Structural brain MRI and cognition in newly diagnosed Parkinson’s disease

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Rennesøy, May 2010

Turi Olene Dalaker
Abstract

Background

Parkinson’s disease (PD) is a common progressive neurodegenerative disorder mainly affecting the elderly. Previously regarded a pure motor disease, PD is now considered a multisystem brain disease with various nonmotor aspects, including cognitive dysfunction. Mild cognitive impairment (MCI) is currently explored as a possible pre-dementia stage in patients with PD. Little is known about the pathology underlying MCI in PD. A full characterization of the various aspects of MCI in PD would be of great value in the search for prognostic factors and potential preventive treatments for disabling dementia in patients with PD.

Volumetric brain magnetic resonance imaging (MRI) is increasingly used in research aiming to find the biological basis for various neurodegenerative diseases, atrophy regarded as the end stage of neurodegeneration. In PD, studies have shown brain atrophy in patients presenting with dementia. A few studies reported loss of brain tissue of midbrain and mesocortex in advanced stage patients with PD and MCI, but no previous study has investigated possible brain atrophy in newly diagnosed patients with PD and MCI.

White matter hyperintensities (WMH) are high signal changes in T2-weighted brain MRI scans as a consequence of vascular damage. WMH has been linked to cognitive dysfunction in both normal aging and neurodegenerative disease, e.g. Alzheimer’s disease. Controlling vascular risk factors resulting in WMH might thus protect
against dementia and other forms of cognitive impairments. Earlier works on WMH in PD are conflicting, but a previous study indicated that WMH load is increased in demented patients with advanced PD. The role of WMH in patients with early PD and MCI is unknown.

**Objective**

The primary aim of this thesis was to test the hypothesis that cognitive impairment in newly diagnosed PD is related to atrophy and/or WMH changes in the brain.

**Methods**

Included subjects are all part of the Norwegian ParkWest study. The ParkWest study is a prospective multicentre cohort study of patients with incident PD from western and southern Norway, caregivers and a control group consisting of age and sex matched normal controls. An MRI examination was part of the baseline study evaluation and was conducted in 182 patients with PD and 109 controls.

By widely used and validated quantitative MRI analysing methods, we performed volumetric global and regional brain segmentation and WMH quantification.

WMH load was estimated by a semi-automated local threshold technique. Total volume and regional distribution of WMH in patients with newly diagnosed PD were compared with findings in age-matched controls. The impact of WMH on cognitive
test performance was investigated both in the unselected PD sample and according to MCI classification.

Global brain MRI atrophy measures were whole brain and total gray/white/cerebrospinal fluid volumes calculated using SIENAX software. Regional atrophy was investigated in a subgroup of patients from one centre using voxel-based morphometry (VBM) and the FreeSurfer software. Ventricular volumes were also calculated.

Neuropsychological tests were chosen to minimize the impact of impaired motor skills and assessed memory, visuospatial and attention-executive domains. Non-demented patients with an observed age-and education corrected z-score deviating more than -1.5 standard deviations from the expected z-score (based on the test performance of controls) in at least one cognitive domain, were classified as having MCI.

**Results**

Our investigations of global brain MRI atrophy parameters (whole brain, total gray and white matter volume) did not show any significant difference between newly diagnosed patients with PD and controls and were not significant predictors of cognitive performance. Furthermore, neither total WMH volume nor regional distribution of WMH was significantly different between patients and controls, irrespectively if patients were presenting MCI. Regional gray matter atrophy
analyses, volume of subcortical gray matter structures and ventricular volume measures may indicate enlargement of fourth, third and left inferior lateral ventricles, but no cortical atrophy, as a sign of MCI in early PD.

**Conclusion**

Based on these studies, WMH do not seem to play a significant role as a neurobiological factor in the cognitive dysfunctions of incident patients with PD. Volume studies could indicate a possible role of atrophy in brainstem, midbrain and temporal regions, but this needs to be explored further in future larger studies.
Publications


Abbreviations

3D - Three-dimensional
AD - Alzheimer’s disease
CSF - Cerebrospinal fluid
DAT - Dopamine reuptake transporter
DLB - Dementia with Lewy bodies
FLAIR – Fluid attenuated inversion recovery
FSL - FMRIB’s Software Library
H&Y – Hoehn and Yahr
MCI - Mild cognitive impairment
MADRS - Montgomery Aasberg Depression Rating Scale
MMSE - Mini-Mental State Examination
MNI - Montreal Neurological Institute
MRI - Magnetic resonance imaging
NEX - Number of excitations
NMR - Nuclear magnetic resonance
PD - Parkinson’s disease
PDD - Parkinson’s disease with dementia
PET - Positron emission tomography
RF - Radio frequency
ROI - Region of interest
SIENA - Structural Image Evaluation, using Normalization, of Atrophy
SIENAX – Structural Image Evaluation, using Normalization, of Atrophy (cross-sectional)

SPECT - Single photon emission computed tomography

SPM - Statistical parametric mapping

TE - Echo time

TI - Inversion time

TR - Repition time

UPDRS - Unified Parkinson’s Disease Rating Scale

VBM - Voxel based morphometry

WMH - White matter hyperintensities
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1. Introduction

Parkinson’s disease (PD) is a common neurodegenerative disease mainly affecting the elderly. With industrialization and the socioeconomic progresses in the past century, population structures are changing. As a consequence of increased prevalence of diseases typical of older age, the potential impact of degenerative brain disorders becomes even more prominent in both human and economic terms. A better understanding of such diseases is important for patients, caregivers and physicians involved, but it is also highly relevant for the general society given all the challenges ahead.

Despite recent advances, much is still unknown regarding pathogenesis and progression of cognitive impairments in PD. The work included in this thesis aims to add more pieces to the puzzle by the use of conventional brain magnetic resonance imaging (MRI). The first general section will give a brief overview of PD and basic MRI principles and analyzing methods, followed by a description of previous work on volumetric MRI and cognitive aspects of the disease. The rest of the thesis describes and discuss structural MRI findings and cognitive impairment in patients with newly diagnosed PD, results obtained from MRI data analyses in the Norwegian ParkWest study.
1.1 Parkinson’s disease

1.1.1 From shaking palsy to multisystem brain disease

PD was first described in 1817 by James Parkinson in his publication “An essay on the shaking palsy”. The essay consisted of six case reports with detailed observations of patients with symptoms of “Involuntary tremulous motion” and “A propensity to bend the trunk forwards and to pass from a walking to a running pace”. Half a century later, Charcot and colleagues further distinguished the disease and suggested the term “Parkinson’s disease”. The pathological hallmark of the disease, the Lewy bodies, was first described in 1913, and the biochemical basis of the disease and advances in dopaminergic treatment was investigated in the 1960’s. In the following years, new knowledge has especially evolved in the genetic and molecular basis of PD, but also clinically with respect to the various nonmotor aspects of the disease.

James Parkinson described the shaking palsy with “the senses and intellect being uninjured” and for a long time PD was regarded a pure motor disease and with a distinct clinical syndrome called parkinsonism. Recent works have shown that PD now must be regarded a multisystem brain disease with widespread and heterogeneous effects on the nervous system resulting in various clinical expressions.
Nowadays PD is considered a chronic, progressive and systemic neurological disease with a number of “non-dopaminergic” symptoms in addition to its well established motor characteristics tremor, rigidity, bradykinesi and postural instability. Cognitive impairment, olfactory dysfunction, dysautonomia, sleep disturbances and neuropsychiatric symptoms are all known possible features of established disease. At present many of these nonmotor symptoms are receiving major attention as possible signs of presymptomatic PD, and intensive efforts are warranted to investigate the basis for PD pathology beyond the nigrostriatal dopamine pathway.

1.1.2 Epidemiology

Idiopathic PD is the second most common neurodegenerative disorder after Alzheimer’s disease (AD). PD affects about 1 % of the population over the age of 60, has increasing prevalence with age and about 4 % of people in the highest age groups suffer from PD.

Prevalence studies are reporting different numbers, but in general community based surveys in Europe found the prevalence between 100 and 200 per 100.000 inhabitants. Age-standardized incidence rated range from 8.6 to 19.0 per 100.000. In the Norwegian Park West study, an age-standardized incidence of 12.6 per 100.000 was calculated. Most studies report incident PD more common in men and in rural
The Norwegian Park West study found an overall male to female ratio of 1.58.

Based on published prevalence studies, Dorsey et al. estimated that in Western Europe’s 5 and the world’s 10 most populous nations the number of individuals diagnosed with PD over 50 years of age will double from 4.1-4.6 million in 2005 to 8.7-9.3 million by 2030.

1.1.3 Aetiology and risk factors

In the majority of PD cases, no obvious cause for the disease is found. PD is now regarded a multifactorial disease, resulting from combinations of hereditary, environmental and endogenous risk factors, but clear causative factors and their relationship with possible susceptibility genes are yet to be explored.

A relationship between age and PD is reported, and the disease is rare in people under 50 years of age. Studies have found that advancing age is associated with faster rate of motor impairment, decreased levodopa responsiveness, more severe gait and postural impairment and more severe cognitive dysfunction. Gilberto Levy proposed a model for the relationship between PD and aging consisting of three elements. The first element suggests a superposition of a topographic gradient of
disease related neuronal loss in brainstem and basal forebrain structures and an aging-related temporal gradient. Furthermore, in PD, unlike other neurodegenerative diseases, advancing age, rather than disease duration, is the most important determinant of clinical progression. Finally, there is an interaction between the effects of age and disease on non-dopaminergic structures. A recent pathology study suggested that two age-related factors are important in the pathological progression in PD: age at onset of symptoms and age-related Alzheimer-type pathology.\textsuperscript{14}

1.1.3.1 Non-genetic risk factors

Many epidemiological studies have investigated the role of pesticides in the pathogenesis of PD, and exposure to such toxins has been found to double the risk of developing the disease. Reports on welding and exposure to various heavy metals are more inconclusive.\textsuperscript{8} There is an inverse association between smoking and PD, and coffee consumption seems to decrease the risk of PD.\textsuperscript{8} Dietary factors such as antioxidants and fatty acids are under investigation, but consistent results are missing. The role of inflammation in the pathogenesis of PD is currently not known.\textsuperscript{8}

1.1.3.2 Genetic risk factors

A meta-analysis showed that the relative risk of PD when having a first-degree relative with PD versus no first degree relative with PD was about 3, and concluded that family aggregation of PD is strong and unlikely to be due to chance.\textsuperscript{15} Genetic
insights in the last decades have expanded the view upon PD as, at least in part, a hereditary disease.

Distinct genetic subgroups of the disease have been identified and provide arguments for PD not being a single clinical entity. Still, monogenetic forms constitute less than 10% of patients with PD, and studies focusing on interaction between susceptibility genes and other factors are so far suffering from methodological weaknesses such as lack of statistical power. Our understanding of genetics in PD is further limited by several factors: the penetrance of mutations are often either reduced or unknown, clinical and pathological expressions are variable, and finally, presence of a genetic mutation indicates a risk for developing PD, but says nothing about timing of disease onset.

1.1.3.3 Vascular factors and idiopathic PD

In AD, a role of vascular pathology in the development of cognitive decline is suggested. Risk factors of cerebrovascular lesions (e.g. ischemic and haemorrhagic stroke, white matter pathology, angiopathy) are among others diabetes mellitus, hypertension, smoking and hyperlipidemia. Many of these risk factors are reversible and might thus represent important targets for intervention aiming to prevent and treat highly disabling dementia.
Work regarding the relationship between cerebrovascular pathology and PD has showed conflicting results with some studies reporting increased frequencies of such events in PD\textsuperscript{18} and others claiming the opposite.\textsuperscript{19} A recent large observational study found increased risk of PD after a previous stroke, and the risk of first-time ischemic stroke in patients with newly diagnosed PD was about 1.5 to 2 times higher than in subjects without PD.\textsuperscript{18} This was in contrast to an earlier cross-sectional study indicating that prevalence of stroke in PD was reduced compared to controls.\textsuperscript{19}

With respect to the clinical impact of vascular pathology, a study on late onset PD showed that presence of minor stroke, diabetes mellitus and ischemic heart disease was significantly associated with more disabling disease,\textsuperscript{20} but a longitudinal study found that smoking status was not significantly associated with disease progression in PD.\textsuperscript{21} The cognitive impact of vascular disease in patients with PD is not established. A post-mortem study reported that cognitive impairment was only associated with severe vascular pathology,\textsuperscript{22} and a clinical observation study reported that established cerebrovascular risk factors were not associated with incident dementia in PD.\textsuperscript{23} The extent and impact of potentially treatable and preventable cerebrovascular pathology in PD is thus uncertain. More research is needed to clarify the importance of vascular brain pathology in the aetiology of both motor and nonmotor aspects of idiopathic PD.
The relationship between cerebrovascular disease and PD is complicated by various issues, and there are factors associated with PD potentially increasing or decreasing the risk of cerebrovascular disease: 1) A reduced prevalence of smokers in PD, smoking being a well known risk factor for vascular pathology, might explain lower frequencies of vascular pathology in PD.\textsuperscript{19} 2) Hyperhomocysteinemia is a risk factor for vascular disease, and antiparkinsonian medication (levodopa) is associated with increased blood levels of homocysteine. This could possibly result in more cerebrovascular disease in patients with PD,\textsuperscript{24} although this was not confirmed in a recent study.\textsuperscript{25} 3) Autonomic dysfunction is common in PD and might have effects on the cardiovascular system causing less vascular disease risk factors in PD.\textsuperscript{26}

Vascular parkinsonism is a clinical entity used to describe a condition where parkinsonism is caused by cerebral vascular disease. The diagnosis is considered when history includes repeated strokes and/or (related) stepwise progression of parkinsonism with compatible neuroradiological findings. In the Norwegian ParkWest study, 1.9 % of incident cases were re-diagnosed during follow-up with vascular parkinsonism. So far, there is no accepted precise definition of vascular parkinsonism, and it might be difficult to distinguish vascular parkinsonism from idiopathic PD. Both the vascular pathologies and PD are diseases of older age, and a recent paper argues that when signs and symptoms are typical for idiopathic PD, coincidence of the two conditions should be considered rather than vascular parkinsonism.\textsuperscript{27}
1.1.4 Neuropathology

The neuropathological hallmarks of PD are gliosis and cell loss in nigrostriatal neurons with the presence of Lewy pathology, and such findings are currently the gold standard for a diagnosis of PD. The resulting dopaminergic deficiency explains dysfunction of movement, behaviour, learning and emotions, but can not account for the full clinical picture seen in patients with PD.

