that lower levels of several CSF biomarkers and poorer UPDRS motor score are linked to synaptic dysfunction. Anyway, the results are conflicting as other studies have not found any association between UPDRS and CSF t- α -syn or A β 42 (137, 188).

The neurogranin analyses need to be confirmed in other studies including assessment of clinical relevance.

Conclusion

The CSF neurogranin levels were significantly lower in the PD group compared to NC and correlated with A β species, t- α -syn and UPDRS motor score, supporting the theory that synaptic function is affected in early PD possibly through presynaptic accumulation of α -syn leading to cognitive and motor impairment.

8 Conclusions

Presently we collect follow-up data after 2 and 4 years. The results should be replicated in larger, population-based longitudinal studies. There is also need for more standardized methods regarding patient and control selections, neuropsychological tests, CSF biomarker analysis and MRI hippocampal subfield segmentation techniques. Disease-specific treatments targeting A β and α -syn are under investigation such as inhibitors of A β accumulation and A β immunotherapies (298).

See *Figure 1: Hypothetical model of pathological mechanisms and biomarkers in Parkinson's Disease cognitive impairment* on page 119 for an overview. Here is a summary of the answers to the seven research questions in this dissertation:

1. We found as the first study, that CSF t- α -syn, A β 38 and 40, T-tau and P-tau are significantly lower, while A β 42 is higher in an early PD group compared to a MCI non-PD group with increased risk of developing AD. This suggests that the CSF biomarkers, even though partly overlapping, may be useful in the distinction between early PD and AD-MCI. There were also strong correlations between the CSF A β species and t- α -syn suggesting interactions between A β and α -syn.
2. We found in line with most other studies, that CSF A β 42 is associated to cognition and especially memory and executive functions in early PD patients, even though all the PD patients had CSF A β 42 levels within the normal range. A β 42 is thus a potential predictive biomarker of dementia in PD probably already in the absence of A β plaques. This suggests early affection of temporal and frontal lobes linked to A β dysmetabolism in PD, possibly through interactions and aggregation of toxic A β and α -syn at the presynaptic terminals leading to synaptic dysfunction. The synaptic dysfunction may arise cortically or strategically in dopaminergic (frontal) and cholinergic (including hippocampus) pathways.
3. We found that different hippocampal subfields, especially subiculum, are reduced also in early PD, as previously shown in longer-lasting PD, before cortical thinning occurs. This suggests that regional hippocampal atrophy is a potential early biomarker preceding cortical gray matter changes.

4. We showed for the first time that there is a relationship between lower CSF A β species and thinner PRC in early PD, suggesting an initial involvement of PRC related to A β dysmetabolism in PD as seen in AD, possibly through interactions with P-tau and α -syn and synaptic dysfunction.
5. We found, in line with other studies, that smaller presubiculum, subiculum, total hippocampus and PRC are related to verbal memory and visuospatial functions also in early PD, suggesting that early affection of hippocampus is related to poorer cognition in PD.
6. This is the first study to show strong correlations between lower CSF A β species, t- α -syn and neurogranin and regional cortical glucose hypometabolism (but not cortical thickness), and these regions correlated with cognition in early PD. This suggests that A β and α -syn dysmetabolism, in the likely absence of A β plaques, cortical Lewy bodies and cortical thinning, affects cortical metabolism in early PD leading to cognitive impairment. This occurs possibly through presynaptic interactions between and accumulation of α -syn and A β species leading to synaptic dysfunction, as reflected by neurogranin as a postsynaptic marker.
7. The CSF neurogranin levels were significantly lower in the PD group compared to NC and correlated with A β species, t- α -syn and UPDRS motor score, supporting the theory that synaptic function is affected in early PD, possibly through presynaptic accumulation of α -syn and A β , leading to cognitive and motor impairment.

Figure 1: Hypothetical model of pathological mechanisms and biomarkers in Parkinson's Disease cognitive impairment

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10 Attachments

10.1 Supplementary material in paper 3

10.1.1 Supplementary methods

Two supplementary control groups were included in addition to healthy controls: non-Parkinson's disease patients with mild cognitive impairment provided two control groups according to CSF A β 42 status. 59 patients with non-Parkinson's disease mild cognitive impairment were recruited from a university-hospital based memory clinic. The inclusion procedure adhere to the Petersen criteria and is extensively documented elsewhere (Selnes *et al.*, 2012; 2013). In addition to using the non-Parkinson's disease mild cognitive impairment group in its entirety, we extracted a subgroup with CSF A β 42 above pathological cut-off (including variance, 700 ng/L) as an additional control group. The same ethical guidelines as for the Parkinson's disease and the healthy control group were followed.

Procedures for CSF samples were the same as for the Parkinson's disease and the healthy control group. FDG-PET/CT imaging was performed as for the healthy control group. For the non-Parkinson's disease mild cognitive impairment group, MRI scans were obtained from a Siemens Espree 1.5 Tesla system. At 1.5 Tesla, one MPRAGE 3D sequence was acquired (TR/TE/TI/FA = 2400/3.65/1000/8°, matrix = 240 × 192), 160 sagittal slices, thickness=1.2 mm, in-plane resolution of 1 mm×1.2 mm. MRI and FDG-PET post processing and statistical operations were performed as for the Parkinson's disease and the healthy control group.

10.1.2 Supplementary results

There were no significant differences in education or gender composition between the Parkinson's disease and non-Parkinson's disease mild cognitive impairment groups, but the A β negative non-Parkinson's disease mild cognitive impairment group was significantly younger. Both mild cognitive impairment groups had significantly lower MMSE scores than the Parkinson's disease group. CSF A β 42 was lower in the non-Parkinson's disease mild cognitive impairment group, whereas P-tau and T-tau were higher. P-tau was also higher in the A β negative non-Parkinson's disease mild cognitive impairment group. (All as compared to the Parkinson's disease group). Group composition, demographics and neuropsychological characteristics are outlined in *Supplementary table 1 and 2 below*).

Supplementary table 1: Overview of demographics and group characteristics

	non-Parkinson's disease mild cognitive impairment	A β negative non-Parkinson's disease mild cognitive impairment
N subjects	59	30
Age, years	62.2 (45-77) ^a	58.7 (45-67) ^b
Females/males	28/31 ^c	11/19 ^d
Education, years	12.6 (7-19) ^e	12.1(7-17) ^f
MMSE	27.7 (24-30) ^g	27.9 (25-30) ^h
Geriatric depression scale score	6.3 (0-15) ⁱ	7.5 (1-15) ^j
CSF A β 38; ng/L	n.a.	n.a.
CSF A β 40; ng/L	n.a.	n.a.
CSF A β 42; ng/L	761 (221-1507) ^k	989 (705-1507) ^l
CSF P-tau; ng/L	82 (35-238) ^m	63 (35-95) ⁿ
CSF T-tau; ng/L	419 (102-1407) ^o	291 (102-564) ^p

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from early Parkinson's disease.

Age: ^a Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: T-test P=0.078. ^b Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: T-test P<0.001.

Gender: ^c Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment between group gender composition differences; Pearson chi-square, P=0.332. ^d Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment between group gender composition differences; Pearson chi-square, P=1.0.

Education: ^e Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=810.5, P=0.516. ^f Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=368.5, P=0.226.

MMSE: ^g Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=498, P=0.001. ^h Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=305, P=0.028.

Geriatric depression scale (available for 39 non-Parkinson's disease mild cognitive impairment, 22 A β negative non-Parkinson's disease mild cognitive impairment): ⁱ Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=121, P=0.000. ^j Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=41.0, P=0.000.

A β 42: ^k Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=503, P=0.001. ^l Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=397, P=0.433.

P-tau: ^m Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=249, P<0.001. ⁿ Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=164, P<0.001

T-tau: ^o Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=549, P=0.004. ^p Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=380, P=0.301.

Abbreviations: A β = amyloid- β ; A β 38 = amyloid- β 1-38; A β 40 = amyloid- β 1-40; A β 42 = amyloid- β 1-42; MMSE = Mini-Mental State Examination; n.a. = not available; P-tau = phosphorylated microtubule-associated protein tau; T-tau = total microtubule-associated protein tau; UPDRS = unified Parkinson's disease rating scale

Supplementary table 2: Neuropsychological characteristics of the groups

Neuropsychological test battery	non-Parkinson's disease mild cognitive impairment	A β negative non-Parkinson's disease mild cognitive impairment
N subjects (missing neuropsychological data)	58 (1)	30 (0)
RAVLT verbal learning of 15 words over 5 trials, T-score	40.8 (17-77) ^a	43.8 (24-77) ^b
RAVLT delayed verbal recall of 15 words after 30 min, T-score	41.4 (21-81) ^c	45.9 (28-81) ^d
RCFT visuo-spatial copy trial, raw score	30.0 (5-36) ^e	31.4 (19.5-36) ^f
RCFT delayed visual reproduction after 30 min, T-score	38.7 (20-59) ^g	43.7 (24-59) ^h
TMT-B divided attention, T-score	44.0 (10-67) ⁱ	45.8 (17-61) ^j
SCW response inhibition, T-score	45.2 (0-63) ^k	45.9 (20-60) ^l
COWAT verbal fluency, T-score	49.2 (21-79) ^m	50.1 (22-71) ⁿ

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from early Parkinson's disease.

RAVLT verbal learning (available for 57 non-Parkinson's disease mild cognitive impairment): ^a Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=459, P=0.002. ^b Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=280.5, P<0.030.

RAVLT delayed verbal recall: ^c Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=461, P=0.001. ^d Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=293.5, P<0.048.

RCFT visuospatial copy trial: ^e Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=588, P=0.038. ^f Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=358.5, P<0.336.

RCFT delayed visual reproduction: ^g Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=538.5, P=0.012. ^h Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=349, P<0.269.

TMT-B divided attention (available for 57 non-Parkinson's disease mild cognitive impairment): ⁱ Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=719.5, P=0.463. ^j Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=333.5, P<0.178.

SCW response inhibition: ^k Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=587, P=0.037. ^l Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=307.5, P<0.078.

COWAT verbal fluency (available for 27 non-Parkinson's disease mild cognitive impairment): ^m Early Parkinson's disease vs. non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=742, P=0.699. ⁿ Early Parkinson's disease vs. A β negative non-Parkinson's disease mild cognitive impairment: Mann-Whitney U=354.5, P<0.419.

Abbreviations: A β = amyloid- β ; COWAT, Controlled Oral Word Association Test; RCFT, Rey Complex Figure Test; RAVLT, Rey Auditory Verbal Learning Test; SCW, Stroop Color Word; TMT-B, Trail-Making Test B

FDG-PET and A β 42 in patients with mild cognitive impairment (both A β positive and A β negative)

As expected, there were clear correlations between CSF A β 42 concentrations and FDG-PET uptake in the non-Parkinson's disease mild cognitive impairment group. The statistical map (Supplementary figure 3 below) shows extensive correlations. In the left hemisphere, three clusters were found (comprising 23% of the left hemisphere surface area), and in the right hemisphere, two clusters were found (comprising 39% of the right hemisphere surface area). The cluster-wise probability was 0.0002 for all clusters, apart from the more frontal cluster in the left hemisphere (shown in Supplementary figure 3 in yellow), for which the cluster-wise probability was 0.004. The coefficient of determination for the effects of CSF A β 42 on FDG uptake in the combined left hemisphere clusters (excluding the effects of age and gender) was R^2 0.163, in the combined right hemisphere clusters R^2 was 0.165.

Further, there were less extensive, distributed correlations between CSF A β 42 concentrations and cortical thickness comprising 2% of the left and 1% of the right hemisphere. The coefficient of determination for the effects of CSF A β 42 on cortical thickness uptake in the combined left hemisphere clusters (excluding the effects of age and gender) was R^2 0.232, in the combined right hemisphere clusters R^2 was 0.161. (Supplementary figure 4 below)

The estimated size of the effects (the coefficient of determination) of CSF A β 42 on FDG uptake was larger in Parkinson's disease than in mild cognitive impairment (R^2 ~0.4 vs. R^2 ~0.16). The PET scans from the two groups were, however from different scanners, they were normalized but still not directly comparable. The effect sizes can as such not be directly compared, but this strongly indicates that CSF amyloid metabolism has an impact on cortical glucose metabolism in early Parkinson's disease.

FDG-PET and A β 42 in A β negative patients with mild cognitive impairment

There were no correlations between CSF A β 42 concentrations and FDG-PET uptake in the A β negative, non-Parkinson's disease mild cognitive impairment group after correction for multiple comparisons. (Also, not corrected for multiple comparisons, no voxels reached the $P < 0.01$ threshold.) Further, there were no correlations between CSF A β 42 concentrations and cortical thickness. (Not corrected for multiple comparisons, a few distributed voxels reached the $P < 0.01$ threshold. Supplementary figure 5 below)

Supplementary figure 1: Correlations between CSF A β 42 concentrations and cortical thickness in the early Parkinson's disease group

Correlations between CSF A β -42 concentrations and cortical thickness in the early Parkinson's disease group, not corrected for multiple comparisons. The threshold is set at $P < 0.01$. Positive correlations show as red and negative correlations as blue. (No significant correlations were seen after correction for multiple comparisons.)

Supplementary figure 2: Correlations between CSF A β 42 concentrations and cortical thickness in the control group.

Correlations between CSF A β 42 concentrations and cortical thickness in the control group, not corrected for multiple comparisons. The threshold is set at $P < 0.01$. Positive correlations show as red and negative correlations as blue. (No significant correlations were seen after correction for multiple comparisons.)

Supplementary figure 3: Correlations between FDG-PET and CSF Aβ42 in mild cognitive impairment

The statistical map shows the clusters in which cortical metabolism in patients with mild cognitive impairment (both Aβ positive and Aβ negative) is correlated with CSF Aβ42. R^2 was 0.163 in the left and 0.165 in the right combined hemisphere clusters (excluding the effects of age and gender). Mean CSF Aβ42 was 989 ng/L (range 705-1507). The clusters comprised 31% of the total surface area.

Supplementary figure 4: Correlations between CSF A β 42 concentrations and cortical thickness in mild cognitive impairment

Correlations between CSF A β -42 concentrations and cortical thickness in the non-Parkinson's disease mild cognitive impairment group. (CSF A β -42 concentrations both above and below pathological cutoff. Mean 762 ng/L, range 221-1507.) The statistical map shows less extensive, spread correlations between CSF A β -42 concentrations and cortical thickness comprising 2% of both hemispheres. R^2 was 0.232 in the left and 0.161 in the right combined hemisphere clusters (excluding the effects of age and gender).