Lewy pathology is a term for α-synuclein immunoreactive neural inclusions and consists of various eosinophilic inclusions in neuronal perikarya (Lewy bodies) in addition to inclusions of neuronal cell processes (Lewy neurites). Lewy bodies are found in the brains of 10% of healthy individuals over 60 years of age, and this is sometimes described as incidental Lewy body disease. A recent study suggests that this might represent pre-symptomatic PD, but the role of non-symptomatic Lewy bodies is not yet fully established.

In 2003 Braak and colleagues presented a new classification system for the pathology of PD. Their hypothesis claims that the disease related pathology develops in a coherent sequence. In the initial two stages lesions involve the anterior olfactory nucleus, the dorsal motor nucleus of the IX/X nerves, the raphe nuclei and the reticular formation. In stages 3 and 4 pathology is confined to the brainstem and anteromedial temporal mesocortex. The principal characteristic of this stage is affection of the substantia nigra and initial clinical recognizable disease. Stages 5 and
6 consist of severe involvement of the brain, including neocortical areas. The six stages are illustrated in Figure 1.1.

**Figure 1.1**

Progression of PD related pathology. Lesions initially occur in the dorsal IX/X motor nucleus and the various brain structures gradually become involved as indicated by the white arrows (left). The gradual decrease in shading intensity is intended to represent the topographic expansion of the lesions during the course of the disease. Simplified diagram (right) showing expansion of lesions (left to right: dm to fc) and the growing severity of overall severity (top to bottom: stages 1-6). dm= dorsal IX/X motor nucleus; co = coeruleus-subcoeruleus complex; sn= substantia nigra; mc= anteromedial temporal mesocortex; hc= high order sensory association areas and prefrontal fields; fc= first order sensory association areas, premotor areas, as well as primary sensory and motor fields. Modified reprint from Braak et al.\textsuperscript{32} with permission from Elsevier.

Several papers have argued against a unified disease progression as suggested in the Braak hypothesis.\textsuperscript{33,34} The staging system was recently validated, and 83 % of subjects showed a distribution of Lewy pathology according to the various stages. Notably 55 % of subjects in Braak’s stages 5 and 6 showed no sign of dementia.
despite widespread cortical affection. In a post mortem study, Halliday et al. identified three distinct subgroups of patients with PD, findings that were not consistent with the Braak concept of a topographical distinct and progressive pathogenesis of the disease. Future studies, combining clinical, neuroimaging and, if available, pathological data, are needed for further validation of the Braak hypothesis.

1.1.5 Clinical manifestations

The clinical picture in PD is heterogenous. The following section aims to give a broad overview of the varied clinical manifestations of PD with special focus on findings in the early clinical recognisable stages of the disease. A newly published study found that both motor and nonmotor features in early PD predicted increased mortality risk, in particular postural instability gait difficulty, cognitive impairment and hallucinations.

1.1.5.1 Motor features

The key motor manifestations of PD are typically unilateral in early disease, and become clinically evident when dopamine levels fall below 60 % in the contra lateral striatum. The four cardinal clinical signs of PD are tremor, rigidity, bradykinesi and
postural instability. These features are not specific for PD, but are also seen in parkinsonian disorders like Multiple System Atrophy, Progressive Supranuclear Palsy and vascular parkinsonism. Tremor is the most common motor symptom in PD and is seen in about 70% of newly diagnosed patients. Axial symptoms are more rare in incident disease, but progress over time, and a postural gait instability dominated PD is in general associated with poorer prognostic outcome. It has been speculated in that this clinical subtype is a result of more extensive underlying pathology.

1.1.5.2 Premotor PD

Lately, there is increasing focus on the premotor phase of PD. This phase of the disease is defined by the period, years or even decades, when various nonmotor symptoms arise preceding the classic motor symptoms PD. The nonmotor symptoms that are believed to be present in this very early stage of PD are olfactory dysfunction, dysautonomia (e.g. bladder dysfunction and constipation) and mood and sleep disturbances. Many of the symptoms could be explained by the Braak hypothesis, but as mentioned above the concept of sequential development of Lewy pathology might not be unitary in all cases of sporadic PD.

By recognizing these symptoms and, if possible, be able to diagnose premotor PD, one hopes to identify subjects at risk before symptomatic degeneration of dopaminergic neurons in substantia nigra has occurred. In the future, combined neuroimaging and for instance olfactory testing in relatives of patients with PD might serve as screening strategies to detect very early disease. The cost effectiveness and
clinical use of such screening tools are currently limited by the lack of effective treatments and knowledge of causative PD pathology.\textsuperscript{16}

1.1.5.3 Nonmotor symptoms

Cognitive deficits, olfactory dysfunction, autonomic disturbances and various neuropsychiatric symptoms are all known nonmotor manifestations of PD.\textsuperscript{40} The most common neuropsychiatric symptoms are depression, anxiety, apathy, fatigue and psychosis (mainly visuo-perceptual symptoms).\textsuperscript{41} Previously often not appreciated, they are now integrated parts of our understanding of PD as a multisystem neurodegenerative brain disease. Many of the nonmotor symptoms associated with PD are considered to be caused by changes in non-dopaminergic transmitter systems,\textsuperscript{7} but the pathology underlying the nonmotor symptoms is debated. According to Braak,\textsuperscript{42} it can be explained by presence of Lewy pathology. The nonmotor symptoms often have a major impact on quality of life in patient with PD,\textsuperscript{43} and both clinical and academic efforts are important in understanding, diagnosing and treating these aspects of PD.
1.1.6 Cognitive impairment

In this thesis, special attention will be given the cognitive deficits in PD. Cognitive dysfunctions are common in PD, and there is a wide range of severity from disabling dementia\textsuperscript{44} to less severe cognitive deficits not affecting daily life activities described as mild cognitive dementia (MCI).\textsuperscript{45}

The pathological basis for cognitive dysfunction in patients with PD is still controversial. Hypotheses include both neurotransmitter deficits (dopamine, serotonin, acetylcholine and noradrenalin) and cortical LB deposits.\textsuperscript{46,47} Several studies have also reported that Alzheimer-type changes are common and are likely to contribute to dementia in PD.\textsuperscript{44} Finally, cerebrovascular pathology might also be present in some subjects.\textsuperscript{22}

1.1.6.1 PD with dementia

Dementia is a common feature of PD with estimated prevalence varying between 24 and 31\%.\textsuperscript{48} Patients with PD have a six-fold increased risk of developing dementia compared to non-demented controls.\textsuperscript{49} PD dementia (PDD) has a major impact on quality of life\textsuperscript{50} and mortality.\textsuperscript{51} PDD is an important risk factor for nursing home placement,\textsuperscript{52} and as a consequence of this, PDD has important health economic implications.
PD with and without dementia can be classified as Lewy body disorders characterized by defects in α-synuclein metabolism and deposits of Lewy pathology. Another disease in this group of neurodegenerative disorders is Dementia with Lewy bodies (DLB). DLB is characterised clinically by dementia combined with idiopathic parkinsonism, persistent visual hallucinations, and cognitive fluctuations. DLB probably accounts for about 15-25 % of late-onset dementias, and is thus the second most common subgroup of degenerative dementias after AD. There is a continuing debate on whether PD, PDD and DLB diagnoses represent different disorders or are just different stages in a spectrum of Lewy body disorders. Per definition, dementia occurring before or within one year after parkinsonism is classified as DLB, whereas PDD is diagnosed if dementia occurs later than one year after onset of parkinsonism. Although these two syndromes share many clinical and pathological features, subtle differences also exist. In the Norwegian ParkWest Study, nine subjects with parkinsonism and initially diagnosed with PD were re-diagnosed as having DLB during follow up diagnostic procedures.

PDD typically involves a variety of cognitive domains including impaired attention, memory, visuospatial, constructional and executive functions, and deficits are found even in early disease stages. Neuropsychiatric symptoms like mood disturbances, hallucinations, delusions and apathy also occur in the majority of patients.
The postural instability and gait difficulty motor subtype of PD have been found to be associated with a faster rate of cognitive decline.\textsuperscript{56,57} Other risk factors for developing dementia are higher age, more advanced clinical disease stage and reduced cognitive ability.\textsuperscript{44,49} In the past few years the concept of MCI has received more attention as a possible pre-dementia stage, and many ongoing studies are trying to elaborate this possible very early stage of PDD. If such a pre-dementia stage can be characterized and diagnosed, it will be of great value in the search for prognostic factors and possible preventive treatments. Knowledge of how to prevent or delay PDD would be very useful for patients and caregivers, but also have major public health benefits.

1.1.6.2 MCI

In the past decade, a variety of definitions for MCI in PD have been used, based on various study designs, cognitive test batteries and cut-off levels for test performance.\textsuperscript{58} MCI was originally a term used for description of the transitional stage between normal cognitive functioning and AD,\textsuperscript{59} but MCI is now increasingly used for other types of disorders, e.g. PD. Still, clinical criteria for MCI in PD have not yet been established.\textsuperscript{45}

MCI in PD typically implies reduced visuospatial, executive and attentional functions contributing to impaired working memory, but deficits in memory are also
common. A study found MCI in about 30% of patients with PD with intact global cognition based on Mini-Mental State performance (MMSE). Increasing age and disease severity, anti-anxiety medication use and daytime sleepiness were independent predictors of the cognitive impairment.

Studies on newly diagnosed patients report frequencies of MCI ranging from 19 to 36%. The variety of findings is probably related to the use of the different MCI classifications and neuropsychological test batteries. Furthermore, the recruitment strategies vary among studies, and thus different PD populations have been included.

As mentioned, the presence of MCI in PD seems to identify patients with a high risk of developing dementia. A clinical observation study including patients with advanced PD found that executive dysfunctions predicted PDD 4 years later. A longitudinal 3.5 year follow-up study on incident PD reported that reduced performance on tests with more posterior cortical basis, along with non-tremor dominant motor subtype, were the most important clinical predictors of global cognitive decline. At follow-up, 57% presented cognitive impairment and 10% dementia. A recently published longitudinal study of patients with early PD, found that 48% of patients had developed cognitive impairment and 9% were demented after 3 years of follow-up. Interestingly, in this study, none of the baseline characteristics predicted cognitive changes in newly diagnosed patients.
1.1.7 Treatment of PD.

The treatment of PD involves dopamine replacement therapy with L-dopa, and several dopamine agonists. Monoamine oxidase inhibitors are also in use. The latter has been shown to have a potential neuroprotective effect and might slow disease progression in addition to increased dopamine levels in the brain. Deep brain stimulation with implementation of electrodes can be helpful for selected patients especially in tremor dominant disease.

As for cognitive symptoms, studies indicate that cholinergic drugs might be useful. A recent small placebo-controlled study show promising results of glutaminergic drugs in PDD.

1.1.8 Diagnosis

Parkinsonism is a clinical diagnosis defined by the presence of the cardinal symptoms of rigidity, bradykinesia, tremor and postural abnormalities. Parkinsonism can be found in various neurological diseases. The diagnosis of idiopathic PD is therefore often a challenge because the symptoms may be subtle at onset, and diagnostic errors include AD, DLB and atypical parkinsonian disorders such as Multiple System Atrophy and Progressive Supranuclear Palsy. A neuropathological study confirmed idiopathic PD in only 76 % of a cohort of 100 patients with clinical PD. The use of
strict diagnostic criteria has increased the accuracy of the clinical diagnosis in parkinsonian syndromes.\textsuperscript{71}

There are several diagnostic criteria in use; based on some combination of the cardinal motor signs and response to dopaminergic treatment. For the current thesis, we have applied the research criteria proposed by Gelb.\textsuperscript{28} The Gelb criteria distinguish between three levels of diagnostic confidence: possible, probable and definite. The diagnosis of definite PD requires histopathological confirmation. (Tables 1, 2 and 3, from Gelb et al.\textsuperscript{28} )

<table>
<thead>
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<th>Group A features: characteristic of Parkinson disease</th>
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<tbody>
<tr>
<td>Resting tremor</td>
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<td>Bradykinesia</td>
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<td>Rigidity</td>
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<td>Asymmetric onset</td>
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<th>Group B features: suggestive of alternative diagnoses</th>
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<tr>
<td>Features unusual early in the clinical course</td>
</tr>
<tr>
<td>Prominent postural instability in the first 3 years</td>
</tr>
<tr>
<td>after symptom onset</td>
</tr>
<tr>
<td>Freezing phenomena in the first 3 years</td>
</tr>
<tr>
<td>Hallucinations unrelated to medications in the first 3 years</td>
</tr>
<tr>
<td>Dementia preceding motor symptoms or in the first year</td>
</tr>
<tr>
<td>Supranuclear gaze palsy (other than restriction of upward gaze) or slowing of vertical saccades</td>
</tr>
<tr>
<td>Severe, symptomatic dysautonomia unrelated to medications</td>
</tr>
<tr>
<td>Documentation of a condition known to produce parkinsonism and plausibly connected to the patient’s symptoms (such as suitably located focal brain lesions or neuroleptic use within the past 6 months)</td>
</tr>
</tbody>
</table>

Table 1. Grouping of Clinical Features According to Diagnostic Utility
Criteria for POSSIBLE diagnosis of Parkinson disease:

At least 2 of the 4 features in Group A* are present; at least 1 of these is tremor or bradykinesia

and

Either None of the features in Group B* is present
Or Symptoms have been present for less than 3 years, and none of the features in Group B* is present to date

and

Either Substantial and sustained response to levodopa or a dopamine agonist has been documented
Or Patient has not had an adequate trial of levodopa or dopamine agonist

Criteria for PROBABLE diagnosis of Parkinson disease:

At least 3 of the 4 features in Group A* are present

and

None of the features in Group B* is present (note: symptom duration of at least 3 years is necessary to meet this requirement)

and

Substantial and sustained response to levodopa or a dopamine agonist has been documented

Criteria for DEFINITE diagnosis of Parkinson disease:

All criteria for POSSIBLE Parkinson disease are met

and

Histopathologic confirmation of the diagnosis is obtained at autopsy (see Table 3)

*Group A and Group B are detailed in Table 1.

Table 2. Proposed Diagnostic Criteria for Parkinson Disease

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Substantial nerve cell depletion with accompanying gliosis in the substantia nigra

At least 1 Lewy body in the substantia nigra or in the locus ceruleus (note: it may be necessary to examine up to 4 nonoverlapping sections in each of these areas before concluding that Lewy bodies are absent)

No pathologic evidence for other diseases that produce parkinsonism (eg, progressive supranuclear palsy, multiple system atrophy, cortical-basal ganglionic degeneration) (note: in excluding other diseases that produce parkinsonism, published consensus criteria should be used when available®)

Table 3. Proposed Criteria for Histopathologic Confirmation of Parkinson Disease
1.1.9 Diagnostic neuroimaging and PD

Neuroimaging is not recommended for routine diagnostic use in PD by the Gelb criteria. In the decade that have passed since these criteria were introduced, the field of imaging in PD has evolved greatly, but PD has remained a clinical diagnose.

Nuclear imaging provides at present the most sensitive tool for early diagnosis of PD and related disorders. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) can provide information regarding various neuron functions, functional metabolism and regional perfusion in the brain using transmitter specific tracers (e.g. dopamine tracers) or unspecific tracers such as glucose. Dopamine transporter imaging (DAT) is in clinical use using SPECT. Loss of dopamine transporter is a marker of reduced number of presynaptic neurons, and DAT-SPECT is suggested as a diagnostic tool in routine clinical practise.

Compared to the nuclear imaging techniques, structural imaging using MRI is in general more available, offers no ionizing radiation and is relatively cheap. With respect to MRI, there are no findings sensitive or specific for the diagnosis of PD. Patients with suspected idiopathic PD are referred for a scan mainly to exclude the various differential diagnoses associated with parkinsonism. Most previous conventional MRI studies have failed to find disease specific abnormalities in early PD, possibly due to limitation of techniques unable to detect the subtle pathology expected in these stages of the disease. The ability for routine MRI to differentiate
PD from controls is therefore modest.\textsuperscript{73} Recently proposed diagnostic tools are not validated and includes among others assessment of the substantia nigra degeneration,\textsuperscript{74} pathologic diffusion in the olfactory tract\textsuperscript{75} and high field MRI.\textsuperscript{76} The new and advanced techniques are promising for better detection of early PD related changes in the brain, but this needs further investigations.

Another widely available and safe imaging modality is transcranial ultrasound. This technique has been used to evaluate the substantia nigra in patients with PD. Hyperechogenicity is found in up to 90\% of patients with idiopathic PD. Increased echo of the substantia nigra has been proposed as a potential risk marker for PD, but this is complicated due to the fact that this finding is seen also in about 10\% of controls. The role of transcranial ultrasound in established disease is limited as it is shown that the finding is not associated with disease progression or clinical severity.\textsuperscript{77}

Potential biomarkers for PD are currently a hot topic.\textsuperscript{78} Such biomarkers may be used as surrogate end points in clinical drug trials in established disease, but are also important in preclinical stages in order to prevent disease in subjects at risk. Until recently, the substantia nigra has received the majority of attention in the search for a neuroimaging biomarker.\textsuperscript{79} The small size of this nucleus and many difficulties in depicting its exact boundaries by current imaging standards, in addition to the fact that clinical expression is found with extensive degeneration, may limit the usefulness of substantia nigra as a site for evaluating disease progression in PD. Advances in techniques and the new knowledge about PD being a more widespread disease has
resulted in new search strategies. Lately, and among many others, both serial volumetric MRI\textsuperscript{80} and metabolic approaches\textsuperscript{81} have been suggested as potential markers of disease progression.

Despite its limited use in clinical diagnostic practise, neuroimaging plays an important role in academic settings. The currently used gold standard in PD research is neuropathology, but such studies often only describe end stage disease and merely provide cross-sectional information. Contrary to this, neuroimaging offers unique in vivo representation of the disease and can also be used in longitudinal designs. Adequate neuroimaging techniques may therefore provide an understanding of PD pathology in the various disease stages, its clinical correlates and the pathological progression over time. Extensive collaborative studies, combining clinical, pathological, genetic, biochemical and imaging findings, are needed in order to achieve such an understanding.
1.2 Magnetic Resonance Imaging (MRI)

1.2.1 History of MRI

Following the discoveries of X-rays by Roentgen in 1895 and radioactivity and radium by Becquerel and the Curies in 1896, the basic techniques of radiology were established and developed. Half a century later, in 1938, Rabi was the first to describe the physical phenomenon of nuclear magnetic resonance (NMR), a discovery that led to the 1944 Nobel Prize in Physics (http://nobelprize.org). Important breakthroughs leading to current MRI technology, were done in the years after the World War II. In 1945 Bloch, and independently Purcell and Pound, discovered the principles of nuclear induction. They showed that one can detect a signal (a voltage in a coil) when a sample is placed in a magnetic field and exposed with a radiofrequency (RF) energy (a RF pulse) of a certain frequency (the Larmor frequency). The detected signal was a result of interaction between nuclei in the sample and the magnetic field. In 1952, Purcell and Bloch received the Nobel Prize in Physics.\(^2\) NMR was further developed as a laboratory spectroscopic technique used for examination of molecular structures in compounds, and great scientists involved in this work were also later honoured with Nobel Prizes (Bloembergen 1981, Ramsey 1989, Ernst 1991, Wüthrich 2002).\(^2\) The clinical use of nuclear magnetic resonance imaging (NMRI) was initiated by the chemist Paul Lauterbur in 1973, and further developed by a physicist named Peter
Mansfield. In 2003 they jointly were awarded the Nobel Prize for physiology or medicine.

Although scientifically accurate, in clinical practice the N was dropped from the acronym NMR due to its negative associations with nuclear energy and weapons, and MRI became the preferred term for diagnostic imaging using NMR. In the decades that have passed since it was first described, MRI has become the most important tool for in vivo investigations of brain structures and function in neurological diseases. It has also increased our knowledge about normal brain function and development.

1.2.2 MRI principles

The MRI system consists of a superconductive stationary magnet providing the main magnetic field, and it is in this large magnet the patient is placed when scanned. The powerful magnet is located in a shielded RF room protecting it from external, unwanted RF radiation. An internal RF system consisting of coils generates excitatory RF pulses used to excite nuclei in the body (transmitter coils) or to detect signals emitted from the NMR process (receiver coils). Strength and spatial location of the emitted signals and the various imaging techniques are made possible through a gradient system, which is also part of the MRI system. Finally, computers detect and transform the magnetic resonance signals into what we now know as MRI. In the following section, I will try to provide a basic explanation of the complicated process behind this fantastic tool.
The main principle behind MRI is based on absorption and emission of energy in the hydrogen nuclei in the body caused by RF pulses. The hydrogen nucleus contains only one proton, and it is therefore also often referred to as a proton. The majority of clinical use of NMR today is based on the hydrogen nuclei. This is due to hydrogen nuclei providing the best magnetic resonance signals, and that they are very numerous in humans as part of the water molecules in our body.\(^8\) Protons have a single positive charge, and they all spin around their own axes. In this way, each proton generates a tiny magnetic field known as a magnetic moment. The many small spinning magnetic moments in our body will try, similar to a bar magnet, to align themselves with the main magnetic field of the MRI scanner. As a result of this, each proton will spin with a frequency (named the Larmor frequency, given by the strength of the magnetic field) either parallel (the lowest energy state) or anti-parallel to the main field. For every million of anti-parallel protons, there are a-million-and-four parallel protons. In MRI one utilizes the net parallel magnetization moment of all the protons to obtain information about the tissue of interest.\(^8\)

In order to measure this magnetic moment, protons need to be exposed to RF pulse stimulation. A 90° RF pulse with Larmor frequency will create resonance resulting in protons being flipped out of their axes in the z-plane of the main magnetic field and down in the x-y transverse plane. The MRI system is created so that we can detect the magnetic moments as vectors in this transverse plane. Within seconds protons will return to their former equilibrium position in the z-plane and in this process they will lose energy detectable as a NMR signal.\(^8\)
The loss of energy (i.e. relaxation) consists of two parallel processes: 1) a dephasing of spins in the x-y plane called the spin-spin relaxation and 2) realignment of protons along the z-axis. In various body tissues, the relaxation process is different based on their molecular structure and their number of protons, and this can be used to differentiate amongst them and provide image tissue contrasts.

1.2.3 Tissue contrast in MRI

1.2.3.1 T1 contrast

As described above, the executing RF pulse will add energy to the protons and flip them away from the z-axis producing a NMR signal. With time, the protons will return to their former low energy state, the NMR signal will gradually decay and disappear. The time it takes for the added energy to diminish, and the protons again to align with the external main magnetic field, is different in various tissues. By definition, the T1 relaxation time is the time it takes for 63 % of the protons to be realigned along the z-axis. Protons’ ability to lose energy to the surrounding environment molecules (the lattice) is referred to as the T1 relaxation property of the tissue, also called spin-lattice relaxation. The T1 relaxation properties depend on the motional frequencies of the molecules contributing to the lattice. In tissue, where this motion is at the Larmor frequency, the T1 relaxation is more efficient and the T1 relaxation time is shorter. A short T1 relaxation time is typically seen when the protons are intermediately bound to their surroundings, e.g. fat, and this is seen as a very bright signal. In both free fluids and in solids, the spin-lattice relaxation is
reduced and the T1 relaxation time is longer (represented with a darker signal). In a T1 weighted MRI scan of the brain, gray matter will appear with intermediate gray signal, white matter will have a lighter white signal and cerebrospinal fluid (CSF) appears dark.\textsuperscript{82} (Figure 1.2)

\textbf{Figure 1.2}

Sagittal T1 weighted brain MRI scan.
1.2.3.2 T2 contrast

The T2 relaxation time is defined as the time it takes until the NMR signal in the transverse x-y plane is reduced to 37% of its value immediately after the executing 90° RF pulse. The flipped protons will continue to produce an NMR signal in the transverse plane as long as they remain in coherent spinning. T2 decay describes the process when the protons change phase, become dephased and lose their transverse magnetization. T2 decay is also different in various tissues, depending on its degree of natural motional frequency, and the T2 relaxation is therefore also named spin-spin relaxation. In tissue consisting of large protons, the T2 relaxation is faster because the flipped protons have reduced capacity to move freely due to large local magnetic field variations. This is seen in solid tissue or in fluids containing proteins, where the transverse relaxation happens fast, the NMR signal decays in a short period of time and the MRI signal appears in shades of gray or dark. In pure water, the protons can move freely around and remain in phase for a long time, resulting in a strong NMR signals. In T2 weighted brain MRI scans, the gray matter will appear light gray, the white matter will have a darker gray signal and cerebrospinal fluid will appear very bright. (Figure 1.3)
1.2.4 Volumetric MRI

Volumetric MRI acquisitions are made available through technical advances in recent years. In three-dimensional (3D) MRI of the entire volume of interest (e.g. the brain) is divided into very thin contiguous slices resulting in numerous cubes, called voxels. Voxel sizes as low as 1x1x1 millimetres are possible. This is considered an advantage as it reduces partial volume effects, but there is a trade-off since it may reduce signal-to-noise ratio. 3D MRI sequences are time consuming and can be more susceptible to artefacts, but are increasingly used also in clinical practise because of the high resolution and great potential of image reformations provided by the raw data.