Supplementary figure 5: Correlations between CSF A β 42 concentrations and cortical thickness in the amyloid-negative, non-Parkinson's disease mild cognitive impairment group

Correlations between CSF A β 42 concentrations and cortical thickness in the amyloid-negative, non-Parkinson's disease mild cognitive impairment group, not corrected for multiple comparisons. The threshold is set at $P < 0.01$. Positive correlations show as red and negative correlations as blue. (No significant correlations were seen after correction for multiple comparisons.)

Supplementary figure 6: Correlations between cortical thickness and CSF neurogranin in early Parkinson's disease

The statistical map shows the clusters in each hemisphere in which cortical thickness in early Parkinson's disease is correlated with CSF neurogranin concentrations. Mean CSF neurogranin was 251 (range 125-556). Cluster-wise $P=0.001$ for the frontal left and $P=0.002$ for parietal left cluster, whereas $P=0.042$ for the parietal right, and $P=0.021$ for the frontal, medial right cluster. Also, there was a right inferior temporal cluster (not visible), $P=0.002$. The clusters comprised 3% of the total surface area. R^2 was 0.655 in the left and 0.630 in the right combined hemisphere clusters (excluding the effects of age and gender).

10.1.3 Supplementary references

1. Selnes P, Aarsland D, Bjørnerud A, Gjerstad L, Wallin A, Hessen E, et al. Diffusion tensor imaging surpasses cerebrospinal fluid as predictor of cognitive decline and medial temporal lobe atrophy in subjective cognitive impairment and mild cognitive impairment. *Journal of Alzheimer's disease : JAD*. 2013;33(3):723-36. (220)

2. Selnes P, Fjell AM, Gjerstad L, Bjornerud A, Wallin A, Due-Tonnessen P, et al.
White matter imaging changes in subjective and mild cognitive impairment.
Alzheimer's & dementia : the journal of the Alzheimer's Association. 2012;8(5
Suppl):S112-21. (299)

10.2 Errata list

Page	Line	Original text	Type of correction	Corrected text
6	21	multiplications and mutations	correction of language	mutations
6	21	GBA	change of text format	<i>GBA</i>
6	21	LRKK2	change of text format	<i>LRKK2</i>
7	19, 25, 26			
8	2			
6	21	PARKIN	correction of language and change of text format	<i>PRKN</i>
7	18, 21			
6	21	SNCA	change of text format	<i>SNCA</i>
7	13			
8	2			
14	17			
6	21	MAPT	change of text format	<i>MAPT</i>
7	15, 25			
14	17			
7	26	transport (32, 34)	correction of language	transport (32, 34).
8	3	dendritic spine loss (17)	correction of language	dendritic spine loss (17).
14	4	functions are common	correction of language	functions is common
18	25	is involved in	correction of language	are involved in

23	1	is important for	correction of language	are important for
25	2	impairment are	correction of language	impairment is
25	16	imparment are	correction of language	impairment is
25	26	stages, there is	correction of language	stages, there are
28	18	quetiapine is often used	correction of language	quetiapine are often used
31	19	was also associated	correction of language	were also associated
31	27	VBM have shown	correction of language	VBM has shown
34	18	MRI have shown	correction of language	MRI has shown
44	21	there were gray matter	correction of language	there was gray matter
49	28	analysis were corrected	correction of language	analysis was corrected
59	25	There were numerically	correction of language	There was numerically
66	1	as this was not	correction of language	as these were not
67	4, 9, 14, 18	a high score represent	correction of language	high score represents
69	22	aacceptable	correction of language	acceptable
74	28	which were smaller	correction of language	which was smaller
75	19	together has	correction of language	together have
89	18	Another limitation which potentially affects	correction of language	Other limitations which potentially affect
93	5	Our results proposes	correction of language	Our results propose
96	15	Our and other's results	correction of language	Our and others' results

96	24	is likely to precede	correction of language	are likely to precede
97	3	causes synaptic	correction of language	cause synaptic
97	8	Our findings suggests	correction of language	Our findings suggest
98	15	reflect and possibly	correction of language	reflects and possibly
99	17	method do not	correction of language	method does not
100	24	was only reduced	correction of language	were only reduced
101	8	is primarily	correction of language	are primarily
101	21	which is linked to visual functions, is found	correction of language	which are linked to visual functions, are found
102	6	were not	correction of language	was not
103	20	there were no	correction of language	there was no
104	22	there were no	correction of language	there was no
104	24	biomarker findings PD	correction of language	biomarker findings in PD
106	1	subiculum were	correction of language	subiculum was
107	14	impairment was	correction of language	impairment were
107	20	with memory og	correction of language	with memory and
109	2	there were no	correction of language	there was no
109	5	Attachments	change of text format	<i>Attachments</i>
110	19	have been shown	correction of language	has been shown
112	24	findings also supports	correction of language	findings also support

112	26	correlation	correction of language	correlations
116	6	needs to be confirmed	correction of language	need to be confirmed
117	5	Disease-specific treatment	correction of language	Disease-specific treatments
140	20	difference	correction of language	differences
140	22	groups were	correction of language	group was
143	19	; in the Parkinson's disease	correction of language	(deleted)

10.3 Papers 1, 2 and 3

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

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Amyloid- β and α -synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease

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ABSTRACT

Introduction: Cognitive impairment in early Parkinson's disease (PD) is common and distinct from early Alzheimer's disease. Predictors and mechanisms are only partially known, but α -synuclein, amyloid- β and tau dysmetabolism may be involved. Our aim was to study associations between cerebrospinal fluid biomarkers (CSF) and cognition in non-dementia PD compared to normal controls (NC) and non-PD patients with mild cognitive impairment (MCI non-PD).

Methods: Patients were classified as having normal, subjective or mild cognitive impairment after cognitive screening. CSF levels of total α -synuclein (t- α -syn), amyloid- β (A β) 38, 40 and 42, total tau (T-tau) and phosphorylated tau (P-tau) were measured in 34 NC, 31 early, non-dementia PD and 28 MCI non-PD patients. A well validated neuropsychological test battery was administered.

Results: In the PD group, 13 had normal cognition, 4 had subjective and 14 mild cognitive impairment. PD patients had significantly lower CSF biomarker levels of t- α -syn, A β 38, 40 and 42, T-tau and P-tau compared to NC. Compared to MCI non-PD, t- α -syn, A β 38 and 40, T-tau and P-tau were also lower, while A β 42 was significantly higher in the PD group. A β 38 and 40 correlated strongly with t- α -syn levels in PD. Lower A β 42 was associated with decreased verbal learning, delayed verbal recall and response inhibition in PD.

Conclusion: While A β 38, 40 and t- α -syn levels are strongly correlated, only lower A β 42 was associated with reduced cognitive functions in early PD, mainly connected to medial temporal lobe-based cognitive functions.

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1. Introduction

Several clinical and biological markers are associated with increased risk of cognitive impairment and dementia in Parkinson's disease (PD) [1]. Mechanisms for mild cognitive impairment (MCI) in PD are not fully known, but the neuropathology is heterogeneous and includes limbic and neocortical Lewy body pathology as well as cortical amyloid β (A β) plaques [2,3]. While low CSF A β 42 is linked to plaques and high CSF tau to neurofibrillary tangles and predict

Alzheimer's dementia decades in advance [4,5], little is known about early CSF biomarkers for dementia in PD (PDD). PD patients, particularly with cognitive impairment, have slightly reduced CSF levels of A β 38, 40 and 42 and reduced or unchanged levels of total tau (T-tau) and phosphorylated tau (P-tau), although higher tau levels have been found in PDD compared with controls [6–8]. CSF α -synuclein may reflect the severity and distribution of Lewy body pathology in the brains of patients with PD, and CSF levels of total α -synuclein (t- α -syn) have been reported low or unchanged compared to controls [6,8].

Reduced A β 42 has been associated with cognitive impairment in memory, phonetic fluency, attention and visuospatial perception in non-dementia PD, and associations between reduced A β 38 and 40

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and memory impairment have been described [7,9–13]. Reduced A β 42 has also been shown to be a predictor of cognitive impairment and dementia in longitudinal studies [11–15]. One study found associations between elevated T- and P-tau and poorer cognition in PD cases with MCI [16], and high P-tau may predict decline in memory and executive functions [17]. Few studies have focused on t- α -syn in relation to cognition in PD; two longitudinal studies found that higher α -synuclein predicted decline in verbal and visuospatial memory and cognitive processing speed, while others have not found this [10,13,14,18,19].

As Lewy bodies are strongly linked to dementia in PD, and common degradation pathways may link A β and α -synuclein metabolism, it is necessary to measure both factors compared to detailed neuropsychological tests to assess relative impact.

Based on previous research, the aims of this study were: 1) to measure CSF A β sub-species, tau and α -synuclein levels, and 2) to investigate their associations with cognitive functions in early, non-demented PD patients.

2. Methods

This cross-sectional study is based on baseline data from an ongoing longitudinal study in a university-hospital based neurological outpatient clinic [20], where all examinations were conducted within a three month period. 31 patients with PD were consecutively recruited during 2011–2014, and examined by a neurologist with training in movement disorders. Inclusion criteria were diagnosis of PD according to the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria [21], Hoehn and Yahr stage < 3 and disease duration $\leq$ 6 years. All patients responded well to dopaminergic medication except 5 patients who had not had an adequate trial. None of the patients reported motor fluctuations or dyskinesias. All subjects had a pathological single photon emission computed tomography (SPECT) using an ioflupane (^{123}I) biomarker (DaTSCAN). Exclusion criteria were dementia (see below for procedure) and other somatic or psychiatric diseases that might contribute to cognitive impairment (including drug abuse, moderate or severe depression, solvent exposure, ischemic stroke and active cancer). Standardized rating scales of motor function (The unified Parkinson's disease rating scale, UPDRS, part III motor examination) and Hoehn & Yahr staging were performed by trained research physicians. Depression was assessed using the 15-item Geriatric Depression Scale. Total daily levodopa equivalent dose (LED) was calculated using conversion formulas yielding standardized LED for each antiparkinsonian drug [22].

We included two non-PD control groups from parallel ongoing studies; one group with non-PD patients with MCI (MCI non-PD) with increased risk of developing Alzheimer's disease (AD) and one group with normal controls (NC). According to the National Institute on Aging - Alzheimer's Association (NIA-AA criteria) [5], all 28 MCI non-PD patients fulfilled the AD-MCI core clinical criteria, whereas 15 of these had AD-MCI high likelihood. Cognitive status was determined from a screening battery as previously described [23], and 21 had amnesic MCI. In addition, an abnormal score on at least one CSF AD biomarker (A β 42, P-tau or T-tau) was required to increase AD likelihood. Exclusion criteria were as for PD and in addition cognitive improvement during follow-up. Subjects included in the MCI non-PD group did not fulfill criteria for PD, Lewy body, frontotemporal or vascular dementia.

34 NC cases were included in the second control group. Four were selected from spouses or relatives of MCI non-PD and PD subjects, whereas 30 subjects were recruited before lumbar puncture in conjunction with orthopedic surgery. Inclusion criteria were normal cognition based on Mini-Mental State Exam (MMSE) $\geq$ 28, Rey Auditory Verbal Learning Test (RAVLT) delayed recall [24], Trail

Making Test-B (TMT-B) [25] and Controlled Oral Word Association Test (COWAT) [25]. The results for the latter tests were converted to T-scores, a normally distributed scale with a mean score of 50 and a SD of 10. A T-score above 35 correspond to above 1.5 SD below the normative mean. Subjects with at least one of the tests below a T-score of 35 were excluded with respect to the NIA-AA criteria [5]. Exclusion criteria were as for PD, in addition to relevant subjective cognitive memory symptoms, PD and AD.

In addition to clinical interview and examination, all PD subjects had cognitive screening for staging as no cognitive impairment, subjective cognitive impairment (SCI), MCI, or dementia (exclusion criterion) as previously described [23]. In PD, raw scores were used to explore associations between neuropsychological tests and CSF biomarkers; Verbal learning and delayed recall (number of learned words of a list of 15 words over 5 trials and number of words recalled after 30 min) were measured by RAVLT [24]. Visuospatial ability and delayed visual reproduction after 30 min were measured by Rey Complex Figure Test (RCFT) [26]. Executive functions were assessed by tests measuring divided attention (TMT-B) [25], response inhibition (Stroop Color Word, SCW) [26] and verbal fluency (COWAT) [25]. High scores on these tests represent good achievement, except for TMT-B and SCW where high scores represent poor performance.

Following lumbar puncture and standardized handling, CSF A β 42, T-tau and P-tau were analyzed with ELISA (Fujirebio Europe, previously Innogenetics) in all patients. CSF A β 42 was considered pathological if $\leq$ 600 ng/L, P-tau if $\geq$ 80 ng/L, and T-tau if $>$ 450 ng/L, according to local cutoffs. CSF A β 38, 40 and t- α -syn were analyzed with electrochemiluminescence using anti-A β 6E10 detection antibody (Meso Scale Discovery, US) in all PD patients, 22 NC and 20 MCI non-PD. T- α -syn analyses were only included if CSF hemoglobin was below 350 ng/mL to avoid the possible effect of hemolysis on the t- α -syn level [27], reducing the number of available cases for this part of the analysis to 24 PD, 15 NC and 15 MCI non-PD.

IBM® SPSS® Version 20 was used for statistical analyses. Comparisons between the three groups (PD, MCI non-PD and NC) were made using Kruskal–Wallis test and post-hoc Mann–Whitney test if significant differences emerged, and Chi Square test or Fisher's Exact test as appropriate. $P \leq 0.05$ was considered statistically significant. Correlations between the CSF biomarkers and the neuropsychological tests were assessed with Spearman Correlation. To adjust for potential confounders, relevant multiple linear regressions with neuropsychological tests as dependent variables and CSF biomarkers as independent variables, were used.

The study protocol was approved by the South-Eastern Norway ethical committee for medical research 24.02.11, last approved change 12.05.14, approval number 2011/99. Written informed consent was obtained from all subjects.

3. Results

In the PD group, mean disease duration from motor symptom onset was 2.5 years (SD 1.2) [range 1–6]. Mean UPDRS part III motor score was 14.9 (SD 6.2) [range 6–31] and median Hoehn and Yahr stage was 1.5 [range 1.0–2.5]. Mean LED was 385.2 (SD 241.5) [range 0–924]. Mean geriatric depression scale score was 1.4 (SD 1.4) [range 0–6]. 13 had normal cognition, 4 had SCI and 14 had MCI, but there were no significant differences in the descriptive data (except for lower MMSE score in MCI) or CSF biomarker levels (data not shown). All PD patients had A β 42 levels above in-house diagnostic cut-off for AD.

There were no significant differences in gender, age or education between the three groups (Table 1). The PD and MCI non-PD groups had lower MMSE scores compared to the NC group. The PD group

had significantly lower, but overlapping levels of Aβ38, 40, 42, t-α-syn, T-tau and P-tau compared to NC. In the MCI non-PD group, Aβ38 and 40 were significantly higher than in PD, while Aβ42 was lower and t-α-syn, T-tau and P-tau were higher than in both PD and NC groups (Table 1, Fig. 1).

In the PD group, t-α-syn values correlated strongly with Aβ38 and 40 (Spearman's rho 0.803 and 0.808, $p \leq 0.01^*$), but did not correlate with Aβ42. There was also a correlation between t-α-syn and Aβ40 in the NC group (Spearman's rho 0.525, $p = 0.044^*$), but not in the MCI non-PD group.

Associations between CSF biomarkers and cognitive tests in the PD group are shown in Table 2. Lower Aβ42 correlated with poorer performance in verbal learning, delayed verbal recall and response inhibition. In multiple linear regression models, a decrease in Aβ42 of 100 ng/L corresponded to a decrease in verbal learning of 3.4 points ($p = 0.001^*$), in delayed verbal recall of 0.7 points ($p = 0.020^*$) and in response inhibition of 4.1 points ($p = 0.026^*$) (Table 2 and Fig. 2). In addition, a decrease in education of 1 year, corresponded to a decrease in verbal learning of 1.69 points ($p = 0.001^*$) and in delayed verbal recall of 0.45 points ($p = 0.002^*$). An increase in UPDRS part III motor score of 1 point, corresponded to a decrease in response inhibition of 1.24 points ($p = 0.010^*$). Gender and age did not have any significant effect on the neuropsychological tests in the regression models. There were no significant correlations between T-tau or P-tau and any of the neuropsychological tests (data not shown).

4. Discussion

Here we explore associations between CSF biomarkers and cognition in early PD with and without MCI, report significant correlations between Aβ38, 40 and t-α-syn as well as differential associations to specific cognitive domains with intra- and extra-medial temporal lobe-based cortical representations.

Although all PD patients had Aβ42 levels above cut-off for AD,

mean levels were significantly lower in PD compared to NC, in accordance with findings in earlier studies [6,8]. Most other studies have found lower levels of Aβ42 in AD dementia as compared to PD [8]. To our knowledge, only one other study has evaluated the levels of Aβ42 in PD compared to a MCI group. They found that longer lasting PD with cognitive impairment without dementia, but not the amnesic MCI group, had significantly lower levels of Aβ42 than controls. They did not compare the levels of Aβ42 in MCI and PD directly [28]. Our study is the first to show that there is also a difference in the levels of Aβ42 in early stages of PD and MCI; even though PD patients had reduced levels of Aβ42 compared to normal controls, levels were not as low as in MCI non-PD patients with increased risk of developing AD.

We found significant associations between lower CSF Aβ42 and poorer verbal learning, delayed verbal recall and response inhibition in PD. Lower CSF Aβ42 may reflect amyloid plaques, and Aβ pathology is known to affect memory and executive network functions. This suggests a link between Aβ pathology and impaired parieto-temporal and frontal lobe functioning also in early PD. This is supported by a magnetic resonance imaging study which found correlations between lower Aβ42 and gray matter volume reductions in mainly temporal and frontal structures which in turn correlated with memory, naming, fluency and visuospatial functions in PD patients with and without dementia [29]. In addition, we found associations between shorter education and poorer verbal learning and recall, while higher UPDRS part III motor score was associated with poorer response inhibition, a measure of cognitive flexibility. The latter may be explained by more advanced disease, or by dopaminergic depletion in both striatum and frontostriatal pathways leading to poorer motor and prefrontal executive functions respectively [30]. Thus, poorer cognitive flexibility in PD may be due to dopamine pathology in addition to Aβ-related pathology which usually leads to amnesia as seen in AD.

To our knowledge only two other studies have focused on the

Table 1

Demographic and clinical characteristics and cerebrospinal fluid biomarker concentrations in the different groups (significant results in bold).

Descriptive	PD	NC	MCI non-PD	p-value ^a
N	31	34	28	—
Gender, female/male %	38.7/61.3	41.2/58.8	57.1/42.9	0.307 ^b
Age at inclusion, years	64.65 (6.53) [47–74]	62.62 (9.80) [40–83]	66.86 (4.45) [58–75]	0.163 ^c
Education, years	13.11 (3.37) [8.0–19.0]	13.97 (3.64) [8.0–21.0]	13.68 (3.63) [8.0–21.0]	0.735 ^c
MMSE score	28.58 (1.59) [22–30]	29.32 (0.77) [28–30]	27.68 (1.68) [23–30]	<0.001 ^{c,d,g}
CSF biomarkers				p-value ^f
Innogenetics, N	31	34	28	—
Aβ42, ng/L	938.48 (156.37) [707–1280]	1047.03 (209.42) [582–1430]	537.00 (184.89) [221–1110]	0.024^{e,g} / <0.001^{e,g} / <0.001^{e,g}
T-tau, ng/L	265.77 (135.27) [119–734]	357.32 (153.15) [114–850]	635.75 (368.44) [224–1407]	0.003^{e,g} / <0.001^{e,g} / 0.001^{e,g}
P-tau, ng/L	44.97 (25.53) [25–155]	54.68 (16.87) [26–110]	117.68 (71.17) [54–403]	0.001^{e,g} / <0.001^{e,g} / <0.001^{e,g}
MSD, N	31	22	20	—
Aβ38, ng/L	1854.68 (784.53) [819.07–4550.95]	2424.45 (495.35) [1382.25–3162.84]	2255.31 (712.58) [1419.64–4389.70]	0.001^{e,g} / 0.035^{e,g} / 0.118^e
Aβ40, ng/L	5080.00 (1790.86) [2212.21–10512.27]	6617.58 (1096.32) [4564.07–8361.94]	6029.45 (1658.50) [3916.08–10633.85]	<0.001^{e,g} / 0.029^{e,g} / 0.039^{e,g}
t-α-syn, N	24	15	15	—
t-α-syn, ng/L	275.68 (77.79) [160.09–476.22]	328.50 (58.15) [229.39–422.04]	425.01 (145.10) [267.39–701.66]	0.017^{e,g} / <0.001^{e,g} / 0.044^{e,g}

Values are presented as mean (SD) [range] unless otherwise stated.

Abbreviations: PD, Parkinson's disease patients; NC, normal controls; MCI non-PD, non-PD patients with mild cognitive impairment; MMSE, Mini-Mental State Examination; CSF, cerebrospinal fluid; Aβ, Amyloid-β; T-tau, total-tau; P-tau, phospho-tau; t-α-syn, total α-synuclein; MSD, Meso Scale Discovery.

^a PD vs. NC vs. MCI non-PD.

^b Pearson Chi-Square test.

^c Kruskal-Wallis test.

^d PD vs. NC 0.022^{e,g}, PD vs. MCI non-PD 0.013^{e,g}, MCI non-PD vs. NC < 0.001^{e,g}.

^e Mann-Whitney test.

^f PD vs. NC/PD vs. MCI non-PD/NC vs. MCI non-PD.

^g Significant $p \leq 0.05$.

Fig. 1. Distribution of the levels of Amyloid- β (A β) 38, 40, 42 and total α -synuclein (t- α -syn) in normal controls (NC), Parkinson's disease (PD) patients and non-PD patients with mild cognitive impairment (MCI non-PD). Round circles and asterisk represent outliers and extreme respectively.

Table 2

Neuropsychological tests in relation to cerebrospinal fluid biomarkers in the Parkinson's disease group (significant results in bold).

Neuropsychological tests	Test scores, mean (SD) [range]	CSF biomarkers	Spearman's rho (p-value)	Multiple regression adjusted effect (95% CI) [p-value]
RAVLT, verbal learning of 15 words over 5 trials	43.97 (11.19) [18–69]	A β 38	0.220 (0.244)	—
		A β 40	0.187 (0.324)	—
		A β 42	0.446 (0.013^a)	0.034 (0.015–0.054) [0.001^a]^b
		t- α -syn	–0.143 (0.516)	—
RAVLT, delayed verbal recall of 15 words after 30 min	8.47 (3.04) [3–14]	A β 38	0.140 (0.461)	—
		A β 40	0.142 (0.454)	—
		A β 42	0.383 (0.037^a)	0.007 (0.001–0.012) [0.020^a]^b
		t- α -syn	–0.153 (0.486)	—
RCFT, visuo-spatial copy trial	31.18 (7.21) [3–36]	A β 38	0.205 (0.277)	—
		A β 40	0.123 (0.518)	—
		A β 42	0.312 (0.093)	—
		t- α -syn	–0.081 (0.712)	—
RCFT, delayed visual reproduction after 30 min	14.48 (6.76) [1–27.5]	A β 38	0.218 (0.248)	—
		A β 40	0.192 (0.309)	—
		A β 42	0.159 (0.403)	—
		t- α -syn	0.037 (0.866)	—
TMT-B, divided attention	138.80 (88.73) [40–349]	A β 38	–0.136 (0.475)	—
		A β 40	–0.161 (0.396)	—
		A β 42	–0.197 (0.297)	—
		t- α -syn	0.030 (0.893)	—
SCW, response inhibition	146.09 (95.96) [40–349]	A β 38	–0.238 (0.205)	—
		A β 40	–0.185 (0.327)	—
		A β 42	–0.374 (0.042^a)	–0.041 (–0.077––0.005) [0.026^a]^c
		t- α -syn	–0.045 (0.838)	—
COWAT, verbal fluency	36.21 (11.23) [18–63]	A β 38	0.285 (0.134)	—
		A β 40	0.233 (0.224)	—
		A β 42	0.263 (0.169)	—
		t- α -syn	0.314 (0.145)	—

N = 30 (29 in COWAT) for A β , and N = 23 for t- α -syn.

Abbreviations: RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; TMT-B, Trail-Making Test B; SCW, Stroop Color Word; COWAT, Controlled Oral Word Association Test; CSF, cerebrospinal fluid; A β , Amyloid- β ; t- α -syn, total α -synuclein.

^a Significant $p \leq 0.05$.

^b Adjusted for gender, age and education in a multiple regression analysis. The regression model with all variables explains 57.8% of the spread of verbal learning and 52.5% of the spread of delayed verbal recall.

^c Adjusted for gender, age, education and Unified Parkinson's disease rating scale part III motor examination. The regression model with all variables explains 44.6% of the spread of response inhibition.

relationship between A β 42 and specific cognitive domains in early PD, reporting similar findings (except impaired response inhibition) [7,10]. In PD of longer duration, associations between A β 42 and executive functions such as phonetic fluency and attention have also been described [9,11]. These findings were not always repeatable, possibly due to differences in the study designs, number of patients, disease stages, neuropsychological tests or A β 42 analysis methods [19,27,31].

The PD patients had significantly lower levels of A β 38 and 40, T-tau and P-tau compared to NC and MCI non-PD, but we did not find any correlation with neuropsychological tests. This conflicts with one study which also found lower levels of A β 38 and 40 in PD compared to NC, but not compared to mild AD dementia, and associations with memory impairment [7]. A large study found that higher P-tau did not correlate with cognition at baseline, but predicted subsequent decline in both memory and executive functions [17]. Another study found associations between higher T-tau and P-tau and a poorer global cognitive screening test in PD with MCI [16]. However, associations between tau and cognition in PD without dementia were not found in other studies [7,9,11–15,27].

We found significantly lower levels of t- α -syn in PD compared to both NC and MCI non-PD. This is in line with most studies comparing PD with NC [6,8]. Most other studies have found higher levels of t- α -syn in AD compared to PD, or AD-MCI and AD compared to NC [6,8,32,33]. To our knowledge this is the first study to compare t- α -syn levels in a group of PD patients with a group of MCI patients with increased risk of developing AD. We found higher t- α -syn in MCI non-PD compared to both NC and PD, suggesting that this measure may help to differentiate between the groups. It has been hypothesized that in PD the CSF level of t- α -syn is decreased because of the sequestering of toxic α -synuclein in Lewy bodies, while in AD the higher t- α -syn level may be due to release of the protein as a result of neuronal injury [32].

To our knowledge only six other studies have examined the relationship between t- α -syn and cognition in PD, and the results have been divergent. We did not find any correlation between t- α -syn and cognition, which is in line with three of the studies on early PD [10,14,19]. Another study found that lower t- α -syn was associated with impaired phonetic fluency in PD with and without dementia and with frontal cortical thinning in PD without dementia [34]. This is in line with the hypothesis that Lewy body pathology contributes to executive dysfunction, possibly due to both frontostriatal dopamine depletion and frontal atrophy. Two longitudinal studies found that higher α -synuclein levels predict cognitive impairment in cognitive processing speed and memory in PD [13,18]. The discrepancies may be due to the same factors as mentioned above for A β 42. A limitation with the present study is that we did not assess phosphorylated or oligomeric α -synuclein. Others have found higher levels of oligomeric α -synuclein in PD [14] associated with reduced MMSE, semantic fluency and visuo-perception in premotor, motor and dementia stages of PD [34].

The mechanisms of how α -synuclein eventually affects cognition is not clear, with the following hypotheses having been proposed: 1) a mechanism compensating for the sequestering of toxic α -synuclein retains functional α -synuclein in the neurons, leading to lower CSF levels and preserved cognition, as opposed to cases where this compensatory mechanism does not occur [18]; 2) sequestering of toxic α -synuclein in Lewy bodies leads to lower CSF levels and normal or slightly impaired cognition, but in more aggressive cases neurodegeneration leads to release of α -synuclein to the CSF resulting in increased levels, along with worsening cognition [13,34].

Our findings are in accordance with two small studies that found heterogeneous neuropathological changes including brainstem, limbic and neocortical Lewy bodies and amyloid plaques in

PD-MCI, which is similar to, but less advanced than in PDD and AD [2,3]. We found that lower A β 42 was associated with poorer cognitive functions in early PD, and A β 38 and 40 were very strongly inter-correlated with t- α -syn in the PD group. This is consistent with the neuropathological studies showing high correlations

Fig. 2. Scatter plots of correlations between relevant neuropsychological tests and Amyloid- β (A β) cerebrospinal fluid biomarkers in Parkinson's disease. High scores on the tests represent good performance, except for response inhibition where high scores represent poor performance.

between cortical amyloid plaques and Lewy bodies [2,3]. This may be due to similarities in α -synuclein and A β processing [35,36]. Thus, cognitive impairment in PD may be due to concomitant Lewy body and AD pathology.