Figure 1.3

Axial T2 weighted brain MRI scan.
1.2.4.1 Volume analyses of structural brain MRI.

In contrast to traditional pathoanatomical methods, morphometric MRI allows in-vivo investigations of the brain, and the field of MRI-based brain morphometry has developed extensively during the last years.\textsuperscript{84}

Previously, and still in most clinical settings, structural brain MRI are analysed qualitatively by investigators reading scans based on their experience and expertise. This lack of objectivity causes problems in research settings when aiming to compare with other work in the field. As a result, many semi-quantitative scales have been developed. An example is the Fazekas’ scale for estimation of ischemic white matter pathology.\textsuperscript{85} With technical developments providing increasing amounts of MRI data (e.g. in research settings) and a need for more accurate and objective analyses, a number of quantitative analysing methods have been developed.

Quantitative volumetric brain MRI analyses can estimate unspecific global scale characteristics, e.g. whole brain volume, or more tissue-specific volumes like total gray matter, total white matter or total CSF volumes. This approach has the advantage of being relatively robust and fast, but offers less detailed information. Another approach is the estimation of local volumetric changes. The advantages of obtaining data on regional changes must often be weighted against a more time-consuming and less robust method. In order to obtain regional quantitative volume data from the brain, manual tracing has been used extensively, e.g. in hippocampal analyses.\textsuperscript{86} Manual tracing is very time consuming as it implies that one has to draw
around anatomical structures on each slice throughout the whole volume.

Development of automatic and time saving computerized methods is therefore an active research area, and several such software packages are now available, e.g. FreeSurfer (http://surfer.nmr.mgh.harvard.edu/).

Manual hippocampal tracing analyses are examples of a region of interest (ROI) approach, where specific brain regions are investigated based on hypothesis on where to expect the pathology. Lately, unbiased computerized methods have been developed where the whole brain is analysed for local pathology detected by statistical evaluation of all the brain voxels, e.g. Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm)

1.2.4.2 General principles of computerized brain volume analyses

Several different approaches exist for estimation of volumetric brain parameters from 3D MRI volume acquisitions, but the various methods have some fundamental concepts and techniques in common. These include magnetic field bias corrections, registration of brains into a reference space and segmentation of brain tissue classes and/or anatomical structures. The many computerized methods available have solved these issues in different ways, and their performances are under continuous investigation. Comparative studies have been published, but the internal validity of the methods has been difficult to establish due to the lack of a gold standard. Most previous validation studies used manual ROI segmentations as source reference.
Recently, developments of realistically simulated data, have been a great improvement in this field of research.\textsuperscript{87}

The choice of method for MRI analysis depends on several factors: the experience and competence of the operator, the computer resources available and, finally, the quality of the MRI data and robustness’ of the algorithms highly influence the success rate of computer-based methods.

1.2.5 White matter hyperintensities analyses

White matter hyperintensities (WMH) are bright signal changes in the white matter seen in T2 weighted images. They are typically seen around the ventricles (periventricular WMH), but also as focal lesions in the deep white matter. WMH are best appreciated on MRI fluid attenuated inversion recovery (FLAIR) sequences. FLAIR is a special T2 weighted sequence where the high signal of ventricular fluid is suppressed resulting in a better contrast between periventricular WMH and intraventricular CSF. (Figure 1.4)
Earlier work estimated the amount of WMH from T2 weighted MRI scans using various visual qualitative rating scales. These scales are easy to implement and relative insensitive to artefacts, but have limitations regarding objectivity and ceiling effects. Recently, computerized volumetric analyses have become more widely used, both fully automatic and semi-quantitative methods. Comparison of the different methods has shown that visual scoring is less sensitive than volumetric assessment, and that automated quantitative segmentation methods are suitable for assessing impact on cognitive function. Others have found that visual rating is as good as the more complex methods in routine clinical practice, but that volumetric assessment should be used in research settings.
1.2.6 MRI and the aging brain

In the past couple of decades, published papers using MRI in the characterization of neurodegenerative diseases are numerous. The pathology coexists with normal age-related processes, and it is therefore important to understand normal brain MRI changes during aging.

1.2.6.1 Whole brain, gray and white matter volumes

Many studies have investigated how normal age changes relate to MRI depicted brain tissue volumes. A large study of more than 2200 participants (age range 34 to 97 years) found that age explained about 50% of differences in total brain volume. Frontal lobe volumes showed the greatest decline with age. Age-related changes were in general small before the age of 50 years. Another study (370 adults age 18 to 97) also showed an age-related decrease in total brain volume, starting from age 30, with later onset of white matter than gray matter loss. Recently, studies have shown consistent and widespread neuroanatomical age-related subcortical and cortical volume differences when analyzing multiple samples using the same method.

Investigating the relationship between changes in hippocampal and cortical gray matter volumes and cognitive functions in healthy elderly, Kramer et al. found in their longitudinal study that reduced volume of hippocampus was associated with decline in episodic memory function and that reduction in cortical gray matter volumes were associated with executive dysfunction.
Results on the impact of gender on longitudinal brain tissue volumes are conflicting. Studies have found no impact of sex after controlling for larger skull size in men. Others show increased gray matter loss in men. Results from a large multisample study concluded that sex had negligible effects on aging in both healthy subjects and in patients with AD.

1.2.6.2 White matter hyperintensities

WMH occur with increased frequencies with higher age in healthy subjects. A population based study showed that, of 1077 subjects between 60-90 years of age, only 5% had no WMH detectable on MRI. Most studies show that women have more WMH changes than men, but there are others reporting that there is no such difference. WMH are also characteristic findings in conditions associated with cerebrovascular risk factors, e.g. vascular dementia, and in inflammatory brain disease like multiple sclerosis. Bright signal white matter lesions on T2 weighted MRI are thus unspecific MRI findings, and the histopathological findings of WMH are different depending on the underlying condition. An early report on incidental WMH showed that punctuate deep and irregular periventricular WMH corresponded to ischemic tissue damage, while smooth periventricular rims of WMH were of non-ischemic origin with demyelination, subependymal gliosis and discontinuities of the ependymal lining. This was questioned in a recent neuropathological paper showing that all age-associated WMH are results of brain vascular damage. Restricted cerebral perfusion in areas with WMH is depicted below in Figure 1.5
Cerebral computer tomography (CT) angiography (left) showing extensive periventricular hypoattenuation (findings corresponding to MRI T2 white matter hyperintensities) and perfusion CT blood flow analyses (ml/100 ml/min) (right) illustrating restricted blood flow in areas of periventricular white matter pathology.

Courtesy of Dr. Mona K. Beyer, Dep. of Radiology, Stavanger University Hospital.
The previously cited study by Kramer and colleagues found that WMH changes in healthy elderly brains also were associated with reduced executive functioning. A recent study showed that age-associated periventricular WMH in non-demented subjects were associated with reduced cortical cholinergic receptor activity. This suggests that disruption of cholinergic projection fibres in periventricular white matter could serve as a substrate for the cognitive deficits associated with WMH. Despite the findings of age-related WMH influencing cognitive status, some studies report that there is no such relationship. These suggest that MRI might be oversensitive when it comes to WMH, and that higher water content in the brain does not necessarily result in loss of function. The association between WMH (especially low grade) and cognition in non-demented individuals is at the present time complex and not fully understood.
1.3 Structural MRI findings in PD

The next section, discussing structural MRI in patients with PD, will be restricted to previous volumetric studies that included non-demented and demented patients in addition to patients with MCI.

As patients included in this thesis are all newly diagnosed, focus will be given to studies of patients in early disease stages. The term early disease stage may, as in this present work, be used as a description of newly diagnosed patients with relatively short disease duration irrespective of disease severity. But the term can be problematic since early PD is also used to describe non-advanced PD not accounting for disease duration. In the following, we have tried to separate between non-advanced disease as in the meaning of limited clinical disease and early disease meaning restricted disease duration.

1.3.1 Morphological analyses of brain MRI in non-demented patients with PD

Few previous studies have investigated changes in whole brain volumes in early PD, and these studies (including 27 and 16 patients) did not find any evidence of significant whole brain atrophy in their patients with PD.\textsuperscript{109,110} In small sample studies of patients with longer disease duration, similar conclusions were drawn.\textsuperscript{110-113} In order to obtain more confident results, the degree of whole brain atrophy in PD needs to be investigated further including larger samples of patients.

The majority of regional structural MRI studies in newly diagnosed PD have been ROI based and focused on the substantia nigra degeneration and its value as a
diagnostic tool. Regarding early non-demented patients with PD, only a couple of studies have addressed regional brain atrophy. Geng et al.\textsuperscript{110} reported reduced putaminal volume in patients (n=16) with limited disease severity and disease duration of about two years when compared to controls, and even more reduction in advanced stage patients with a mean disease duration of about five years (n=8). The volume of putamen was correlated with disease severity. This study also found pallidal atrophy in advanced disease, but there was no significant volume loss in substantia nigra or nucleus caudatus in any disease stage. Bruck and colleagues\textsuperscript{114} found hippocampal and prefrontal lobe atrophy in 22 unmedicated non-demented patients with PD (mean disease duration 1.7 years), and that impaired memory was related to hippocampal atrophy and reduced sustained attention to prefrontal tissue loss.

A manual ROI approach was also used to achieve a better in vivo understanding of the brain changes in non-demented patients with longer disease duration. Alegret et al.\textsuperscript{115} found that ventricular (third and lateral) enlargement in rather advanced patients with mean disease duration of about 14 years was correlated with memory and frontal lobe functions, and that reduced putaminal volume was associated with poorer motor functions.

Many previous ROI studies have focused on the medial temporal lobes and the majority in patients with relatively long disease duration. The reason for this is that
degenerative changes in the hippocampus are established as pathologic hallmarks of AD and there is a need to develop imaging tools that can differentiate between the various neurodegenerative diseases. The first such study described in 1996 hippocampal atrophy in both non-demented and demented patients with PD. The degree of atrophy was less than in AD, but more than in a group of patients with vascular dementia.\textsuperscript{116} Hippocampal atrophy was also found in a later study in patients with PD, although to a lesser degree than in patients with PDD suggesting a progressive pattern.\textsuperscript{117} Bouchard and colleagues detected age related hippocampal atrophy in patients with PD, both non-demented and demented subjects.\textsuperscript{118} Still, all these findings are in contrast to a study reporting that hippocampal atrophy in non-demented patients with PD was not statistically significant.\textsuperscript{119}

In the past few years, several operator independent VBM studies have investigated various aspects of PD, but again few studies exist that include patients with short disease duration. Nagano-Saito reported no differences in gray matter densities comparing cognitively intact patients with PD and normal controls. Only when analysing a subgroup of rather advanced stage patients (mean disease duration 4.9±4.3 years, Hoehn & Yahr (H&Y) stage >2), atrophy was found in bilateral orbitofrontal cortex, left ventrolateral cortex and left parahippocampal gyrus.\textsuperscript{120} Recently, Ibarretxe-Bilbao et al. found significant gray matter loss in the right amygdala and bilateral orbitofronal cortex in a VBM study of early stage non-demented patients with PD (disease duration 3.1±1.6 years and H&Y 1.7±0.4)
compared to controls. The atrophy in orbitofrontal cortex was associated with impaired recognition of emotions and decision-making impairment in PD.\textsuperscript{121}

The studies of patients with longer disease duration show conflicting results, and VBM studies have failed in detecting consistent significant regional brain atrophy in non-demented PD. Burton et al.\textsuperscript{122} showed reduced gray matter volume in the frontal lobe of 31 patients with PD (disease duration about 44 months, mean age 75 years and mean Unified Parkinson’s Disease Rating Scale (UPDRS) III 26), while Beyer et al.\textsuperscript{123} found reduced gray matter in the superior temporal region in a group of 20 patients with PD (disease duration 12 years, mean age about 73 years and mean H\&Y 2.4). Other studies report atrophy in nucleus caudatus and associated ventricular enlargement,\textsuperscript{124} hippocampus and anterior cingulate,\textsuperscript{125} intra-parietal sulcus,\textsuperscript{126} superior temporal and frontal gyrus\textsuperscript{127} and cerebellum.\textsuperscript{128}

Most previous volumetric studies have focused on possible presence of gray matter atrophy in patients with PD. A recent study might represent a somewhat change of focus in VBM studies. In this study comparisons of 26 early, untreated patients with PD and 14 healthy controls revealed no significance in local gray matter volumes. In PD, however, there was reduced white matter volume in the right temporal lobe.\textsuperscript{129} The clinical significance of this finding is at present time unknown especially since there were no correlations between the local white matter volumes and neuropsychological tests reflecting memory and visuospatial processing.
Another newly published VBM study also reported white matter atrophy in addition to lack of gray matter loss in patients with PD.\textsuperscript{130} Interestingly, the atrophy cluster was located in the brainstem and thus somewhat in accordance with the Braak hypotheses.\textsuperscript{32} In this study the included patients had limited disease severity (H&Y stage I and II), but had a rather long disease duration (mean duration since first diagnosis about 6 years). These preliminary results are very interesting, but need to be validated in future, larger studies.