Limitations of the study are the relatively small sample sizes, especially in the t- α -syn analysis, as well as the exploratory goals in the study with the number of analyses limiting hypothesis testing. The diagnosis of early PD without longitudinal follow-up to exclude atypical parkinsonism may always be somewhat uncertain, but in addition to positive DaTSCANS, all the PD patients fulfilled established diagnostic criteria [21]. Strengths of the study are the comprehensive early-stage non-dementia PD patient classification, well validated neuropsychological tests and mapping of CSF A β sub-species and α -synuclein. All the examinations were conducted within a three month period in the PD group. To our knowledge this is the first study to show that there is a difference in the levels of t- α -syn and A β 38, 40 and 42 in early PD compared to a MCI group with increased risk of developing AD, but this must be confirmed in larger studies.

Conflict of interest

Ane Løvli Stav: none.

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Contributors

DA and TF contributed to the concept and design of the study, ALS and EA collected the data, ALS analyzed the data, and all authors contributed to the interpretation of data. ALS, DA and TF drafted the article, and all authors revised it critically. All authors have approved the final article.

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ARTICLE OPEN

Hippocampal subfield atrophy in relation to cerebrospinal fluid biomarkers and cognition in early Parkinson's disease: a cross-sectional study

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Cognition is often affected early in Parkinson's disease (PD). Lewy body and amyloid β ($A\beta$) pathology and cortical atrophy may be involved. The aim of this study was to examine whether medial temporal lobe structural changes may be linked to cerebrospinal fluid (CSF) biomarker levels and cognition in early PD. PD patients had smaller volumes of total hippocampus, presubiculum, subiculum, CA2–3, CA4–DG, and hippocampal tail compared with normal controls (NCs). In the PD group, lower CSF $A\beta$ 38 and 42 were significant predictors for thinner perirhinal cortex. Lower $A\beta$ 42 and smaller presubiculum and subiculum predicted poorer verbal learning and delayed verbal recall. Smaller total hippocampus, presubiculum and subiculum predicted poorer visuospatial copying. Lower $A\beta$ 38 and 40 and thinner perirhinal cortex predicted poorer delayed visual reproduction. In conclusion, smaller volumes of hippocampal subfields and subhippocampal cortex thickness linked to lower CSF $A\beta$ levels may contribute to cognitive impairment in early PD. Thirty-three early PD patients (13 without, 5 with subjective, and 15 with mild cognitive impairment) and NC had 3 T magnetic resonance imaging (MRI) scans. The MRI scans were post processed for volumes of hippocampal subfields and entorhinal and perirhinal cortical thickness. Lumbar puncture for CSF biomarkers $A\beta$ 38, 40, 42, total tau, phosphorylated tau (Innogenetics), and total α -synuclein (Meso Scale Diagnostics) were performed. Multiple regression analyses were used for between-group comparisons of the MRI measurements in the NC and PD groups and for assessment of CSF biomarkers and neuropsychological tests in relation to morphometry in the PD group.

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INTRODUCTION

Cognitive impairment (CI) is a common non-motor feature in Parkinson's disease (PD). Approximately, 25% of non-demented PD cases have mild CI (MCI), which may precede dementia.¹ Research on the role of central nervous system structural affection and associated molecular mechanisms for cognitive decline in PD is needed for early diagnosis and intervention.

Imaging studies in PD have shown different patterns of magnetic resonance imaging (MRI) cortical gray matter atrophy. In PD without dementia, little or no regional thinning has been found in hippocampus, temporal, limbic, frontal, parietal, or occipital cortex, but there are more pronounced changes in PD-MCI and widespread atrophy is described in PD with dementia.^{2–4} Hippocampal atrophy has been associated with CI, especially poorer memory, in PD with and without dementia.³

Learning, memory, and visuospatial functions are related to hippocampus and subhippocampal regions (including entorhinal and perirhinal cortices, entorhinal cortex (ERC), and perirhinal cortex (PRC)) of the medial temporal lobe.^{5,6} The hippocampal subfields are in varying degree present within the head, body, and tail of the hippocampus.⁷ The hippocampus proper includes the dentate gyrus (DG) tightly connected to CA4, and the remaining subfields of cornu ammonis (CA3–1)⁵ filled by densely packed

pyramidal cells⁶ similarly layered as the neocortex. As described in primate models, associative cortical input is transferred mainly through PRC and ERC, and processed through the hippocampal subfields by way of the highly plastic connections in the polysynaptic loop to CA1, subiculum, and presubiculum.⁶ These output structures project back to ERC, PRC and prefrontal, thalamic, parahippocampal, and retrosplenial regions.⁵

MCI and Alzheimer's disease (AD) have been linked to medial temporal lobe affection including CA1–4, DG, subiculum, presubiculum, ERC, and PRC^{8–12} that are related to poorer memory functions.^{9–11} Selective vulnerability of different hippocampal regions in PD with and without dementia have been reported in a few studies that found atrophy in the hippocampal head,^{13,14} CA1–4, DG, subiculum, the whole hippocampus, and ERC in association with poorer memory functions.^{2,15–18}

The etiology of CI in PD is not known in detail, but the neuropathology is ultimately heterogeneous involving both cortical Lewy bodies containing α -synuclein and amyloid β ($A\beta$) plaques.¹⁹ Neuropathological changes in the hippocampus have been shown in postmortem PD brains with and without dementia, including Lewy neurites, especially in CA2 (refs. 20,21), Lewy neurites, Lewy bodies, and neurofibrillary tangles in PRC and ERC²² in addition to $A\beta$ deposition in ERC.²¹

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Table 1. Descriptive data of the study participants

Descriptive	NC	PD	P-value
N	32	33	—
Age at MRI (years)	62.9 (10.0) (40–82)	64.9 (7.0) (48–74)	0.533 ^a
Gender (male/female (%))	44/56	64/36	0.108 ^b
Education (years)	13.5 (2.8) (8–18)	13.1 (3.3) (8–19)	0.663 ^a
MMSE score	29.3 (0.7) (28–30)	28.6 (1.6) (22–30)	0.019 ^a
Duration of motor symptoms (years)	—	2.7 (1.5) (1–6)	—
Hoehn and Yahr stage (median)	—	1.5 (1–2.5)	—
UPDRS part III motor score	—	14.4 (6.5) (3–31)	—
Levodopa equivalent doses	—	392.2 (249.4) (0–924)	—
Geriatric depression scale	—	1.2 (1.4) (0–6)	—
N	23	31	—
Aβ38 (ng/l)	—	1878.6 (775.1) (819.1–4550.9)	—
Aβ40 (ng/l)	—	5125.4 (1772.9) (2212.2–10512.3)	—
Aβ42 (ng/l)	1007.6 (187.2) (692.0–1290.0)	943.0 (154.6) (707.0–1280.0)	—
T-tau (ng/l)	374.9 (188.1) (154.0–893.0)	271.1 (132.8) (119.0–734.0)	—
P-tau (ng/l)	55.7 (18.3) (37.0–110.0)	45.6 (25.3) (25.0–155.0)	—
N	—	24	—
T-α-syn (ng/l)	—	282.6 (74.4) (174.3–476.2)	—

Abbreviations: Aβ, amyloid-β; MRI, magnetic resonance imaging; MMSE, Mini-Mental State Examination; NC, normal controls; PD, Parkinson's disease patients; P-tau, phosphorylated tau; T-tau, total tau; t-α-syn, total α-synuclein; UPDRS, unified Parkinson's disease rating scale.

Values are presented as mean (s.d.) (range) unless otherwise stated.

^aMann–Whitney test.

^bPearson χ^2 -test.

*Significant $P \leq 0.05$.

Aβ, tau, and α-synuclein neuropathologies are reflected as neurochemical changes in the cerebrospinal fluid (CSF), and CSF Aβ42, total tau, and phosphorylated tau have been established as biomarkers for AD diagnosis.²³ On a group level, amyloid species (Aβ42, 40, and 38) are lower than in controls and associated with CI in PD.^{24–29} In most studies, tau levels are reduced or unchanged in PD without dementia.^{25,29} CSF total α-synuclein (t-α-syn) is reduced or unchanged in PD,^{25,29} but its association with CI is not yet clarified.^{27–31}

In AD and normal controls (NCs), links between brain atrophy and CSF biomarkers have been reported, supporting the hypothesis that the biomarkers reflect regional or global neurotoxicity related to protein dysmetabolism.⁸ Studies in AD have found associations between lower CSF Aβ42 and CA1 deformation, and hippocampal atrophy and higher T- and phosphorylated tau, and hippocampal, parahippocampal, and ERC atrophy.^{8,32} However, very few studies have assessed the relationship between CSF biomarkers and atrophy in PD. Aβ species and total tau have been associated with ventricular enlargement, but not with hippocampus volume.³³ Associations between lower Aβ42 and higher tau and frontal, parietal and temporal cortical atrophy were found in PD with dementia,³⁴ and lower Aβ42 in non-demented PD patients was prospectively associated with parietal and occipital thinning.²⁶ An association between low t-α-syn and frontal cortical thinning in PD without dementia has also been reported.³¹ To our knowledge, no other study has evaluated the hippocampal subfields in relation to CSF biomarkers in PD.

The aims of this study were to measure structural changes of hippocampal subfields and subhippocampal regions and explore possible associations with CSF biomarkers and neuropsychological functions in early, non-demented PD patients with and without MCI. We hypothesized that PD patients have smaller hippocampal subfield volumes compared with NC, and that these changes are associated to CSF biomarkers of neurodegeneration as well as to learning, memory, and visuospatial functions.

RESULTS

Comparisons of descriptive and MRI subfields data between PD and NC groups

Descriptive data and CSF measurements of the study participants are presented in Table 1. The PD patients were in early stages with mild to moderate motor symptoms, 13 cases had normal cognition, 5 had subjective CI (SCI), and 15 had MCI, and all had Aβ42 levels above in-house diagnostic cutoff for AD. The PD and NC groups were similar regarding age, education, and distribution of gender. Mini-Mental State Exam was significantly lower in PD compared with NC.

Table 2 presents the MRI volume and thickness data in PD and NC. Total hippocampus, presubiculum, subiculum, CA2–3, CA4-DG, and hippocampal tail were significantly smaller in the PD group, but after adjusting for multiple comparisons only the subiculum and hippocampal tail remained significantly smaller. Before correction for multiple comparisons, none of the 34 cortical thickness variables were significantly thinner in the PD patients compared with the NC group (data not shown).

Relationship between the MRI subfields data, CSF biomarkers, and cognition in the PD group

Associations between CSF biomarkers and MRI variables in the PD group are shown in Table 3. Lower CSF Aβ38 and 42 were predictors for thinner PRC; a decrease in 2 s.d. of Aβ38 (1550.2 ng/l) and Aβ42 (309.2 ng/l) corresponded to a decrease in PRC thickness of 0.25 and 0.23 mm, respectively. There were no other significant relationships, and no association between Aβ42 and PRC in the NC group ($P=0.558$).

Finally, we assessed the MRI variables and CSF biomarkers as predictors of the neuropsychological tests (Table 4). Lower Aβ42, and smaller presubiculum and subiculum in combination with lower Aβ42 predicted poorer verbal learning and delayed verbal recall; 2 s.d. decrease in Aβ42 (312.98 ng/l), presubiculum (0.184% of intracranial volume (ICV)), and subiculum (0.211% of ICV) corresponded to a decrease in verbal learning of 10.02, 8.41, and 8.13 points, and a decrease in delayed verbal recall of 1.88, 1.94, and 2.07 points, respectively. The regression models with Aβ42

Table 2. Comparison of MRI variables between NC and PD

MRI variables	NC	PD	NC versus PD ^a
	mean (s.d.) (range)	mean (s.d.) (range)	Adjusted mean difference (95% CI) (P-value)
Total hippocampus (% of ICV)	6.0 (1.0) (4.4–8.2)	5.24 (0.74) (3.7–6.8)	–0.492 (–0.834 to –0.149) (0.006)*
Presubiculum (% of ICV)	0.68 (0.13) (0.46–0.97)	0.58 (0.096) (0.41–0.78)	–0.060 (–0.106 to –0.013) (0.014)*
Subiculum (% of ICV)	0.93 (0.15) (0.69–1.25)	0.81 (0.11) (0.60–1.10)	–0.079 (–0.131 to –0.026) (0.004)**
CA1, (% of ICV)	0.49 (0.075) (0.38–0.70)	0.45 (0.056) (0.33–0.60)	–0.021 (–0.050 to –0.008) (0.147)
CA2–3, (% of ICV)	1.49 (0.24) (1.15–2.01)	1.33 (0.21) (0.95–1.93)	–0.100 (–0.198 to –0.002) (0.046)*
CA4-DG (% of ICV)	0.83 (0.14) (0.62–1.14)	0.74 (0.11) (0.53–1.05)	–0.056 (–0.109 to –0.003) (0.038)*
Hippocampal tail (% of ICV)	0.55 (0.10) (0.38–0.76)	0.46 (0.07) (0.32–0.62)	–0.058 (–0.095 to –0.021) (0.002)**
PRC (mm)	3.39 (0.24) (2.9–4.0)	3.45 (0.25) (3.0–4.1)	0.078 (–0.048 to 0.205) (0.220)
ERC (mm)	3.53 (0.25) (3.1–4.2)	3.65 (0.27) (3.0–4.2)	0.116 (–0.019 to 0.251) (0.091)

Abbreviations: CA, cornu ammonis; DG, dentate gyrus; ERC, entorhinal cortex; ICV, intracranial volume; MRI, magnetic resonance imaging; NC, normal controls; PD, Parkinson's disease patients; PRC, perirhinal cortex.

^aMultiple linear regression analysis with MRI as dependent variable, and group (PD or NC), gender, and age as independent variables.

*Significant $P \leq 0.05$.

**Significant $P \leq 0.0056$ after adjusting for multiple comparisons by Bonferroni.

Table 3. CSF biomarkers as predictors for MRI variables in the PD group

MRI variables	A β 38	A β 40	A β 42	T-tau	P-tau	t- α -syn
Total hippocampus (% of ICV)	(0.201)	(0.193)	(0.374)	(0.638)	(0.857)	(0.250)
Presubiculum (% of ICV)	(0.511)	(0.413)	(0.726)	(0.930)	(0.574)	(0.587)
Subiculum (% of ICV)	(0.909)	(0.797)	(0.579)	(0.470)	(0.339)	(0.973)
CA1, (% of ICV)	(0.645)	(0.554)	(0.709)	(0.151)	(0.106)	(0.832)
CA2–3, (% of ICV)	(0.537)	(0.470)	(0.532)	(0.741)	(0.715)	(0.250)
CA4-DG (% of ICV)	(0.724)	(0.623)	(0.792)	(0.678)	(0.506)	(0.218)
Hippocampal tail (% of ICV)	(0.787)	(0.499)	(0.948)	(0.630)	(0.272)	(0.807)
PRC (mm)	0.00016 (0.00003–0.00028) (0.018)*	(0.065)	0.00075 (0.00018–0.00133) (0.012)*	(0.250)	(0.779)	(0.828)
ERC (mm)	(0.774)	(0.990)	(0.176)	(0.520)	(0.074)	(0.497)

Abbreviations: A β , amyloid- β ; CA, cornu ammonis; CI, confidence interval; DG, dentate gyrus; ERC, entorhinal cortex; ICV, intracranial volume; PD, Parkinson's disease patients; P-tau, phosphorylated tau; t- α -syn, total α -synuclein; PRC, perirhinal cortex; T-tau, total tau.

$N = 31$ (24 for t- α -syn).

Multiple linear regression analysis with MRI as dependent variable and CSF biomarkers, as predictors adjusting for age and gender (independent variables). Values are presented as adjusted effect (95% CI) (P-value).

*Significant $P \leq 0.05$.

and presubiculum (subiculum) explained 71.0% (69.5%) and 63.4% (63.6%) of the spread of verbal learning and delayed verbal recall, respectively. The combination of CSF and MRI biomarkers were better predictors of cognition than considered separately. Smaller total hippocampus, presubiculum, and subiculum predicted poorer visuospatial copying; 2 s.d. decrease in total hippocampus (1.397% of ICV), presubiculum, and subiculum corresponded to a decrease in visuospatial copying of 7.91, 6.21, and 7.36 points, respectively. Thinner PRC and lower A β 38 and 40 predicted poorer delayed visual reproduction separately; 2 s.d. decrease in PRC (0.507 mm), A β 38 (1557.17 ng/l), and 40 (3567.48 ng/l) corresponded to a decrease in delayed visual reproduction of 5.78, 4.67, and 3.57 points, respectively, but this was no longer significant when combining PRC with A β 38 or A β 40 in the same regression analysis. Lower A β 42 only predicted poorer response inhibition, and no MRI variables predicted executive functions.

DISCUSSION

The present study is the first study to assess hippocampal subfields and subhippocampal regions in relation to CSF biomarkers in PD patients, proposing A β species as early predictors of PRC thickness and as predictors of CI in conjunction with hippocampal subfields. It confirms selective vulnerability of the hippocampal area in early PD patients without dementia. Specifically, we found that subiculum and the hippocampal tail

were significantly smaller in PD compared with NC. When exploring the association with cognitive domains in the PD group, we found that smaller subiculum and presubiculum predicted poorer verbal learning and delayed verbal recall, and together with A β 42, they predicted cognition even better. Lower total hippocampus, presubiculum, and subiculum volumes were associated with poorer visuospatial copying. None of the hippocampal subfields showed associations with the CSF biomarkers. Our findings are in line with studies in primate models and patients with amnesic MCI that have shown associations between hippocampus, subiculum, and presubiculum, and memory and visuospatial functions.^{5,6,9,11}

One earlier study in PD using the same subfield segmentation technique found volume loss in CA2–3, CA4-DG, subiculum, and the whole hippocampus, and this correlated with verbal learning.¹⁵ This is partly in line with our study, and the discrepancies may be due to different PD sample types. For example, our sample had shorter disease duration, fewer with MCI, no one had hallucinations, and there were partly different neuropsychological tests.

Another study in early PD with and without CI found associations between radial distances as a measure of atrophy in CA1, CA3, and subiculum, and poorer verbal recall and recognition, suggesting that these functions are impaired at least partly due to structural hippocampal changes.¹⁶ They did not find any significant associations between CSF amyloid or tau

Table 4. MRI variables and A β 42 as predictors of neuropsychological tests in the PD group

Predictors	RAVLT verbal learning	RAVLT delayed verbal recall	RCFT visuospatial copy	RCFT delayed visual reproduction	TMT-B divided attention	SCW response inhibition	COWAT verbal fluency
A β 42	0.032 (0.012–0.051) (0.003) ^a	0.006 (0.000–0.012) (0.049) ^a	(0.431) ^b	(0.263) ^a	(0.159) ^a	–0.039 (–0.077 to –0.002) (0.040) ^{a,b}	(0.628) ^b
A β 38	(0.323) ^a	(0.198) ^a	(0.388) ^b	0.003 (0.000–0.007) (0.038) ^a	(0.342) ^a	(0.143) ^b	(0.649) ^b
A β 40	(0.281) ^a	(0.182) ^a	(0.569) ^b	0.001 (0.000–0.003) (0.049) ^a	(0.369) ^a	(0.192) ^b	(0.870) ^b
Total hippocampus	(0.130) ^c	(0.079) ^c	5.66 (1.66–9.66) (0.007) ^{a,b}	(0.815) ^d (0.814) ^e	(0.573) ^a	(0.404) ^f	(0.845) ^b
Presubiculum	45.70 (12.75–78.64) (0.009) ^{a,c}	10.52 (0.282–20.76) (0.044) ^{a,c}	33.77 (5.54–62.00) (0.021) ^{a,b}	(0.355) ^d (0.386) ^e	(0.103) ^a	(0.052) ^f	(0.948) ^b
Subiculum	38.55 (7.54–69.57) (0.017) ^{a,c}	9.79 (0.420–19.16) (0.041) ^{a,c}	34.90 (10.27–59.52) (0.007) ^{a,b}	(0.184) ^d (0.216) ^e	(0.231) ^a	(0.139) ^f	(0.324) ^b
CA1	(0.514) ^c	(0.518) ^c	(0.431) ^b	(0.512) ^d (0.512) ^e	(0.958) ^a	(0.668) ^f	(0.259) ^b
CA2–3	(0.363) ^c	(0.166) ^c	(0.067) ^b	(0.875) ^d (0.906) ^e	(0.858) ^a	(0.671) ^f	(0.421) ^b
CA4-DG	(0.350) ^c	(0.136) ^c	(0.071) ^b	(0.873) ^d (0.923) ^e	(0.776) ^a	(0.855) ^f	(0.389) ^b
Hippocampal tail	(0.058) ^c	(0.108) ^c	(0.255) ^b	(0.745) ^d (0.894) ^e	(0.943) ^a	(0.294) ^f	(0.384) ^b
PRC	(0.709) ^c	(0.516) ^c	(0.993) ^b	11.41 (2.16–20.66) (0.018) ^a (0.079) ^d (0.052) ^e	(0.370) ^a	(0.504) ^f	(0.591) ^b
ERC	(0.256) ^c	(0.216) ^c	(0.646) ^b	(0.225) ^d (0.183) ^e	(0.350) ^a	(0.159) ^f	(0.707) ^b

Abbreviations: A β , amyloid- β ; CA, cornu ammonis; COWAT, Controlled Oral Word Association Test; DG, dentate gyrus; MRI, magnetic resonance imaging; PD, Parkinson's disease patients; PRC, perirhinal cortex; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; SCW, Stroop Color Word; TMT-B, Trail-Making Test-B.

N = 30 (29 for COWAT).

Multiple linear regression analysis with neuropsychological tests as dependent variables and MRI variables, and CSF biomarkers as predictors adjusting for age, gender, and education (in addition to UPDRS for RCFT visuospatial copy, SCW, and COWAT).

Values are presented as adjusted effect in points on neuropsychological tests (95% CI) (P-value).

^aAdjusting for age, gender, and education.

^bAdjusting for UPDRS, age, gender, and education.

^cAdjusting for A β 42, age, gender, and education.

^dAdjusting for A β 38, age, gender, and education.

^eAdjusting for A β 40, age, gender, and education.

^fAdjusting for A β 42, UPDRS, age, gender, and education. *Significant $P \leq 0.05$.

species and hippocampal radial distance thickness.³³ The discrepancies with our study may be due to the use of different segmentation techniques, radial distance mapping, and neuropsychological tests.

Even though PRC thickness was not different in PD compared with NC, lower A β 38 and 42 were significant predictors for thinner PRC in the PD group only. Both lower A β 38, 40, and thinner PRC predicted poorer delayed visual reproduction, but these associations disappeared when analyzed together. This may indicate that there is a relationship between A β species and PRC and delayed visual reproduction, but the findings are not consistent and more research are needed with larger sample sizes to confirm the result. To our knowledge, this is the first study to assess PRC thickness in PD.

PRC is one of the first areas affected by neurofibrillary tangles in pre-dementia AD,³⁵ and is also affected in PD.²² A β may contribute to hyperphosphorylation of tau, which is the main component of neurofibrillary tangles.³⁶ PRC have been linked to visual recognition memory,⁵ and CSF A β 42, visual recognition memory, and PRC are all affected early in patients with amnesic MCI and AD.^{10,12,37} A study in early PD found correlation between low CSF A β 42 and poorer delayed visual pattern recognition

memory in early PD.²⁸ On the basis of this and our findings that lower A β 42 and 38 are predictors for thinner PRC in early PD, we hypothesize that A β pathology leads to thinning of PRC, possibly through development of neurofibrillary tangles, and subsequently impaired visual recognition memory. As the present study has a low number of cases with potential bias possibilities, it is important to explore this theory in a larger study population and also include tests of visual recognition memory.

A recent amyloid PET study in cognitively normal elderly showed that cortical A β load was related to functional MRI disruption of functional connectivity of the PRC, supporting the hypothesis that dysfunction in PRC may be a very early sign of cortical amyloid deposition and predict memory impairment and dementia.³⁸ However, longitudinal follow-up is needed to test this hypothesis in more detail.

As hippocampal subfields and subhippocampal regions are affected and related to poorer memory functions and CSF A β 42 and T- and phosphorylated tau in MCI and AD,^{8–12,32} our findings are not unique to early PD, but may reflect concomitant AD or at least shared A β and tau pathology.

ERC has been reported to be smaller in PD and is associated with poorer learning and memory.^{2,17,18} We found no such

association and the discrepancy may be due to longer disease duration in other studies that most of the patients included already had MCI or dementia or also different neuropsychological tests used in these studies.

Although α -synuclein is a synapse protein and a major component of Lewy bodies, the role in CI is unclear,^{27–31} but a possible enhancing interaction with A β has been proposed.^{39,40} We found no association between t- α -syn CSF levels and cognition or hippocampal subfield atrophy. More studies are needed to explore the role of t- α -syn and cognition, and CSF studies should measure other α -synuclein species as well.

There was no cortical gray matter thinning in other brain regions, suggesting that differential hippocampal atrophy precedes gray matter changes in early PD. This is in line with another study describing hippocampal atrophy as a biomarker of initial CI in PD.⁴

This is the first study to assess hippocampal subfields and subhippocampal regions with 3T MRI in relation to CSF biomarkers and cognition in early PD patients. Another strength of this study is CSF t- α -syn measurement in addition to the traditional biomarkers.

3-T MRI high-field strength employed here allows more detailed imaging of hippocampal substructure. All segmentations were manually inspected, and the image and segmentation quality and consistency were considered good. The segmentation technique was automated, and whether manual segmentation is superior to automated is debated,^{41,42} but we only included the larger hippocampal structures with the best Dice overlap coefficients with manual segmentation.⁴¹ Anatomical subfields differences may arise due to different segmentation techniques, and a unified segmentation protocol is needed.

Another limitation of the study is the low number of participants. Power calculations for the hippocampal subfields to detect a difference of 7.5% between PD and NC, based on a power of 80%, a significant alpha of 0.05 and s.d.'s for the different hippocampal subfields available from another study,¹⁵ required 19–41 subjects in each group. Power calculations for subiculum and presubiculum only demanded more participants than in our study. This suggests that the number of participants is adequate to see the most relevant differences. The cross-sectional design is another limitation, and subsequent longitudinal data from this study might inform about the relation between progression of CSF biomarkers, atrophy, and cognition.

In conclusion, regional hippocampal atrophy is present early in PD patients and related to impairments in verbal learning and memory and visuospatial functioning, and probably connected to A β pathology. The perirhinal cortex might be the first affected area in PD contributing to impairment of visuospatial memory.

MATERIALS AND METHODS

Subjects

The patients in this cross-sectional study were consecutively recruited from a university hospital based neurological outpatient clinic from 2011 to 2014 (ref. 29).

Thirty-three patients diagnosed with PD according to the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria,⁴³ Hoehn and Yahr stage < 3 and disease duration $\leq$ 6 years were included. Neurological examination and diagnosis, the unified PD rating scale part III motor examination and Hoehn and Yahr staging were performed by trained research physicians and movement disorder specialists. All PD patients had pathological ioflupane (¹²³I) single-photon emission computed tomography (DaTSCAN) to support the clinical diagnosis of PD. The 15-item Geriatric Depression Scale was used to assess depression. Total daily levodopa equivalent dose was calculated.⁴⁴ Exclusion criteria were dementia, and other severe somatic or psychiatric comorbidity that could contribute to CI, including cancer under treatment, ischemic stroke, drug abuse, solvent exposure, and moderate to severe depression.

Cognitive screening tests were performed in all PD patients and used to categorize the patients into groups of no CI, SCl, MCI, or dementia (exclusion criterion) as described earlier.⁴⁵ Associations between hippocampal subfields and neuropsychological tests were explored in 30 patients using raw scores as previously described;²⁹ verbal learning and delayed recall (number of learned words of a list of 15 words over five trials and number of words recalled after 30 min) were measured by Rey Auditory Verbal Learning Test. Visuospatial ability and delayed visual reproduction after 30 min were measured by Rey Complex Figure Test. Aspects of executive functions were assessed by tests measuring divided attention (Trail-Making Test-B), response inhibition (Stroop Color Word), and verbal fluency (Controlled Oral Word Association Test, COWAT). High scores on these tests represent good achievement, except for Trail Making Test-B and Stroop Color Word where high scores represent poor performance.

Thirty-two neurologically healthy NCs were recruited from volunteers, spouses, or relatives of participating patients ($n = 16$) and from subjects undergoing elective orthopedic surgery in spinal anesthesia ($n = 16$). Inclusion criteria were as previously described²⁹ normal cognition based on Mini-Mental State Exam $\geq$ 28, Rey Auditory Verbal Learning Test delayed recall, Trail Making Test-B, and Controlled Oral Word Association Test. Exclusion criteria were as for PD, in addition to relevant subjective cognitive memory symptoms, PD and AD.