In summary, studies investigating volumetric changes in the brains of patients with newly diagnosed PD are few and inconsistent. Furthermore, results from studies of more advanced stage patients with PD and of patients with longer disease duration without cognitive impairment, are not conclusive. It must be noted that the above-mentioned structural MRI studies are limited by many factors. In early disease the diagnosis of PD might be difficult and patients with other parkinsonian disorders could possibly have been included. Secondly, all studies included few patients which might mask significant results given the known heterogeneity in PD. Finally, lack of consistency in MRI protocols and analyses makes it difficult to compare the various studies in order to draw general conclusions.
1.3.2 MRI brain atrophy in patients with PDD

Results from previous structural MRI studies on patients with PD and dementia are restricted by many of the same limitations as outlined above, but in general, more consistent significant brain MRI changes have been found than in non-demented patients. Manual ROI studies have detected atrophy of hippocampus, \textsuperscript{116-119} amygdala, \textsuperscript{118,119} and enthorhinal cortex. \textsuperscript{131} VBM studies have also reported strong (results corrected for multiple comparisons) findings of atrophy in patients with PDD compared with controls in hippocampus\textsuperscript{132} and right cuneus in addition to left inferior parietal region. \textsuperscript{133} Other studies report widespread atrophy using less stringent statistical tresholds. \textsuperscript{122,123,125} Despite all efforts, a specific pattern of gray matter atrophy in PDD is yet to be established.

A recent VBM study reported findings from hippocampal volume analyses in patients with advanced PD, including PDD and non-demented patients with and without visual hallusinations. \textsuperscript{132} Ibarrextxe-Bilbao and colleagues here found that the PDD group had hippocampal gray matter reductions in the entire hippocampus, while non-demented patients with visual hallucinations showed significant reductions only in the hippocampal head. Non-demented patients without visual hallucinations did not have any gray matter tissue loss.
1.3.3 MRI brain atrophy in patients with PD and MCI

Along with the growing interest of establishing possible characteristics of a pre-dementia stage in PD, a few recent structural MRI studies have looked for brain changes in patients with PD and MCI. The first two studies in the field were both published in 2007. Meyer et.al\textsuperscript{134} reported using ROI methods and visual rating, that parkinsonian patients (including both PD and DLB) with MCI was characterized by third ventricular enlargement, but also a certain degree of atrophy in the entorhinal cortex when compared to MCI subjects who later converted to AD. When compared to normal controls, parkinsonian patients with MCI also showed frontal and temporal lobe and hippocampal atrophy in addition to frontal horn ventricular enlargement. In this study parkinsonian patients with MCI did not differ from parkinsonian patients with dementia for any MRI variable.

Beyer and colleagues\textsuperscript{123} showed in a VBM study gray matter atrophy in left frontal and both temporal lobes comparing patients with MCI with patients with normal cognition, but results were not corrected for multiple comparisons and were no longer present when age and gender was included as covariates in analyses. Both previous studies included old patients with advanced disease. Patients were few, and the studies differed in methods regarding PD diagnosis, neuropsychological assessment and MRI analyses and results must therefore be interpreted with caution.
1.3.4 Longitudinal volumetric MRI studies in PD

Longitudinal studies of whole brain volume loss in PD are conflicting. A two year follow-up study using semiautomatic methods indicated significant percentage whole brain loss in 8 non-demented PD patients (-0.81 %/year) compared to 10 controls (-0.04 %/year). Increase in ventricular size was not significantly different between groups, and visual inspection showed diffuse gyral atrophy in the temporal, parietal and frontal cortex in patients with PD. Brain volume loss was correlated with measurements of global cognitive functions and symptom duration. Later, Burton et al. found rates of brain atrophy over one year no different between 18 patients with PD (0.31±0.69 %/year) and 24 age-matched controls (0.34±0.76 %/year). Interestingly, this latter study found brain atrophy rates significantly increased in 13 patients with PDD (1.12±0.98 %/year), but there were no significant correlations between increased atrophy rates and age or dementia severity.

A longitudinal VBM study detected gray matter loss in 8 advanced stage patients with PD without dementia over a period of about two years in limbic, paralimbic and temporo-occipital regions. Eleven patients with PDD showed decrement in mainly the neocortex. Later, another VBM study found no evidence of gray matter atrophy in PD patients after a follow-up of about 1.4 years. Finally, a recent VBM study found increased (over a period of about 29 months) brain atrophy and conversion to PDD in patients with PD and visual hallucinations compared to patients with no such hallucinations.
For the PDD group, longitudinal MRI has been suggested as a marker for disease progression and possibly for detection of effects of disease-modifying treatment in clinical trials, but further studies investigating longitudinal evolution of brain atrophy in PD are clearly needed. A better understanding of the natural course of the pathological processes is necessary in addition to an improved understanding of the various patient subgroups in which different rates of atrophy may predict various clinical phenotypes.

1.3.5 MRI WMH studies in PD

The influence of WMH in PD is at present not established. The majority of previous studies have used various semi-quantitative rating scales, and most studies have investigated possible correlations between WMH and disease severity. Lately, studies have also focused on the relationship between WMH and cognitive dysfunction. The pathophysiological explanation for clinical influence of WMH is not completely understood, but disruption of white matter fibre tracts has been suggested. A possible link between WMH and gait disability could be due to interrupted cortical-subcortical tracts involved in gait and balance. The same mechanism has been proposed for periventricular WMH affecting cognition by disconnecting major cholinergic tracts.

In 1995 Piccini and colleagues published a study showing, by use of semi-quantitative rating, that 102 non-demented patients with PD had more periventricular
WMH than controls, and that the patients with periventricular WMH had more advanced disease at shorter duration of symptoms than patients without periventricular WMH. An association between disease severity and WMH was also the conclusion of a recent study including 141 patients with PD; Lee et al. found that WMH was correlated especially with axial symptoms and independently associated with the postural instability and gait difficulty motor subtype of PD. Still, these studies are contrasted by work reporting that WMH were not significantly related to PD motor symptoms.

Regarding the influence of WMH on cognitive functions in patients with PD, previous studies have also shown conflicting results. A couple of newly published studies found that vascular risk factors and WMH changes did not contribute to cognitive impairments in PD. Earlier work by Beyer et al. found that demented patients with PD had higher total WMH deep, periventricular and frontal deep WMH load than non-demented patients and concluded that vascular factors thus might influence cognitive status in advanced PD. It must be noted that the total PD group was not different from controls with respect to WMH. In this study deep WMH were significantly associated with the MMSE performance. Another study also suggested vascular pathology as possible explanation for PDD and showed that subjects with parkinsonian dementia had higher mean severity of WMH compared to NC. Still, these latter cross-sectional findings are not supported by the only longitudinal study in the field. Burton et al. showed, as the only study utilizing automated volume quantification, higher baseline mean total WMH volume in AD,
but not in PD with dementia or DLB compared to normal controls. WMH progression over one year was no different between groups, and change in WMH volume did not correspond to change in global cognitive performance.

With respect to previous work on the impact of WMH on MCI in PD, Meyer et al. reported that in their small sample of parkinsonian MCI patients did not differ from controls or demented parkinsonian patients with respect to vascular brain lesions.\textsuperscript{134} Recently, Rodriguez- Oroz and colleagues\textsuperscript{25} published a cross-sectional study showing no difference in WMH burden in patients with advanced PD, irrespectively of cognitive group status; cognitively normal, having MCI or suffering from dementia.

WMH are possible preventable contributing factors to cognitive dysfunction in patients with PD. Given the limited number of previous studies in this important field, it is evident that more studies are needed.
2. Hypotheses

The aim of this thesis was to test the hypothesis that cognitive impairment in newly diagnosed PD is related to brain atrophy and/or WMH changes in the brain. By studying a large number of unselected patients with incident PD from a community-based study we wanted to search for a possible neurobiological basis for their cognitive deficits. To achieve this, we applied various widely used and previously validated structural MRI methods providing us with different levels of information.

**Paper I**

In other neurodegenerative diseases with dementia, especially AD, whole brain atrophy is a surrogate marker for cerebral pathology even in early disease stages.\(^\text{148}\) Furthermore, age-related vascular changes in the brain represented as WMH, are also associated with cognitive dysfunction.\(^\text{149}\) Based on this, we hypothesized that global loss of brain tissue and/or high WMH load is present and may be contributing factors to the cognitive deficits observed in a large unselected cohort of patients with early PD.

**Paper II**

Previous VBM studies have found regional gray matter atrophy in advanced PD, in PD with dementia and PD with MCI.\(^\text{122,123}\) We therefore hypothesized that MCI and
reduced cognitive performance in patients with early PD also would be associated with VBM detectable focal gray matter loss.

**Paper III**

In a sample of patients with advanced PD, our group previously found that frontal deep, periventricular and total deep WMH were increased in advanced stage demented patients with PD compared to non-demented patients, although the whole PD cohort did not differ from controls with respect to total WMH.\(^{144}\) WMH might be especially associated with frontal lobe domains including executive function, attention and processing speed and working memory.\(^ {149-151}\) To further explore the cognitive impact of WMH in patients with PD, we tested the hypothesis that WMH, total volume and according to regional distribution, contribute to MCI and the attention/executive cognitive dysfunctions in patients with newly diagnosed PD.

**Paper IV**

The Braak hypothesis suggests that PD pathology in very early clinical stages should be mainly concentrated in brainstem nuclei, forebrain and to some extent the temporal mesocortex.\(^ {152}\) Neuropathological work has found that brainstem pathology is associated with dementia in patients with PD.\(^ {153}\) In this paper we tested the hypothesis that MCI in newly diagnosed PD is associated with subcortical brain tissue loss detectable on structural MRI.
3. Methods

3.1 Subjects

All subjects included in this thesis are part of the Norwegian ParkWest study. This is a population-based multi-center prospective longitudinal cohort study of patients with incident PD from Western and Southern Norway. The study comprises patients, their caregivers and a control group with similar age and sex distribution. This sample is to be followed prospectively for 10 years.

3.1.1 Patients

A total of 265 patients met the diagnostic criteria of PD, and 212 consented to participate in the 10-year longitudinal ParkWest study (diagnostic procedure as outlined below in section 3.2). As discussed in section 1.3, we have defined our patients as patients with early stage PD in the sense that they are newly diagnosed, irrespectively of their degree of clinical disease expression.

3.1.2 Controls

In the Norwegian ParkWest study an age-and sex matched control group was also recruited. The controls consisted of subjects in the following preference order:
patient’s spouse, a friend in the same age-group as the patient, or unrelated persons from social clubs for the elderly in the study area. Exclusion criteria for controls were dementia, parkinsonian symptoms at clinical examination and previous or current treatment with anti-parkinsonian medication. In addition, the controls should be able to carry out the baseline and planned examination programme.

3.1.3 MRI cohort

An MRI examination was performed as part of the baseline assessment in 182 patients with PD, and in 109 of 205 controls at four centres: Stavanger (45 patients and 42 controls), Haugesund (11 patients and 2 controls), Bergen (106 patients and 37 controls), and Arendal (20 patients and 28 controls).

The number of scanned controls in Bergen, was limited due to economical restrictions. With respect to patients lacking a representative MRI scan, the reasons for this were wrong or incomplete protocol (n=8), claustrophobia (n=6), poor health incompatible with MRI (n=4), MRI contraindications e.g. pacemaker (n=5) or they refused to be scanned (n=2). For unknown reasons, five patients with PD did not have a MRI scan.

Mean time interval between baseline clinical and neuropsychological evaluation and MRI scan was 32 (standard deviation ± 44) days in the patient group and 174 (standard deviation ± 144) days for controls.
Before all image analyses, we excluded subjects with gross brain pathology known to influence segmentation outputs, e.g. large cortical infarcts (n=4) or arachnoid cyst (n=1). We also excluded subjects with major scan artefacts. Due to the use of different MRI series in the various analyses, this varied among the different studies using 3D T1 weighted series (Paper I, II and IV) and FLAIR (Paper I, II and III). Furthermore, the final number of subjects included in each study depended on requirements and performance of the imaging analysis tools. Detailed descriptions of the final study sample for the various studies can be found in each separate paper.
3.2 Diagnostic procedure of PD

In the Norwegian ParkWest study a comprehensive case ascertainment system, using all available resources both in hospital-based and community-based health care, resulted in a population-representative cohort of all residents in the study area who were diagnosed with incident PD between November 1st, 2004, and August 31st, 2006. All patients were diagnosed with PD according to research criteria as described in section 1.1.8. The final diagnosis was made, in a four-step procedure after an average follow-up of 28 months, through an independent evaluation and final consensus by two senior movement disorder specialists. In making the diagnosis, the study neurologists took all available patient material; medical history, clinical examination, imaging data and a possible response to dopaminergic treatment into consideration. None of the included patients had a history of marked cognitive decline before signs of motor symptoms or within the first year of the disease.
3.3 Clinical assessment tools

In all subjects we recorded the demographic variables age, gender and years of education. Additional clinical and neurological assessments were carried out as described below.