Written informed consent was obtained from all participants. The study was approved by the South-Eastern Norway ethical committee for medical research 24 February 2011, last approved change 12 May 2014, approval number 2011/99.

Cerebrospinal fluid analysis

Thirty-one PD and 23 NC had lumbar puncture. Concentrations of A β 42, total tau, and phosphorylated tau were analyzed with commercially available enzyme-linked immunosorbent assay kits (Fujirebio Europe, previously Innogenetics, Gent, Belgium). In PD patients, A β 38, 40, and t- α -syn levels were determined by electrochemiluminescence using anti-A β 6E10 detection antibody (Meso Scale Diagnostics, Rockville, MD, USA). As red blood cells contain α -synuclein antigenicity, a cutoff value of 350 ng/ml for hemoglobin was determined, and samples with higher hemoglobin concentrations were excluded from the further analysis as previously recommended,⁴⁶ reducing the number of cases to 24 PD for this sub-analysis.

MRI acquisition, segmentations, and analyses

All participants had Philips Achieva 3-T MRI scanning (Philips Medical Systems, Best, The Netherlands). A single three-dimensional turbo field echo sequence was acquired for morphometric analysis with the following sequence parameters: repetition time/echo time/inversion time/flip angle = 4.5 ms/2.2 ms/853 ms/8°, matrix = 256 $\times$ 213, 170 slices, thickness 1.2 mm, in-plane resolution of 1 $\times$ 1.2 mm. Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.3.0 (<http://surfer.nmr.mgh.harvard.edu/>) as previously described.¹² This includes segmentation of the subcortical white matter and deep gray matter volumetric structures,⁴⁷ and parcellation of the cortical surface according to a previously published parcellation scheme.⁴⁸ This labels cortical sulci and gyri, and thickness values are calculated in the regions of interest (ROI). The thickness values of the ERC and PRC were calculated using methods based on ultrahigh resolution *ex vivo* applied to *in vivo* MRI, as implemented in FreeSurfer.^{49,50} Further, an automated procedure for segmentation of the subfields of the hippocampus, as implemented in FreeSurfer, was employed.⁴¹ From the hippocampal segmentation, we chose to analyze volume of the following subregions; subiculum, presubiculum, cornu ammonis sector 1 (CA1) separately, sector 2 and 3 combined (CA2–3), sector 4 combined with the dentate gyrus (CA4-DG), and the hippocampal tail, the posterior part of hippocampus that cannot be segmented into the different subfields.⁴¹ In addition, we analyzed the 34 cortical thickness regions of interests⁴⁸ to differentiate this to hippocampal changes.

Statistical analysis

Statistical analyses were performed by using IBM SPSS version 20 (Armonk, NY, USA). Continuous variables of demographical and clinical data were compared by Mann–Whitney *U*-tests, and for categorical variables (gender) χ^2 -test was used. The sum of the left and right side MRI subfield volume variables were calculated and were normalized to ICV by dividing the

volume variables by ICV. The average of the left and right side PRC, ERC, and 34 cortical thickness variables, and the normalized subfield volume variables were used as dependent variables in multiple linear regression analyses with group (PD or NC), gender, and age as independent variables. The multiple comparisons were corrected for by Bonferroni. They were further assessed as dependent variables in multiple regression analyses with CSF biomarkers as predictors adjusting for age and gender (independent variables) in the PD group. Finally, we performed multiple regression analyses with neuropsychological tests as dependent variables and MRI variables as predictors adjusting for age, gender, and education. CSF biomarkers, unified PD rating scale part III, and levodopa equivalent dose that proved to be significant predictors of a neuropsychological test were included in the multiple regression analysis together with the MRI variables. P -values ≤ 0.05 were considered significant.

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CONTRIBUTIONS

DA and TF contributed to the concept and design of the study. ALS and EA collected the data, and ALS and KKJ analyzed the data. AB was responsible for set-up and quality control of the MRI protocol. LK and PS were responsible for processing of MRI data. EH was responsible for the implementation of neuropsychological tests. All authors contributed to the interpretation of data. ALS and KKJ drafted the article, and all authors revised it critically. All authors have approved the final article.

COMPETING INTERESTS

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Impaired synaptic function is linked to cognition in Parkinson's disease

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Abstract

Objective: Cognitive impairment is frequent in Parkinson's disease, but the underlying mechanisms are insufficiently understood. Because cortical metabolism is reduced in Parkinson's disease and closely associated with cognitive impairment, and CSF amyloid- β species are reduced and correlate with neuropsychological performance in Parkinson's disease, and amyloid- β release to interstitial fluid may be related to synaptic activity; we hypothesize that synapse dysfunction links cortical hypometabolism, reduced CSF amyloid- β , and presynaptic deposits of α -synuclein. We expect a correlation between hypometabolism, CSF amyloid- β , and the synapse related-markers CSF neurogranin and α -synuclein. **Methods:** Thirty patients with mild-to-moderate Parkinson's disease and 26 healthy controls underwent a clinical assessment, lumbar puncture, MRI, ¹⁸F-fludeoxyglucose-PET, and a neuropsychological test battery (repeated for the patients after 2 years). **Results:** All subjects had CSF amyloid- β 1-42 within normal range. In Parkinson's disease, we found strong significant correlations between cortical glucose metabolism, CSF A β , α -synuclein, and neurogranin. All PET CSF biomarker-based cortical clusters correlated strongly with cognitive parameters. CSF neurogranin levels were significantly lower in mild-to-moderate Parkinson's disease compared to controls, correlated with amyloid- β and α -synuclein, and with motor stage. There was little change in cognition after 2 years, but the cognitive tests that were significantly different, were also significantly associated with cortical metabolism. No such correlations were found in the control group. **Interpretation:** CSF A β , α -synuclein, and neurogranin concentrations are related to cortical metabolism and cognitive decline. Synaptic dysfunction due to A β and α -synuclein dysmetabolism may be central in the evolution of cognitive impairment in Parkinson's disease.

Introduction

Cognitive impairment is an important nonmotor symptom of Parkinson's disease (PD).¹ Mean duration from clinical disease onset to dementia is 10 years, but cognitive impairment has been demonstrated already at the time of diagnosis.²

Several factors argue for early involvement of synaptic pathology in PD. Many of the proteins relevant in PD pathophysiology are involved in synaptic physiology (reviewed in³). α -synuclein is considered the core pathological substrate of PD, and in addition to accumulation in Lewy bodies, a major deposition site of CNS oligomeric α -synuclein is the presynapse, accompanied by transsynaptic dendritic spine loss.^{4,5} The presynaptic terminal is the main site of function for both amyloid precursor protein (APP) and α -synuclein. α -synuclein is linked to synaptic-vesicle complex formation and APP to synaptic-vesicle docking and priming. Neurogranin, a substrate of protein kinase C, is abundantly expressed, and located preferentially at the postsynaptic dendritic spine plasma membrane.⁶

Intracellular metabolism of these proteins is performed by overlapping sets of enzymes and subcellular compartments.^{7–10} Presynaptic activity and endocytosis influence release of amyloid- β (A β) species to the interstitial fluid.¹¹ APP is initially cleaved at the presynaptic terminal to several biologically active metabolites, including A β 1–38 (A β 38), A β 1–40 (A β 40), and A β 1–42 (A β 42), the latter particularly prone to brain parenchymal deposition.^{12,13}

Most studies of CSF A β species in PD have reported slight reductions.^{14,15} We and others have found a correlation between CSF A β 42 levels and memory performance in PD,^{15,16} also in subjects with normal CSF A β 42 levels (thus without evidence for amyloid plaques).^{15,17} These changes relate to hippocampus subfield volumes¹⁸; experimental studies show detrimental preplaque A β effects on synaptic function,¹⁹ and human A β turnover decreases from approximately 40 years of age,²⁰ suggesting preplaque effects of A β on cognition and synaptic physiology.

MRI studies show that slight gray matter atrophy is involved in PD cognitive decline.²¹ Studies combining neuroimaging and CSF in PD are scarce, but CSF A β correlate with reduced gray matter volume and associated neuropsychological function.^{22,23} FDG-PET studies have identified a widespread, specific pattern of reduced glucose metabolism in PD,²⁴ most prominent in the frontoparietal and occipital cortices, even in the early stages of the disease.²⁵ Cerebral glucose metabolism reflects cortical activation and synaptic density.²⁶ Several studies associate this hypometabolism with cognitive impairment, suggesting that hypometabolism in PD primarily reflects cognitive deterioration.^{27,28} Though a very recent

postmortem study suggests that loss of synaptic markers predicts cognitive decline in Lewy body diseases,²⁹ to the best of our knowledge, no direct links have been established between reduced glucose metabolism in PD and independent synaptic markers.

Because (1) cortical glucose metabolism is reduced and correlates with cognitive impairment in PD, and (2) CSF A β species are decreased and correlate with neuropsychological performance, and amyloid- β release to interstitial fluid may be related to synaptic activity; we hypothesize that synapse dysfunction is a common link between cortical hypometabolism, CSF amyloid- β concentrations, and presynaptic deposits of α -synuclein. We thus expect to find a correlation between levels of CSF A β species and relative glucose metabolism, and also to possible markers of synaptic dysfunction or loss, such as CSF α -synuclein and neurogranin.

Materials and Methods

Subjects

This study is based on an ongoing longitudinal follow-up of patients in a university hospital-based neurological clinic. Thirty nondemented patients with mild-to-moderate stage PD were recruited between 2011 and 2014 and examined by a neurologist with extensive experience in movement disorders, as previously described.¹⁵

The patient group and inclusion criteria in this study is the same (aside from one subject excluded due to missing FDG-PET) as the one described in ref 15. In short, inclusion criteria were diagnosis of PD according to the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria,³⁰ Hoehn and Yahr stage <3, and disease duration <6 years. All PD subjects had a pathological dopamine transporter single photon emission computed tomography (DaT-SPECT). Exclusion criteria were dementia (see below for procedure) and other somatic or psychiatric diseases that might contribute to cognitive impairment. The Unified Parkinson's Disease Rating Scale (UPDRS), part III motor examination and Hoehn & Yahr staging were performed. Patients had their medication as usual when assessed. Total daily levodopa equivalent dose was calculated. All PD subjects underwent cognitive assessment employing a well-validated neuropsychological test battery thoroughly described previously.^{15,31} The battery covers memory: *Delayed verbal recall* (Rey Auditory Verbal Learning Test (RAVLT)),³² and *Delayed visual reproduction* (Rey Complex Figure Test (RCFT)), (Meyers & Meyers, 1995) and aspects of executive functions: *Psychomotor speed* (Trail Making Test-A (TMT-A)), *Divided attention* (Trail Making Test-B (TMT-B)) (Reitan & Wolfson 1985), *Word fluency* (Controlled Oral Word

Association Test (COWAT)) (Benton & Hamsher 1989), and *Response inhibition* (Stroop Color-Word, D-KEFS) (Delis et al., 2001). All examinations were conducted within a 3-month period, and repeated after 2 years for the PD subjects.

In addition to PD patients, a healthy control group was included. Healthy controls were recruited from spouses of included patients (three subjects), and the remaining controls were recruited before lumbar puncture in conjunction with orthopedic surgery. Twenty-six subjects assented and successfully underwent MRI and FDG-PET. Exclusion criteria were as for PD, in addition to relevant subjective cognitive memory symptoms.

All subjects gave their written consent, and the Regional Committee for Medical and Health Research Ethics South-East evaluated (based on the Norwegian Health and Research Act and the Helsinki Declaration of 1964; revised 2013) and approved the study. All further study conduct was based on and in line with these guidelines.

CSF analysis

CSF samples were collected from all participants by lumbar puncture, performed between 9 and 12 AM, and consecutively after inclusion. Following lumbar puncture and standardized handling, CSF A β 42 > 700 ng/L were considered normal.

CSF A β 38, A β 40, and total α -synuclein were analyzed with electrochemiluminescence immunoassays (Meso Scale Discovery, US) in all PD patients. Due to possible effects of hemolysis, total α -synuclein were only included if CSF hemoglobin was <350 ng/mL. α -synuclein values for five patients were thus excluded.

CSF neurogranin concentration was measured using a previously described in-house ELISA.⁶ Briefly, an assay based on the monoclonal antibody Ng7 (epitope including amino acids 52–65 on neurogranin) was used for capture, a polyclonal neurogranin anti-rabbit antibody (ab23570; Upstate Biotechnology, Lake Placid, NY, USA) for detection, and full-length neurogranin protein as calibrator. We were able to measure neurogranin concentrations in all included PD patients. For 19 of the 26 included controls there was sufficient CSF available to assess neurogranin concentrations, and we added an additional 11 controls for which there was no available PET data. (The neurogranin healthy control material, $N = 30$, mean age 61.5 [range: 40–78; SD: 9.34], 18 females.)

FDG-PET and MRI imaging

FDG-PET/CT imaging was performed with a Siemens Biograph 64 PET/CT scanner for the PD group, and a Siemens Biograph 16 PET/CT scanner for the healthy

control group. All subjects fasted at least 4 h before image acquisition, and had an intravenous bolus of 218 MBq (± 23 MBq) injected, 45 min prior to scanning. A low-dose CT scan for attenuation correction was acquired. Patients were scanned for 15 min in three-dimensional mode using one bed position (axial range = 16 cm). Acquired data were corrected for random events, dead time, attenuation (CT-derived μ -map), scatter (model based), and decay. PET volumes were reconstructed using an iterative algorithm (OSEM 2D, four iterations, eight subsets), and a postreconstruction three-dimensional Gaussian filter of width 3.5 mm full-width at half maximum was applied. The axial image format was 256×256 (pixel size: 2.67 mm $\times$ 2.67 mm), with a slice thickness of 2.00 mm. Blood glucose at FDG injection time was measured routinely, and the patient was excluded if blood glucose exceeded 8.0 mmol/L.