3.3.1 Assessment of parkinsonism and disability

For the papers in this thesis, we rated the severity and stage of PD using the UPDRS\textsuperscript{154} and the H\&Y scale.\textsuperscript{155}

The UPDRS\textsuperscript{154} was developed in 1987 and evaluates four components of PD: mentation, behaviour and mood (part I), activities of daily living (part II), severity of motor symptoms (part III) and therapy complications (part IV). Of these, we used the subscore III as an assessment of severity of parkinsonism in our patients with PD.

The H\&Y scale\textsuperscript{155} was published in 1967 as a tool to evaluate disease progression, measured by impairment and disability of movements, balance and gait. The scale ranges from 0 (no motor symptoms) to 5 (bilateral parkinsonism and not able to walk).
3.3.2 Assessment of depression

We estimated severity of depressive symptoms using the Montgomery-Aasberg Scale (MADRS).\textsuperscript{156} The MADRS was recently validated in patients with PD.\textsuperscript{157}

3.3.3 Assessment of cognitive impairment

In the Norwegian ParkWest study, global cognitive status was screened with the MMSE.\textsuperscript{145}

A diagnosis of dementia associated with PD was made according to proposed consensus criteria.\textsuperscript{44} The diagnosis was based on neuropsychological testing, clinical history from a standardized caregiver questionnaire (the IQCode),\textsuperscript{158} in addition to the clinical examination.

Assessment of possible cognitive changes in patients with early PD was done using neuropsychological tests exploring cognitive domains that we know are affected in PD. The neuropsychological battery was chosen with tests that were not or only minimally affected by motor performance:

*The California Verbal Learning test II (CVLT-II)*\textsuperscript{159} evaluated verbal memory. CVLT-II consists of 16 words which were read five times, and after each time the test
subject is asked to recall as many words as possible. We included total immediate recall (sum of trials 1-5), short-delay and long-delay free recall (after 20 minutes) scores in our analyses.

The Silhouettes subtest from the Visual Object and Space Perception Battery (VOSP)\textsuperscript{160} tests visuospatial abilities. In this test, the subject is asked to identify the outline of 30 objects, which are rotated through varying degrees from the lateral axis.

The Semantic verbal fluency tests how many names of animals the subject can generate in 60 seconds.

The Stroop test\textsuperscript{161} is a test of selective attention and executive functions. In the interference part of the Stroop test, the test subject is asked to name the color of the word (which is printed in colored ink different from the printed name, 3\textsuperscript{rd} card). Psychomotor speed was examined using the sum of words produced during the color (colored patches, 1\textsuperscript{st} card) and the word (printed words, 2\textsuperscript{nd} card) conditions.

The Serial 7 test from the MMSE\textsuperscript{145} was also used to assess attention and executive functions. In this test the subject is asked to subtract serial 7’s from 100.

Based on these tests, three cognitive domains were calculated: a) memory (CVLT-II), b) attention and executive functions (Serial 7 from MMSE, Semantic verbal fluency, Stroop words, colors and color-words) and c) visuospatial functioning (VOSP silhouettes). Since age, sex and education affect performance on cognitive tests,
multiple regression analyses were performed using data from the healthy control subjects for each of the cognitive domains. The resulting regression formulae were used to calculate expected z-scores for the patients with PD based on age, sex and education.

3.3.4 MCI classification

MCI was classified based on performance in the three cognitive domains. Using a modified version of the criteria for MCI,\textsuperscript{59} subjects without dementia, but with an observed age-and education corrected z-score deviating more than -1.5 standard deviations from the expected z-score in at least one cognitive domain, were classified as having MCI. We refer to Aarsland et al. for further details.\textsuperscript{64}
3.4 MRI and image analyses

Quantitative image analyses were performed at the Buffalo Neuroimaging Analysis Center (BNAC), Buffalo, NY, USA during the period from June 2007 to June 2008. Operators blinded to clinical characteristics and tests results performed the MRI analyses. As principal investigator on all the papers included in this thesis, T.O. Dalaker was involved in all MRI analyses, although with excellent help from knowledgeable co-authors at the BNAC.

3.4.1 MRI imaging protocol

The MRI scans were performed at four imaging centres using a common protocol. A 1.5 T scanner was used in three centres (Philips Intera in Stavanger and Haugesund and Siemens Symphony in Bergen) and a 1.0 T Philips Intera system was used in Arendal. The protocol included a coronal 3D T1 weighted sequence used for brain volume calculations, and an axial FLAIR sequence used for classification of WMH. Sequence parameters for each MRI site were as follows:

For the 3D T1 weighted sequence: Stavanger: repetition time [TR]/echo time [TE] 10.0/4.6 msec, flip angle 30.0 degrees, 2 mm slice thickness with 1 mm spacing between slices (1 mm slices with no gap), Number of excitations [NEX] 2, Matrix 256x256; Haugesund: TR/TE 20.0/4.6, flip angle 30.0, 1 mm slice thickness with 1 mm spacing, NEX 1, Matrix 256x256; Bergen: TR/TE 2130.0/3.9, flip angle 15, 1
mm slice thickness/ no gap, NEX 1, Matrix 256x256, Arendal TR/TE 25/6.9, flip angle 30.0, slice thickness 2 mm with 1 mm spacing, NEX 1, Matrix 256x256.

**For the FLAIR sequence:** Stavanger: TR/TE 6000/100, inversion time [TI] 2000, 4 mm slice thickness/1 mm gap, NEX 2, Matrix 256x256, Haugesund: TR/TE/TI 6000/110/2000, 4 mm slice thickness/1 mm gap, NEX 2, Matrix 256x256, Bergen: TR/TE/TI 8400/103/2500, 4mm slice thickness/0.4 mm gap, NEX 2, Matrix 256x204, Arendal: TR/TE/TI 6000/100/1900, 4 mm slice thickness/1 mm gap, NEX 2, Matrix 256x256.

### 3.4.2 WMH analyses

WMH were outlined on each axial FLAIR image slice using a reproducible, semi-automated local thresholding technique with Java Image Manipulation software (version 4.0, Xinapse Systems, Northants, UK, [http://www.xinapse.com](http://www.xinapse.com)). All WMH were contoured by a single rater (TOD) with similar reproducibility, as previously reported. Reproducibility of the technique was calculated as a coefficient of variation (100 % x standard deviation/mean). After one month of training, testing showed an average inter rater reproducibility of 3.8 % and an intra rater reproducibility of 1.8 %.

An example of this technique is shown in Figure 3.1
Figure 3.1

Axial FLAIR scans (upper raw) and correspondent lesions masks (lower raw) are presented, showing WMH contoured with Java Image Manipulation software.

In paper I, we calculated total volume of WMH in each subject, and performed group and regression analyses as described in the article.

In paper II, binarized WMH masks, were used to eliminate voxels of misclassified WMH, from the gray matter segmented images (please see section 3.4.3.2 VBM).
In paper III, we investigated the spatial distribution and the total volume of WMH, and in this study we performed voxel-wise statistics of WMH masks in addition to standard statistical models including the total WMH volumes. Our voxel-wise approach can be summarized as follows: First, we co-registered all FLAIR images to the Montreal Neurological Institute standard-space image (MNI152 template) using a 12-parameter affine model via the FLIRT linear registration tool developed by The Analysis Group at the Oxford Centre for Functional MRI of the Brain (FMRIB). The resulting transformation matrices were then applied to the corresponding WMH mask images to bring these into the same standard space. Prior to statistical comparison, all standard-space WMH mask images were smoothed using a Gaussian kernel with a standard deviation of 4 mm. Voxel values were then used as dependent variables in a mass-univariate application of the general linear model to address spatial-specific group differences and to assess the relationship between attention-executive functions and spatially specific presence of WMH within the total PD sample. Statistical significance was assessed using non-parametric permutation testing, and we used the threshold-free cluster enhancement technique rather than the more standard a priori specification of a cluster forming threshold.

### 3.4.3 Brain volume analyses

In the following section, I will give an overview over the volumetric methods used. Due to the complexity of the theories underlying them, and the fact that it would be beyond the scope of this thesis, I will and cannot describe them in detail. Specific information can be found in the reference literature of each method.
3.4.3.1 SIENAX

FMRIB has developed many methods for analysis of neuroimaging data. The majority of their techniques can be freely downloaded from FMRIB’s Software Library (FSL) (http://www.fmrib.ox.ac.uk/fsl).\textsuperscript{163}

Included in FSL are FMRIB’s tools for brain change analysis, both for longitudinal and cross-sectional studies. SIENA (Structural Image Evaluation, using Normalization, of Atrophy) provides a quantitative method for estimation of intra-subject temporal change in brain size, and SIENAX is the cross-sectional version of the software used for atrophy measurements between subjects at a single time point.\textsuperscript{166} In our initial unselected investigation of our ParkWest MRI cohort (paper I) we used SIENAX as a tool for estimations of global atrophy changes in incident PD patients compared to control subjects. SIENAX is fast and fully automated. Both SIENAX and SIENA was developed with a focus on robustness and the software has been shown to perform well also using different MRI field strengths, scanners and slice thickness.\textsuperscript{166} SIENAX provides us with information regarding global brain size changes. Such changes may reflect widespread tissue loss or could be a result of localised processes. SIENAX is thus an efficient method for estimations of degree of degeneration, but it cannot establish regional extent of changes.

Briefly SIENAX consists of the following steps:
1. Deskulling of the brain using Brain Extraction Tool (BET), also part of FSL, resulting in a brain and skull output from each image.

2. Registration and normalization of the individual brain and skull images to MNI152 standard space using FLIRT. Here the brain is used for initial registration and final translation and rotation, and the skull is used for scaling and skew optimization.

3. Applying dilated standard MNI152 brainmask in order to optimize deskulled output.

4. Tissue segmentation and bias correction of original deskulled brain (gray matter, white matter, and CSF) using FMRIB's Automated Segmentation Tool (FAST). Segmentation volumes are then multiplied by a volumetric scaling factor derived from the normalization procedure to give rise to normalized brain volumes.

5. Volumes are reported normalized to skull using normalization parameters derived from the registration process, and can now be used in statistical quantitative analyses.

In our analyses we applied FSL version 3.3.11, including SIENAX 2.4, BET 2.1 and FLIRT 5.4.2. Group analyses compared global tissue volumes between controls and patients with PD, and using multiple regression analyses we investigated the impact of MRI volumes on cognition in PD.

An example of the SIENAX segmentation output is given in Figure 3.2.
3.4.3.2 Voxel based morphometry

We used VBM in our MRI analyses of regional gray matter atrophy in paper II. VBM is an automated technique consisting of a few main steps.

1. The MRI scans are segmented into gray matter, white matter and CSF, bias corrected and transformed into stereotactic space by a computerized algorithm.
2. Segmented tissue masks are smoothed in order to increase statistical validity by rendering data more normally distributed and reducing the effective number of statistical comparisons. Furthermore, smoothing compensates for the nonexact nature of the spatial normalization.

3. Voxel by voxel statistical analysis is performed. Standard VBM approach evaluates changes in tissue concentration and/or volumes (on modulated images) using parametric statistics within the framework of the general linear model.

For our study we performed VBM using SPM5, (http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB 7.4.0 (R2007a), (Mathworks, Natick, MA). We applied a modified unified segmentation procedure\textsuperscript{170} VBM method (http://dbm.neuro.uni-jena.de/vbm) adjusted to take into consideration WMH that could be wrongly classified as gray matter.\textsuperscript{173} Such WMH correction was recently highly recommended in studies with an elderly population.\textsuperscript{174}

We first created study- and site-specific tissue priors modified to eliminate the possible influence of WMH on gray matter segmentation. 3D-T1 images were first normalized to the MNI152 template. During this step, the images were weighted by their WMH to exclude the lesions from the normalization procedure (nulling the WMH and setting the remaining to 1). Because of the elderly study sample, we did not use the MNI tissue probability maps priors in this first segmentation. The
segmentation process resulted in gray matter-, white matter- and CSF-segmented images for each subject. Normalized gray matter, white matter and CSF images were then again conditionally averaged (areas of WMH were not included). The resulting gray matter, white matter and CSF-averaged images were smoothed with an 8-mm full-width at half-maximum (FWHM) gaussian kernel and used as tissue probability maps during the final image segmentation.

3D-T1 images were again segmented in native space into gray matter-, white matter- and CSF-segmented images, using the study-specific tissue priors. During the normalization step, all images were again weighted by their WMH masks. Afterwards, normalized WMH masks were applied to the segmented images to remove possible remaining misclassified voxels. And then, finally, modulated gray matter images were smoothed with an 8-mm full with a FWHM gaussian kernel.

Gray matter volumes were compared between groups (MCI PD vs. non-MCI PD, MCI PD vs. controls and non-MCI PD vs. controls) using a factorial design controlling for total intracranial volume and UPDRS III (PD only). For all analyses we used a 1 -1 contrast, in addition to a reverse -1 1 contrast. We also performed regression analyses (+1 contrast) between gray matter volumes in the total PD sample and cognitive domain performances (memory, visuospatial and attention-executive) Statistical significance level was set at a height threshold of p<0.05 false discovery rate correction.
3.4.3.3 FreeSurfer.

FreeSurfer is a freely available software application developed at the Athinoula A. Martinos Center for Biomedical Imaging (www.nmr.mgh.harvard.edu/martinos). It consists of a set of tools for automatic reconstruction of the brain’s cortical surface and volumetric segmentation from structural MRI data (www.surfer.nmr.mgh.harvard.edu). The reconstructed brain surface can be used in combination with functional MRI data, but is also used for measurements of cortical thickness related to neurological diseases and as a tool for understanding of brain development and function.

We applied FreeSurfer (version 3.2) in order to investigate the degree of subcortical atrophy in our newly diagnosed PD patients. This method gives detailed volumetric data of a number of brain structures: cerebral white matter, cerebral cortex, ventral diencephalon, lateral ventricle, inferior lateral ventricle, cerebellum white matter, cerebellar cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, third ventricle, fourth ventricle, brain stem and CSF. The subcortical segmentation process is described in a detailed methodological publication.175 Initially, the T1 weighted MRI scan is registered to the Talairach space,176 and the brain is segmented from non-brain tissue using a hybrid algorithm combining watershed algorithms and deformable surface models.177 The next stage is an initial volume labelling followed by correction of inhomogeneities in the magnetic field followed by a non-linear volumetric alignment to Talairach space. After this pre processing, the final volume labelling is performed. This step is based on both subject
specific intensity values and prior information from a subject-independent probabilistic atlas based on a manually labelled training set. Each voxel in the brainmask is assigned a label according to its relationship with the label atlas, its spatial relation with neighbour labels and its measured intensity. In Paper IV, we performed group comparisons of the subcortical volumes using multivariate analysis of covariance statistics (MANCOVA) controlling for total intracranial volume, age and gender.
4. Results

Paper I
We investigated the extent of whole brain, global gray matter and white matter atrophy and total WMH volume, and found that our unselected cohort of patients with PD did not differ significantly from controls. Furthermore, we showed that none of the MRI measurements were significant predictors of neither global cognition nor specific cognitive domains (attention-executive, memory and visuospatial) in PD.

Paper II.
Aiming to investigate if MCI in patients with early PD was characterized by region-specific gray matter atrophy, group analyses comparing patients with and without MCI and controls showed no regional gray matter tissue differences. We also showed that there were no significant correlations between reduced local gray matter volume and impaired cognitive test performance.

Paper III
In this paper we showed that there were no significant differences between the control subjects and PD patients with or without MCI with respect to total volume or spatial distribution of WMH. In addition, there was no significant relationship between total volume or spatial distribution of WMH and attention-executive functions in patients with PD.
Paper IV

We found that patients with PD and MCI had significantly larger fourth ventricle, third ventricle and left inferior lateral ventricle volumes than controls. Compared to patients without MCI, left inferior lateral and third ventricular volumes were significantly larger in patients with MCI. The difference in fourth ventricular volume did not reach statistical significance, but we showed that volume of fourth ventricle was significantly correlated with cognitive performance only in PD patients presenting MCI and not in cognitively intact patients or controls. No other significant MRI subcortical volume group differences were found.
5. Discussion

5.1 Main findings

1. There was no significant loss of whole brain, total gray matter or total white matter volumes in newly diagnosed patients with PD compared to age- and sex matched control subjects (paper I).

2. There was no significant local gray matter atrophy in newly diagnosed patients with PD when comparing with elderly subjects without PD, irrespectively if patients with PD were presenting MCI or not (paper II).

3. Total whole brain, gray matter, white matter and lateral ventricular volumes were no significant predictors of global cognition in patients in early PD when controlling for possible confounders, especially age (paper I).

4. Localized gray matter volume was not significantly correlated with memory, visuospatial or attention-executive function when controlling for age, sex, education and motor severity (paper II).
5. Total lateral ventricular volume was larger, but only trend significant (p=0.059), in patients with PD compared to controls (paper I), but a subgroup analysis of patients with MCI showed significantly larger fourth, third and left inferior lateral ventricular volumes compared to controls and larger third and left inferior lateral ventricle compared to patients with no MCI (paper IV). Differences in fourth ventricular volume are illustrated in Figure 5.1.

**Figure 5.1**

Coronal Freesurfer segmentation output of A) normal healthy control (Female, 65 years, fourth ventricle volume 2.23 ml) and B) patient with Parkinson’s disease classified as having mild cognitive impairment (Female, 68 years, fourth ventricle volume 2.77 ml) demonstrating differences in fourth ventricle volume.

Color legend: pink – gray matter; white – left cerebral white matter; neon green – right cerebral white matter; gray – brain stem; yellow – hippocampus; light green – thalamus, violet – lateral ventricles; light blue- caudate; brown – cerebellum gray matter; light brown – cerebellum white matter; dark green - fourth ventricle.
6. Total WMH volume was not significantly increased in patients with PD compared to controls, and total WMH volume was not a significant predictor of global cognition in PD (paper I).

7. Total volume or regional WMH distribution was not significantly different in patients with PD (both with and without MCI) than in controls, and neither higher total WMH volume nor WMH spatial distribution was significantly associated with reduced attention/executive performance in patients with PD (paper III).
5.2 Methodology

5.2.1 Study design

The ParkWest study\textsuperscript{10} is a population based study, and this design offers several advantages to clinic-based studies. The ParkWest study aims to include all residents with incident PD in the study area and in this way capture the full spectrum of incident PD. This is in contrast to hospital-based studies, often including more severely affected patients and thus introducing a potential selection bias.

Our study is a multi centre study, a challenging design with respect to both planning and execution of the study protocol. Careful training and regular meeting of involved personnel has been conducted to secure a high level of both intra- and inter-centre validity. Still, use of different MRI scanners is shown to affect volumetric analyses with as much as 10 times.\textsuperscript{178} In the ParkWest study, the MRI was done on the same scanner in each centre during the entire study period, and a common study imaging protocol was used. In order to reduce bias of the multiple scanners, we have controlled for centre in articles including all subjects using semi-automated WMH analyses and the relatively robust SIENAX method\textsuperscript{166} (paper I and paper III). For paper II, we a priori decided to use data from one centre (Stavanger) due to recommendations that VBM should be used for scans acquired with the same scanner.\textsuperscript{171} FreeSurfer is highly dependent on gray and white matter tissue contrast in the MRI data (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferBeginnersGuide).
To avoid centre bias, and based on visual inspection revealing the best imaging quality in scans from Stavanger, we only included scans from this centre also in paper IV. This has recently been shown to be a good decision; results from an evaluation of the FreeSurfer subcortical segmentation performance concluded that results within scanner platforms are reliable, but combing data across field strengths and platforms introduces a bias.\(^{179}\)

The data used for this thesis are the baseline results from the ParkWest study. Cross-sectional results cannot provide answers on causality, only associations between variables of interest. This is thus a limitation of the study, but hopefully the longitudinal continuation of the study can provide more and new knowledge about both causality and progression of cognitive impairments and brain changes in PD.

### 5.2.2 Selection of the study population

PD is a clinical diagnosis, and the definite diagnosis according to Gelb et al.\(^{28}\) can only be made through histopathological examinations post mortem. Idiopathic PD may be difficult to diagnose in early stages. In this study, the diagnosis of PD was given by specialists in movement disorders to improve diagnostic accuracy.\(^{180}\) Before the final diagnosis, patients had about two years of clinical follow-up and repeated examinations. Known differential diagnoses, e.g. dementia with Lewy bodies, were carefully excluded with as much confidence as possible in early disease based on current criteria. Still, we cannot be 100 % confident that some patients with time may
end up with diagnoses such as Progressive Supranuclear Palsy and Multiple System Atrophy.

Control subjects were selected based on age and sex and recruited from spouses and friends of the patients and in this way provide the same socioeconomic and cultural background. Diagnoses with high prevalence in the elderly, e.g. cerebrovascular disease, were not exclusion criteria in order to recruit a control/reference cohort with similar co-morbidity as observed in the general population. Thus, the findings in our study likely reflect differences between representative groups of patients with PD and elderly subjects.

With respect to the MRI cohort there are a few issues that deserve to be discussed. All patients were intended to have a MRI scan, but due to MRI contraindications and poor health, an examination could not be performed in some subjects. This may have introduced biases. One potential bias could be exclusion of patients with cardiovascular disease potentially resulting in WMH, due to pacemaker being a contraindication of MRI. Still, we believe this is a minor issue in our cohort since statistical comparison of patients with and without MRI showed no significant difference in vascular risk (rated as presence of one or more of the following: hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, peripheral vascular disease, coronary heart disease, previous stroke or current smoking), p =0.217, Pearson Chi-Square. Furthermore, patients without MRI could potentially be older subjects with more advanced disease (motor and/or cognition) making the MRI
examination an impossible task. We do find that patients without an MRI scan are older (mean age 73 years vs. 67 years, independent samples t-test p=0.003), have higher UPDRS III score (mean 29.1 vs. 22.4, Mann-Whitney test p=0.024) and H&Y stage (mean 2.2 vs. 1.9, Mann-Whitney test p=0.051), but total MMSE score did not differ significantly (26.9 vs. 27.7 Mann-Whitney test p=0.188).

A potential caveat may also be subjects excluded as a result of major MRI artefacts. Good quality scans are dependent on that subjects are able to lie still in the scanner. It is therefore also possible that subjects excluded due to movement artefacts are older patients with more advanced disease. Similar selection bias is introduced by technical software factors since segmentation of subjects with major atrophy may fail (due to registration and normalisation errors), and these subjects are left out of the statistical analyses. As an illustrations of this is presented in paper I, where we showed that patients with unacceptable SIENAX segmentations (some due to major atrophy experienced by visual inspection) were somewhat older than patients with acceptable segmentations.

In general, exclusion of subjects is unfortunate and should be avoided as it results in reduced power in addition to the potential biases mentioned above. Still, we believe that this is unavoidable given the population based nature of the study and the know limitations of the MRI technique and today’s analysing methods.
5.2.3 Neuropsychological assessment

The neuropsychological evaluations used for this work has several advantages. The tests were chosen because they are believed to be little affected by motor impairments, and the majority of patients were tested before they started ant-dopaminergic treatment. Furthermore, the cognitive classifications were done taking age, sex and education under consideration, and whenever possible using published research criteria, e.g. for dementia in PD. A possible criticism of the cognitive aspects of this research is the rather limited neuropsychological test battery.

The MCI approach in the ParkWest study needs to be validated. As mentioned in section 1.1.6.2, there is at present time no established MCI classification in PD. As a result of different methodology and sensitivity of applied neuropsychological tests, frequency of MCI differs among previous incident cohorts. A previous long-term follow-up study of PD patients with MCI indicates that MCI, especially certain subtypes, is a risk factor of PDD. The prognostic value of the MCI classifications needs important validation through longitudinal research, and it will thus be very interesting to follow the ParkWest study cohort in the next 10 years.

Much work is currently investigated in defining standardized diagnostic MCI criteria in PD, and several task forces are working on the subject (D. Årsland, personal communication). Potential differences lie in the choice of number and type of neuropsychological tests and in the cut-off applied to define impairments compared to controls. In the near future, collaborative effort and validation studies will
hopefully result in guidelines available for new clinical studies and meta-analyses. Standardized MCI criteria are also of utmost importance for future imaging studies in this field of research. Neuroimaging may be used as a tool in investigations of the underlying pathology causing the heterogeneous clinical expressions of MCI in PD. Depending on the outcomes of studies, imaging can potentially become a valuable aid in future clinical drug trials aiming to improve deficits or delay progression of cognitive dysfunction in patients with PD.

5.2.4 Brain volumetric methods

Volumetric MRI in research is used to estimate extent of tissue loss and brain morphometry in normal brains as well as in pathological conditions. Loss of brain tissue (i.e. atrophy) is regarded as the end stage of neurodegeneration. Volumetric MRI is used as a biomarker in studies of Alzheimer’s disease\(^{148}\) and multiple sclerosis\(^ {182}\). Recently 3D patterns of MRI atrophy were validated as a surrogate marker of the neurodegenerative aspects of AD atrophy correlating with neurofibrillary tangle pathology\(^ {183}\). In PD research, the usefulness of such investigations is not yet established. Analyses of newly diagnosed patients potentially provide information about the extent of pathological processes very early in the disease. Neuropathological studies in newly diagnosed patients are sparse. MRI studies investigating atrophy in early PD are therefore necessary to increase our understanding of how the brain pathology acts and develops, knowledge important in, for example, future drug trials aiming to prevent tissue loss.
Despite potential advantages, there are a few general methodological issues to be addressed regarding volumetric brain MRI analyses. As discussed in section 5.2.1, several technical factors should be considered when planning and executing studies since accuracy of results is influenced by image quality, technical protocols and use of different MRI scanners. In our study we have tried to take this into account, and when choosing methods for testing of our hypotheses, we had to consider the robustness of the software in addition to the quality of the MRI data.

We applied automated volumetric MRI methods, and thus minimized operator dependent biases possibly affecting previous manual ROI studies. Still, it is well known for especially VBM, that results may vary considerably based on methodological options available for analysing parameters like smoothing filter size and normalization template. Our adapted VBM approach is thoroughly described in section 3.4.3.2. The rationale for our decisions was based on established reference literature, and previous comparable research in addition to web site advices by Dr. C. Gaser, a leading expert in MRI morphometry. Options also exist for the presentation of significant anatomic labels resulting from VBM studies. We decided to present our results according to Brodmann area and the Talairach and Tournoux atlas, similar to comparable previous studies, e.g. Beyer et al. We recognize that this approach has been criticised given its inaccuracy and may be suboptimal.
In addition to the above issues regarding methodology, biological factors are also known to influence the outcomes of atrophy studies, e.g. normal aging. A discussion of age as a potential confounder is recommended when reporting MRI studies, and is accordingly included in all the included papers of this thesis.