For both PD and control subjects, MRI scans were acquired on a Philips Achieva 3 Tesla system. A single three-dimensional turbo field echo (TFE) sequence was acquired for morphometrics. Cortical reconstruction and volumetric segmentation was performed with FreeSurfer.^{33,34} The exact sequence parameters and reconstruction methods are described elsewhere.³⁵

Whole head PET volumes were coregistered with the anatomical volume using a rigid body spatial registration as implemented in the Spatial Parametrical Mapping (SPM 8, Wellcome Trust Centre for Neuroimaging, UCL, UK) co-registration tool. PET volumes were resliced into 1 mm MRI. For each subject, a voxel-based intensity normalization to the mean uptake in the cerebellar white matter was performed. (Prior to intensity normalization, the cerebellar white matter mask was eroded to avoid influence due to partial volume effects, inaccurate segmentation, or coregistration). Intensity normalized volumes were used for all further analyses. Because there is both global cerebral and cerebellar cortical hypometabolism in PD, neither global cortical mean nor cerebellar cortical normalization were suitable. We therefore chose to normalize the FDG-PET uptakes against mean cerebellar white matter.²⁵

Statistical analysis

Statistical maps were generated using the general linear model as implemented in FreeSurfer. Auxiliary statistical analyses were performed using IBM SPSS version 22 (Chicago, IL). Statistical maps of correlations (while correcting for effects of age and gender) between normalized FDG-PET uptake (or cortical thickness) and each CSF biomarker concentration were created. This operation was performed separately in the PD and control groups. Also, similar statistical maps of correlations between cortical

thickness and CSF biomarker concentrations were created. These statistical operations on global data and surface maps were generated by computing a general linear model of the effects of CSF biomarker concentrations on FDG uptake (or cortical thickness) at each vertex, while controlling for effects of age and gender. A cluster-wise correction for multiple comparisons was employed, based on ref 36. A cluster-forming threshold of $P < 0.01$ was chosen, and only clusters with a cluster-wise $P < 0.01$ (Bonferroni corrected for examining both hemispheres) were kept.

The coefficient of determination, R^2 was determined for the relation between CSF biomarkers (separately) and FDG uptake or cortical thickness (in pertinent areas as determined above). The effects of age and sex were regressed out of mean FDG uptake or cortical thickness, and the resulting residual was entered in a simple regression model as dependent variables, with the CSF biomarker concentration as explanatory variable.

To assess the relationship with cognitive function, mean FDG uptake (corrected for age and sex) in the pertinent areas as determined above for each CSF biomarker was also correlated (Spearman) with T-scores of neuropsychologic tests.

According to examination of histograms and as assessed by the Kolmogorov–Smirnov test of normality, CSF neurogranin concentrations were not normally distributed in neither the PD nor the control group. We therefore assessed group differences using Mann–Whitney U . There were no significant correlations (Spearman's rho) between CSF neurogranin concentrations and age or sex (neither within the PD group nor within the control group). We did therefore not correct for age or sex when comparing the groups. One outlier in the PD group with values 10–20 times the mean in the group, was removed, but included in additional analyses for reference.

Within the PD group, the distribution of CSF neurogranin, A β 38, 40, and 42 concentrations as well as UPDRS scores were borderline normally distributed according to examination of histograms and as assessed by the Kolmogorov–Smirnov test of normality. We therefore chose to assess the correlation between these variables with Spearman correlations. (The estimations of these correlations were repeated with parametric Pearson correlations with very similar results, data not shown.)

Two-year follow-up cognitive data were available for the PD subjects. To assess the association between cognition, time, and FDG-PET clusters as determined above, a mixed models approach was used: each of the examined neuropsychological variables were entered as dependent variables accounting for repeated measures (in separate models) with age, the FDG-PET cluster corresponding to CSF A β 42, and also an interaction between time and the

FDG-PET cluster (to model the association between FDG-PET and change over time) as independent variables. A priori, the FDG-PET cluster corresponding to CSF A β 42 was chosen for this analysis because it is one of the larger, representative clusters and the well-documented role of A β 42 in synaptic pathology.

Results

Demographical, CSF, and neuropsychological characteristics of the groups

There were no significant differences in age, gender composition, education, or MMSE between the PD and the healthy control group. Neuropsychological function was in the normal range for both groups, except slightly below normal on a measure of divided attention in the PD group. Group composition and demographics are outlined in Table 1 and neuropsychological characteristics in Table 2.

Relationship between the CSF biomarkers

CSF neurogranin concentration was significantly lower in the PD group as compared to the control group (Mann–Whitney $U = 211$, $P = 0.001$). (Not excluding the outlier, the difference was still significant (Mann–Whitney $U = 150$, $P = 0.006$).) (Fig. 1).

There were no significant correlations between CSF neurogranin concentration and age or sex in either of the diagnostic groups (in the PD group, respectively, $\rho = 0.192$; $P = 0.319$, $\rho = -0.260$; $P = 0.173$, in the control group, respectively, $\rho = -0.224$; $P = 0.235$, $\rho = 0.283$, $P = 0.130$). There was a significant correlation between α -synuclein and age ($\rho = 0.533$, $P = 0.006$) in the PD group.

CSF neurogranin concentrations were significantly correlated with the concentrations of CSF A β 38 ($\rho = 0.694$; $P < 0.001$), A β 40 ($\rho = 0.653$; $P < .001$), A β 42 ($\rho = 0.434$; $P = 0.019$), and α -synuclein ($\rho = 0.759$; $P \leq 0.001$). CSF neurogranin concentrations were significantly inversely correlated with UPDRS score ($\rho = -0.369$; $P = 0.039$), as was α -synuclein ($\rho = -0.483$, $P = 0.020$). There were no significant correlations between UPDRS scores and concentrations of CSF A β 38, 40, or 42.

FDG-PET and A β in patients with PD

There was a significant correlation between CSF A β 42 concentrations and FDG-PET uptake in PD; lower CSF A β 42 concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 2) shows decreased cortical metabolism in mainly parietal, frontal, and occipital areas. For both hemispheres, there was one frontal and one

Table 1. Overview of demographics and group characteristics.

	Parkinson's disease	Healthy controls
N subjects	30	26
Age	65.0 (47–74) years	65.0 (47–81) ¹
Females/males	11/19	13/13 ²
Education	13.0 (8–19) years	12.6 (8–20) ³
MMSE	28.6 (22–30)	28.7 (26–30) ⁴
Geriatric depression scale score	1.3 (0–6)	1.4 (0–6) ⁵
Duration from motor symptom onset	2.6 (1–6) years	n.a.
UPDRS part III motor score	15.4 (6–31)	n.a.
Hoehn and Yahr stage	1.55 (1–2.5)	n.a.
Total daily levodopa equivalent dose	390.0 (0–924)	n.a.
CSF A β 38; ng/L	1877 (819–4551)	n.a.
CSF A β 40; ng/L	5125 (2212–10512)	n.a.
CSF A β 42; ng/L	943 (707–1280)	987 (634–1290) ⁶
CSF total α -synuclein; ng/L	278 (160–476) ⁷	n.a.
CSF neurogranin; ng/L	251 (125–556) ⁸	397 (119–988) ^{9,10}

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from Parkinson's disease.

A β , amyloid- β ; A β 38, amyloid- β 1–38; A β 40, amyloid- β 1–40; A β 42, amyloid- β 1–42; MMSE, Mini-Mental State Examination; n.a., not available; UPDRS, Unified Parkinson's Disease Rating Scale.

¹Age: Parkinson's disease versus Controls: *T*-test *P* = 0.989.

²Gender: Between-group gender composition differences; Pearson chi-square, *P* = 0.315.

³Education: Parkinson's disease versus Controls: Mann–Whitney *U* = 349, *P* = 0.498.

⁴MMSE: Parkinson's disease versus Controls: Mann–Whitney *U* = 380, *P* = 0.864.

⁵Geriatric depression scale: Parkinson's disease versus Controls: Mann–Whitney *U* = 357.5, *P* = 0.757.

⁶A β 42: Parkinson's disease versus Controls: Mann–Whitney *U* = 329, *P* = 0.312.

⁷*N* = 25. Missing for five subjects with Parkinson's disease.

⁸Neurogranin: One outlier in the Parkinson's disease group excluded, see methods section.

⁹*N* = 30 for Controls, see methods section.

¹⁰Parkinson's disease versus Controls: Mann–Whitney *U* = 211, *P* = 0.001.

posterior (comprising parietal, occipital, and some temporal vertices) cluster. There were no correlations between CSF A β 42 concentrations and cortical thickness in PD.

In addition, there was a positive correlation between CSF A β 38 concentrations and FDG-PET uptake in PD. The statistical map (Fig. 3) shows the widespread clusters, most notably posterior lateral areas in which cortical metabolism correlated with CSF A β 38 concentrations.

There were also slight correlations between CSF A β 40 concentrations and FDG-PET uptake in PD. The

Table 2. Neuropsychological characteristics of the groups.

Neuropsychological test battery	Parkinson's disease		Healthy controls
	Baseline	Follow-up	
N subjects (missing neuropsychological data)	28 (2)	29 (1)	26 (0)
RAVLT delayed verbal recall of 15 words after 30 min, T-score	50.4 (31–75)	53.0 (25–71)	47.3 (32–67) ¹
RCFT delayed visual reproduction after 30 min, T-score	46.5 (20–75)	60.1 (22–80)	n.a.
TMT–A psychomotor speed, T-score	41.0 (26–62)	47.5 (32–62)	n.a.
TMT–B divided attention, T-score	39.9 (10–59)	47.0 (27–66)	50.2 (28–65) ²
SCW response inhibition, T-score	50.7 (27–67)	50.7 (20–70)	49.0 (20–63) ³
COWAT verbal fluency, T-score	48.1 (33–69)	52.0 (32–73)	48.7 (25–69) ⁴

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from Parkinson's disease at baseline.

A β , amyloid- β ; COWAT, Controlled Oral Word Association Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; SCW, Stroop Color-Word; TMT–A, Trail Making Test–A; TMT–B, Trail Making Test–B.

¹RAVLT delayed verbal recall: Parkinson's disease versus Controls: Mann–Whitney *U* = 297, *P* = 0.24.

²TMT–B divided attention (available for 25 controls): Parkinson's disease versus Controls: Mann–Whitney *U* = 207.5, *P* = 0.011.

³SCW response inhibition: Parkinson's disease versus Controls: Mann–Whitney *U* = 345.5, *P* = 0.936.

⁴COWAT verbal fluency (available for 25 controls): Parkinson's disease versus Controls: Mann–Whitney *U* = 331.5, *P* = 0.728.

statistical map (Fig. 4) shows small posterior lateral clusters in both hemispheres in which cortical metabolism correlated with CSF A β 40 concentrations.

FDG-PET and A β in healthy controls

There were no correlations between CSF A β 42 concentrations and cortical FDG-PET uptake in the control group. (Not corrected for multiple comparisons, still no voxels reached the *P* < 0.01 threshold). Further, there were no correlations between CSF A β 42 concentrations and cortical thickness in the control group. (Not corrected for multiple comparisons, with the threshold set at *P* < 0.01 some small, distributed areas were detected.)

FDG-PET and α -synuclein in patients with PD

There was a significant correlation between CSF total α -synuclein concentrations and FDG-PET uptake in PD;

Figure 1. Median CSF neurogranin concentrations. The median concentration of CSF neurogranin in patients with mild-to-moderate Parkinson's disease and healthy controls, error bars represent 95% confidence intervals. (Mean CSF neurogranin in the Parkinson's disease subjects was 251 (range: 125–556), and in healthy controls, 397 (range: 119–988).)

lower CSF total α -synuclein concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 5) shows decreased cortical metabolism in several cortical clusters. Cluster-wise $P = 0.0002$ for the more frontal left clusters and the lateral right clusters, and $P = 0.021$ for the remaining left, and $P = 0.028$ for the remaining right cluster.

There were no correlations between CSF total α -synuclein concentrations and cortical thickness in PD.

FDG-PET and neurogranin in patients with PD

There was a significant correlation between CSF neurogranin concentrations and FDG-PET uptake in PD; lower CSF neurogranin concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 6) shows decreased cortical metabolism in several cortical clusters. Cluster-wise $P = 0.008$ for the lateral left and $P = 0.002$ for the medial left cluster, whereas $P = 0.0002$ for the single, larger right cluster.

There was a significant correlation between CSF neurogranin concentrations and cortical thickness in PD. (Not shown). There were five small clusters, cluster-wise $P = 0.001$ for a frontal left and $P = 0.002$ for a parietal left cluster, whereas $P = 0.042$ for a parietal right, and $P = 0.021$ for a frontal, medial right cluster. Also, there was a right inferior temporal cluster, $P = 0.002$. The clusters comprised 3% of the left and 3% of the right hemisphere. R^2 was 0.655 in the left and 0.630 in the right combined hemisphere clusters.

Relationship between FDG-PET clusters and cognition in patients with Parkinson's disease

For the cortical areas in which glucose metabolism was correlated with either CSF $A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, total α -synuclein, or neurogranin, there were strong correlations between mean FDG uptake and most tests in the neuropsychological test battery, including measures of executive function (word fluency, response inhibition) and verbal and visual recall (Table 3). P -values for COWAT verbal fluency, SCW response inhibition, RAVLT delayed verbal recall, and RCFT delayed visual reproduction correlations with FDG-PET CSF-based clusters were all <0.01 . Correlations to TMT-B divided attention were not significant.

Relationship between FDG-PET clusters, cognition, and cognitive decline in patients with Parkinson's disease

For the cortical areas in which glucose metabolism was correlated with CSF $A\beta_{42}$, there were strong correlations ($P \leq 0.002$) between mean FDG uptake and all tests in the neuropsychological test battery at baseline (as assessed by the mixed model approach described above). Only TMT-A ($F = 5.684$, $P = 0.27$) and COWAT verbal fluency ($F = 4.431$, $P = 0.48$) were significantly changed from baseline to follow-up, and for these tests, FDG-PET cluster uptake was associated with change. (The interaction between repeated neuropsychological testing and cluster FDG-PET uptake is a measure of this. $F = 5.035$, $P = 0.36$ for TMT-A and $F = 5.041$, $P = 0.36$ for COWAT verbal fluency). TMT-A performance and verbal fluency improved, and for both higher FDG-PET uptake was associated with better performance at follow-up.

Discussion

Combining clinical, imaging, and CSF-proteomic techniques, this study links cortical glucose metabolism to $A\beta$ metabolism, CSF α -synuclein and neurogranin and cognitive impairment in PD. To the best of our knowledge, this is the first study to explore regional correlations between FDG-PET, $A\beta$ species, and cognition, and also relate these measures to an independent synaptic marker. The study results support our initial hypothesis, that is, that synaptic dysfunction is related to both reduced glucose metabolism and reduced levels of $A\beta$ species, putatively related to presynaptic deposition of α -synuclein. The highly significant associations to a wide range of cognitive functions, including memory and aspects of executive functions, demonstrate the clinical relevance of our

Figure 2. Correlations between FDG-PET and CSF A β 42 in Parkinson's disease. The statistical map shows two clusters in each hemisphere, one frontal (yellow) and one posterior (turquoise) cluster, in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF A β 42 concentrations. Mean CSF A β 42 was 943 ng/L (range 707–1280). Cluster-wise $P = 0.0001$ for all clusters. The clusters comprised 36% of the total surface area (27% of the left and 45% of the right hemisphere surface area). R^2 was 0.389 in the left and 0.433 in the right combined hemisphere clusters (excluding the effects of age and gender).

findings. Although there was little change in cognitive performance from baseline to follow-up in the PD on group level, performance changes were significantly associated with FDG-PET.

We describe correlations between CSF A β 38 and A β 42 concentrations and FDG-PET in parietal association, occipital and lateral frontal areas in PD even though levels of A β 42 were within normal range for all included PD subjects. Similar associations were not found in healthy controls. Furthermore, within these same areas there were strong FDG-PET correlations to CSF neurogranin concentrations. CSF neurogranin was also strongly correlated with concentrations of the A β species, and were significantly reduced compared to healthy controls at a group level. The broad cortical areas involved are mirrored by broad and highly significant cognitive associations.

As described in the introduction, the reduced FDG-PET signal, the lowered CSF A β species and the reduced

levels of α -synuclein and neurogranin may all be linked to reduced synaptic activity. In AD, increased CSF neurogranin concentrations probably reflects synaptic degeneration with loss of integrity of postsynaptic spines, that results in increased CSF levels, and this is hypothesized specifically for Alzheimer's disease.^{37,38} In PD, cortical presynaptic pathology has been demonstrated in the form of accumulation of α -synuclein oligomers, accompanied by loss of dendritic spines but other postsynaptic injury was not described.⁵ This is consistent with our present findings of a reduced CSF neurogranin in the present PD population as compared to healthy controls. Thus, though synapses are a focus of both the Alzheimer and PD processes, the different mechanisms of attack may explain the marked differences in neurogranin levels observed in these diseases.³⁸ The strong association observed here between CSF α -synuclein and neurogranin is in line with such a mechanism, but other factors such as altered degradation or transport mechanisms may also be implicated. Late in

Figure 3. Correlations between FDG-PET and CSF A β 38 in Parkinson's disease. The statistical map shows the three clusters in the left hemisphere and the two clusters in the right hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is positively correlated with CSF A β 38 concentrations. For the left hemisphere, cluster-wise $P = 0.0001$ for the two mainly lateral clusters, whereas for the mainly medial (precuneal; green) cluster, cluster-wise $P = 0.0074$. For the right hemisphere, cluster-wise $P = 0.0001$ for the larger, posterior cluster, whereas cluster-wise $P = 0.00170$ for the frontal. The clusters comprised 36% of the total surface area (38% of the left hemisphere surface area, and 33% of the right). R^2 was 0.319 in the left and 0.304 in the right combined hemisphere clusters (excluding the effects of age and gender). Mean CSF A β 38 was 1877 ng/L (range: 819–4551).

the disease-course PD cases suffer from more extensive multifactorial neurodegeneration, including amyloid pathology. The present cohort consisted of early cases (Table 1, mean disease duration 2.6 years), whereas mean disease duration in the PD cohort described in ref. 38 was 8 years. If active amyloid-associated neurodegeneration causes the rise of CSF neurogranin in Alzheimer's disease, this may possibly explain why the latter cohort also had higher values than the present cohort. Thus, the present findings suggest that several disease markers and clinical parameters like UPDRS and cognitive function are linked to synaptic dysfunction in PD, and putatively demonstrate a clinical relevance of CSF neurogranin in PD via its correlation with the UPDRS motor scale.

Though reduced FDG-PET uptake points to cortical involvement and the described cortical presynaptic accumulation of α -synuclein points to a cortical focus, we

cannot exclude subcortical pathology as contributing factors. The nucleus basalis of Meynert (in the basal forebrain) has widespread projecting cholinergic neurons to the entire neocortex, and is known to degenerate in PD (as well as Alzheimer's disease), leading to cortical dysfunction and predicting cognitive decline.^{39–41} Corticopetal dopaminergic pathways mainly project to the frontal cortex. In PD, reduced dopaminergic and cholinergic transmission has been demonstrated in a pattern coinciding well with the frontal and posterior clusters demonstrated in the present work.⁴² As such, the present findings in the PD group could, hypothetically, be construed as an effect of reduced dopaminergic (the frontal cluster) and reduced cholinergic transmission (the posterior cluster) in accordance with the dual syndrome hypothesis. However, here we describe very strong links between cognition, cortical glucose metabolism, and CSF

Figure 4. Correlations between FDG-PET and CSF A β 40 in Parkinson's disease. The statistical map shows one cluster in the left hemisphere and two clusters in the right hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is positively correlated with CSF A β 40 concentrations. For the left hemisphere cluster and the right hemisphere occipital cluster, cluster-wise $P = 0.0001$. For the right hemisphere parietal cluster (shown in blue), cluster-wise $P = 0.0061$. The clusters comprised 6% of the total surface area (8% of the left hemisphere surface area, and 5% of the right). R^2 was 0.277 in the left and 0.250 in the right combined hemisphere clusters (excluding the effects of age and gender). Mean CSF A β 40 was 5125 ng/L (range: 2212–10512).

A β species, both potentially linked to a cortical etiology. Also, neurobiological underpinnings linking A β and α -synuclein physiology in the presynaptic terminal, evidence for cortical presynaptic buildup of α -synuclein aggregates and postsynaptic spine retraction is in line with our demonstration of links to CSF neurogranin and α -synuclein, and primary cortical etiology. Although the present findings do not primarily address mechanistic links, decades of preplaque A β pathology including oligomerization may well influence presynaptic α -synuclein metabolism, and there is experimental evidence that A β 42 may escalate production of toxic α -synuclein oligomers – directly disrupting SNARE-dependent vesicle function.^{43,44}

Although we find strong links to A β , a reverse causality cannot be excluded, in that synaptic dysfunction could be due to presynaptic α -synuclein aggregates slightly reducing A β in proportion to the degree of α -synuclein-dependent neurodegeneration.^{5,45} Alternatively, defective

presynaptic proteolysis and lysosomal degradation could affect both A β and α -synuclein metabolism.^{7,8,46} In either case, postsynaptic spines would be affected secondarily, likely leading to the observed reduced levels of neurogranin. Combined, pre- and postsynaptic dysfunction would result in loss of clinical and cognitive function as measured by the UPDRS and the cognitive measures. Putatively, these hypotheses could be tested or differentiated by also measuring presynaptic markers, like CSF synaptosomal-associated protein 25 (SNAP25, a component of the trans-SNARE complex)⁴⁷ or synaptotagmin, expected to be changed if vesicle fusion is truly hampered.

There are limitations to the current work that needs to be addressed. The PET scans of the PD and control groups were acquired on two similar, but not identical scanners. However, the scan and image reconstruction parameters, and also the postprocessing procedure were

Figure 5. Correlations between FDG-PET and CSF total α -synuclein in Parkinson's disease. The statistical map shows the clusters in each hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF t- α -syn concentrations. Mean CSF t- α -syn was 278 (range: 160–476). Cluster-wise $P = 0.0002$ for the more frontal left clusters and the lateral right clusters, and $P = 0.021$ for the remaining left, and $P = 0.028$ for the remaining right cluster. The clusters comprised 13% of the total surface area (8% of the left and 19% of the right surface area). R^2 was 0.337 in the left and 0.339 in the right combined hemisphere clusters (excluding the effects of age and gender).

identical between the scanners and all analyses were conducted within, and not between, groups. Care should nevertheless be taken when comparing the results between groups. Also, the sample size in the present work is limited, and our findings need to be reproduced in other populations (including drug naive), and although fluctuations are relatively uncommon at the present disease stage (Hoehn and Yahr stage <3 , disease duration <6 years) the subjects should preferably have been tested at a standardized time in relation to medication. The sample size for the FDG-PET analyzes with α -synuclein and neurogranin were even smaller than for $A\beta$, and one might expect more widespread, significant clusters with a larger sample. Further, although FDG uptake reflects synaptic function, there exists no direct or absolute measure of synaptic function, and this should be taken into account when interpreting the present results.

All PD subjects had normal levels of CSF $A\beta_{42}$, and were thus unlikely to harbor amyloid plaques. However, a

presence of soluble, synaptotoxic $A\beta$ oligomers cannot be excluded and could be an alternative explanation for the present findings related to $A\beta$. Measuring $A\beta$ oligomers in the CSF of patients with PD may be a way to test this alternative explanation.

We were able to investigate the correlations between cortical metabolism and the alternative amyloid species $A\beta_{38}$ and $A\beta_{40}$ (considered less prone to aggregate) in the PD group, and interestingly the correlations between FDG-PET and $A\beta_{38}$ were comparable to those with $A\beta_{42}$ (whereas the correlations with $A\beta_{40}$ were more sparse). As described earlier,¹⁸ CSF $A\beta$ species and α -synuclein concentrations correlate strongly, strengthening the supposition that presynaptic α -synuclein and $A\beta$ metabolism may interact to produce the current findings.

On group level, the PD patients performed as well as presumptively healthy controls on neuropsychological tests except on a measure of divided attention (functions that may be affected both by impaired dopaminergic and

Figure 6. Correlations between FDG-PET and CSF neurogranin in Parkinson's disease. The statistical map shows the clusters in each hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF neurogranin concentrations. Mean CSF neurogranin was 251 (range: 125–556). Cluster-wise $P = 0.008$ for the lateral left and $P = 0.002$ for medial left cluster, whereas $P = 0.0002$ for the single, large right cluster. The clusters comprised 10.5% of the total surface area (4% of the left and 16% of the right surface area). R^2 was 0.330 in the left and 0.346 in the right combined hemisphere clusters (excluding the effects of age and gender).

Table 3. Correlations between cognition and mean FDG-PET in cortical clusters related to CSF biomarkers in Parkinson's disease.

Neuropsychological test battery	FDG-PET CSF biomarker-based cortical clusters				
	$A\beta_{42}$ ¹	$A\beta_{38}$ ¹	$A\beta_{40}$ ¹	α -synuclein ²	Neurogranin ³
RAVLT delayed verbal recall of 15 words after 30 min, T-score	0.566 (0.001)	0.615 (<0.001)	0.651 (<0.001)	0.561 (0.004)	0.616 (<0.001)
RCFT delayed visual reproduction after 30 min, T-score	0.608 (<0.001)	0.655 (<0.001)	0.667 (<0.001)	0.551 (0.005)	0.605 (0.001)
TMT-A divided attention, T-score	0.650 (<0.001)	0.602 (<0.001)	0.607 (<0.001)	0.727 (<0.001)	0.504 (0.005)
TMT-B divided attention, T-score	0.277 (0.145)	0.248 (0.195)	0.283 (0.136)	0.249 (0.252)	0.184 (0.350)
SCW response inhibition, T-score	0.617 (<0.001)	0.613 (<0.001)	0.621 (<0.001)	0.549 (0.005)	0.585 (0.001)
COWAT verbal fluency, T-score	0.499 (0.006)	0.499 (0.006)	0.539 (0.003)	0.653 (0.001)	0.488 (0.008)

Entries are Spearman's rho Correlation Coefficient (P -value). Bold entries are significant.

$A\beta$, amyloid- β ; COWAT, Controlled Oral Word Association Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; SCW, Stroop Color-Word; TMT-B, Trail Making Test-B. ¹⁻³Names refer to the clusters significantly correlated with that CSF biomarker.

¹ $N = 30$ for RAVLT, RCFT, and SCW. $N = 29$ for TMT-B, COWAT, WAIS-III.

² $N = 24$ for RAVLT, RCFT, SCW, and COWAT. $N = 23$ for TMT-B and WAIS-III.

³ $N = 29$ for RAVLT, RCFT, and SCW. $N = 28$ for TMT-B, COWAT, and WAIS-III.

cholinergic networks and frontoparietal hypometabolism⁴⁸). The present results may have been more informative were the included PD subjects more cognitively impaired, or if the time from baseline to follow-up was longer. However, there were strong correlations between cortical glucose metabolism and cognitive function in the PD group. Although there was sparse change in cognition at 2-year follow-up, cortical metabolism was significantly associated with this change. We hypothesize that baseline cortical glucose metabolism will predict further cognitive change and predict time from disease onset to dementia.

Herein, we describe a correlation between cortical glucose metabolism, cognitive functions, cognitive change, CSF A β species, neurogranin, and α -synuclein in PD. The findings were robust even though CSF A β 42 was within normal levels for all included subjects with PD, suggesting that cortical synaptic function is related to cognitive functioning, and is affected in PD. If confirmed, the present results will have important implications for understanding the role of synaptic dysfunction, A β and α -synuclein in the evolution of PD. We further find lower CSF concentrations of neurogranin in these patients with mild-to-moderate PD, but further studies are needed to assess whether this may be of clinical relevance. Additionally, the findings question the previously postulated association of reduced CSF α -synuclein concentration in PD with Lewy body pathology, in that reduced CSF α -synuclein concentration may reflect synaptic degeneration and reduced release.

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Author Contributions

PS drafted the manuscript, contributed to the conception and design of the study, acquisition and analysis of data. ALS contributed in revising a substantial portion of the manuscript and acquisition of data. KKJ contributed in revising a substantial portion of the manuscript and acquisition of data. AB contributed to the conception and design of the study, and acquisition of data. CC contributed to the acquisition and analysis of data. EA, LK, and ISA revised the manuscript and contributed to the acquisition of data. EH contributed to the conception and design of the study, and acquisition of data. HZ, KB,

DA, and TF contributed in the conception and design of the study as well as acquisition of data and revision of the manuscript. All authors approved the final draft.

Conflicts of Interest

Dr. Selnes, Dr. Stav, Dr. Johansen, Dr. Bjørnerud, Dr. Kalheim, Dr. Almdahl, Dr. Hessen, and Dr. Auning have nothing to disclose. Dr. Coello reports personal fees from Imanova Ltd, outside the submitted work. Dr. Zetterberg reports personal fees from Roche Diagnostics, non-financial support from Eli Lilly, personal fees from Pharmasum Therapeutics, outside the submitted work; and HZ is co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Blennow reports personal fees from Fujirebio Europe, personal fees from Roche Diagnostics, personal fees from IBL International, outside the submitted work; and Co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Aarsland reports personal fees from Novartis, personal fees from H Lundbeck, grants from Astra-Zeneca, outside the submitted work. Dr. Fladby reports grants from Norwegian Research Council, grants from EU-JPND, during the conduct of the study; in addition, Dr. Fladby has a patent PCT/EP2009/001210 issued, and a patent PCT/US2011/062233 pending.

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