The exact biological basis of morphometric brain changes are not known. At present a histopathological validation of the volumetric MRI findings in PD is lacking, and we can therefore not be sure what is causing the previously reported brain volume changes. A recent study by Burton et al. investigated the pathological correlates of medial temporal lobe atrophy in subjects with histological verified diagnoses of AD, DLB and vascular cognitive impairment. The study reports that of 23 subjects with DLB, nine had PD clinically for more than one year prior to development of the psychiatric features of DLB. In their sample they found that medial temporal lobe atrophy was a significant predictor of AD tangle pathology, but not Lewy body like-inclusions in the hippocampus. The cellular substrate for the morphometric brain changes previously reported in patients with PD may thus represent pathology beyond the Lewy bodies diagnostic of the disease. These are very interesting findings, and more studies comparing antemortem MRI scans and histopathology are highly needed.
5.2.5 WMH evaluation

We have used a well known semi-automated method for estimation of WMH load. This method differs from most previous WMH studies in PD using semi-quantitative rating scales.\textsuperscript{85,88,89} As described in section 1.2.5 these scales may have some limitations, and comparison of different methods has shown that visual scoring is less sensitive than volumetric assessment.\textsuperscript{90} We did not have techniques available for fully automatic WMH lesion estimations, but believe that our semi-automatic method and especially the voxel-wise comparison approach is a methodological improvement compared to previous studies.

5.2.6 Statistics

For statistical comparisons of demographic and clinical data, we have employed generally accepted methods according to the distribution of data. We have used a standard, but rather liberal, statistical significance threshold in all papers (p<0.05) given the explorative nature of our work. Our studies are among the first population based structural MRI studies of patients with early PD and should provide hypotheses for future research. If more conservative thresholds were to be used, this would inflate the risk of making type II errors and thus potentially limit generation of such new hypotheses. Although this explorative approach, we have considered and taken into account important issues regarding thresholding and statistical methods of voxel-based statistics. Voxel-based MRI analyses are mass-univariate based including large
numbers of statistical comparisons, and results should therefore be controlled for multiple comparisons.\textsuperscript{172} In both our voxel-based papers (paper II and III), we have addressed this, and results are accordingly corrected for multiple comparisons.

We are aware that our non-significant volumetric findings could be a result of underpowered analyses, especially in group analyses of paper II and IV including only subjects from one centre. As stated in the papers, results must therefore be interpreted with caution. Still, our sample size is comparable to previous and current studies in the field. The limited sample size is especially evident for our MCI group, and we were not able to perform any MCI subgroup analyses. The characteristics of a small heterogeneous MCI group might be difficult to depict, but still results from paper IV indicate that mesocortex, brainstem and midbrain pathology might be a common trait of MCI in newly diagnosed PD. Future studies should, if possible, include more subjects especially given the limited expected effect size.\textsuperscript{189}

In all our volumetric analyses we have corrected for the impact of aging on brain morphometry. Given the potential special role of age on disease progression in PD, this issue needs to be discussed in detail. As mentioned, prevalence studies report a clear relationship between age and PD, and the disease is rare in people under 50 years of age.\textsuperscript{8} Studies have found that advancing age is associated with faster rate of motor impairment, decreased levodopa responsiveness, more severe gait and postural impairment and more severe cognitive dysfunction.\textsuperscript{13} If age interacts with disease specific neurodegenerative processes in PD, as suggested by Levy et al.,\textsuperscript{13} correcting
for age in statistical models might remove disease specific findings. In volumetric brain MRI studies of neurodegenerative diseases, age correction is necessary to establish effects not being a result of normal aging. Taken together, these factors represent another future challenge in PD imaging research. We have not been able to address this statistically in the presented papers, and a probability might therefore exist that we have removed disease specific brain changes when correcting for age.
5.3 Results

5.3.1 WMH

We found no significant association between WMH and cognition in PD. In addition, we did not show any significant difference in volume and distribution of WMH in patients with PD compared to age-matched controls. These results therefore indicate that vascular brain pathology is not a major contributor to the cognitive dysfunctions seen in newly diagnosed patients PD. Our findings are thus in line with a previous clinical observation study from our group claiming that there is no association between cerebrovascular risk factors and incident dementia in PD.²³

Results in paper III are somewhat in conflict with an earlier study from our group regarding WMH in patients with PD.¹⁴⁴ In this paper, Beyer and colleagues found that demented patients with PD had significantly more WMH in frontal, periventricular and deep white matter compared to non-demented patients. The differences were driven by lower levels of WMH in non-demented patients than control subjects, and the combined PD group was not significantly from different from controls with respect to WMH. The semi-quantitative rating scale used in this former study is different from our quantitative volumetric approach, but our voxel-wise analyses on regional distribution of WMH should be a good method for detection of significant distribution differences of WMH in patients with PD and various degree of cognitive dysfunction.
An important consideration in the interpretation of our WMH findings is that the previous works include older patients with longer disease duration. It is thus possible that development of age-associated WMH will have more impact on cognition as the disease progresses, at least in some subjects.

Our results do indicate higher lesion load in patients with PD and MCI compared to patients with no MCI and controls, but the difference did not reach statistical significance. It is possible that analysis of various MCI subgroups would provide information about the extent of vascular pathology in the different aspects of MCI in early PD, but this was not performed in the present study and could be an interesting topic for future studies.

We found significantly increased frequency of diabetes mellitus in our MCI group (paper III). Diabetes mellitus is a risk factor for WMH progression, and it will be very interesting to investigate how the WMH progression relates to the various vascular risk factors in the cognitive subgroups of the ParkWest study. Even though WMH was not a significant contributor to cognitive dysfunction at present time, only longitudinal follow-up can provide answers to how cardiovascular risk, baseline WMH volume and age will impact WMH progression and cognitive status in our PD cohort as the disease progresses.
5.3.2 Brain volume analyses

5.3.2.1 Whole brain, total gray matter and total white matter volumes

Results from global brain atrophy analyses (i.e. SIENAX\textsuperscript{166}) reflect the degree of widespread neurodegenerative pathology leading to loss of brain tissue. Our analyses did not reveal any significant group differences between our unselected incident PD cohort and age-matched controls. These results thus suggest that in newly diagnosed patients with PD, widespread atrophy is limited. This is in line with previous studies including smaller samples of patients with PD.\textsuperscript{109,112} Still, pathology may be present not reflected by our method, as indicated in a diffusion study of early PD.\textsuperscript{109} Furthermore, local gray matter changes may exist in such a degree or distribution that it is not reflected using global volume estimations.

5.3.2.2 Ventricular volume analyses

Cerebral ventricular volume analyses provide an indirect measure of brain atrophy. Our relatively crude global methods cannot estimate potential regional pathology in detail. But findings, if present, may provide indications of such, generate hypotheses and motivate future studies. In paper I, normalized lateral ventricular volume was the variable showing largest difference between patients and controls, with a trend towards significantly increased volume in patients with PD. This finding could be a sign of pathology in surrounding brain, possibly on a regional basis as indicated in Cordato et al.\textsuperscript{126} A recent study showed a strong relationship between asymmetric lateral ventricular enlargement and PD motor asymmetry and progression.\textsuperscript{191} Future
studies should investigate the various aspects of lateral ventricular morphology in patients with PD as a marker of brain tissue pathology.

We were able to detect significant differences between patients with PD and MCI and controls with respect to fourth ventricular, third and left inferior lateral volumes. We choose to interpret also this as a possible indirect sign of pathology in adjacent brain. Comparing patients with and without MCI, we showed significant differences in third and left inferior lateral volumes. Enlargement of fourth ventricular volume could therefore be a disease specific sign rather than pathology related to cognitive impairment. Still we chose to interpret the strong correlations between memory performance and fourth ventricular volume only within the MCI group, as indications of a role of brainstem pathology also in cognitive dysfunctions in newly diagnosed PD. Interestingly, a recent study also found brain stem atrophy in a sample of patients with early PD.\textsuperscript{130} Again, the studies could be in line with the Braak hypothesis,\textsuperscript{32} but as discussed above in section 1.1.4 the validity of this hypothesis is debated, and more research is clearly needed.

5.3.2.3 Regional gray matter volume analyses

We looked for regional gray matter changes using VBM in a subgroup of our patients, and we were unable to detect significant changes in patients compared to controls irrespectively if they were presenting MCI or not. In the correlation analyses, cognitive test performance was not significantly associated with localized gray matter volume. Our results are somewhat different previous comparable studies,\textsuperscript{123,134} but as
described in paper II, we believe that the conflicting results can be explained by a number of factors. First, our patients were less cognitively impaired with a higher MMSE score. Secondly, previous studies included samples of older subjects with longer disease duration and more advanced PD than in our current study. Thirdly, all three studies were based on small cohorts. Given the clinical and pathological heterogeneity in PD, small sample sizes may lead to different findings. These factors, in addition to important methodological differences in MRI analysis and MCI classification, are likely to explain the conflicting results. We believe that our present findings, with the limitations of a relatively small cohort especially for the group analyses, provide us with important new knowledge of how to investigate the biological basis for early cognitive changes in PD. Our VBM study indicated that the neurodegenerative process underlying PD at this stage is subtle. In the years to come, research aiming to detect the cerebral pathology in newly diagnosed, and preferably premotor, PD has to continue the search for neuroimaging techniques able to visualize such discrete cerebral changes.

Our FreeSurer analysis in paper IV was in line with the VBM analysis, and none of the subcortical gray matter volumes were significantly different between groups. This is different from previous ROI based studies of early non-demented patients with PD. We did find smaller volumes in PD patients compared to controls in most of the examined structures, so an explanation of the non-significant findings could again lie in a possible lack of power.
5.3.3 Clinical implications

Our results provide knowledge regarding the potential impact of vascular brain changes and cognitive dysfunction in PD. Based on our work there is little evidence that prevention and control of WMH risk factors will reduce the frequencies of cognitive impairment in early clinical stages. Still, given our cross-sectional design, results of causality cannot be drawn, and results must therefore be interpreted with caution.

Findings from our atrophy studies suggests that the degree of tissue loss in early PD (with and without cognitive impairment) is less than shown in for instance AD, and the clinical use of conventional structural MRI in PD is therefore still limited to exclusion of differential diagnoses. Our results, and previous published papers in the field, may indicate a certain degree of pathology on a group level and provide hypotheses for future research, but at present the individual diagnostic value is very limited.
6. Conclusion and future directions

The lack of significant global atrophy findings in early PD is not surprising given results from previous smaller studies, but we have now replicated the findings in our large population based sample of patients. Work included in this thesis provides more knowledge about PD, contrary to for instance AD, as a disease with little tissue loss in early disease stages. Results on tissue specific and subcortical atrophy are limited by a possible lack of power, but altogether our findings could indicate brain pathology in accordance with the Braak hypothesis. This needs to be investigated in future studies including larger cohorts and preferably longitudinal designs.

All our findings must be seen in the light of contemporary, similar studies investigating other neurodegenerative disease with known associated cognitive dysfunction. At present time, structural MRI in PD does not seem to be the most potent potential imaging biomarker, but the reason for this might be that most previous studies have used methods validated against AD. Based on our preliminary results, future studies searching for the biological basis for cognitive dysfunction in early PD should also focus on developing imaging techniques and analysis methods for better investigation of brainstem and midbrain.

Furthermore, a shift of focus from mainly investigations of gray matter pathology to studies investigating white matter atrophy and disruptions of white matter integrity could contribute important knowledge as indicated by recent findings in Martin et
This also needs to be explored in the years to come. We have investigated the association between total white matter volume, ventricular size (an indirect measure of white matter atrophy) and WMH and cognition in early PD, but potential sources for new information could, for instance, be more use of diffusion MRI of white matter tracts in the brain. In general, combining various imaging parameters (e.g. morphometry, diffusion and functional techniques) may result in new knowledge and increased diagnostic sensitivity of MCI in PD, as recently shown in a multimodal imaging study of MCI in AD. An interesting focus for future studies lies in exploring the various cognitive and motor subtypes in PD. As clinical and histopathological research are giving us more and more information about the disease heterogeneity in patients with PD, the neuroimaging community needs to approach this and search for a biological basis for the clinical expressions. This can be accomplished in large, longitudinal population based studies, and our hope is that future investigations of the ParkWest cohort can bring us closer to fulfilling this aim.

To the best of our knowledge, our work is the first in analyzing MRI scans of patients with early PD using various volumetric methods. In the years ahead, more studies of this kind would be valuable in the exploration of MRI detectable pathology in PD brains. Volumetric MRI findings should preferably be compared to post mortem atrophy studies and histopathological findings (although we acknowledge that this is often not available in early disease stages). In this way, the sensitivity and validity of
the various volumetric tools could be established, essential knowledge for future work in this field of science.

A final comment regarding future structural MRI research, especially voxel-based, is emphasizing the need for consistent reporting and methodology. This is valuable especially when comparing findings from various studies. Previous studies reported their results using a wide spectrum of thresholds and methods, often in small sample sizes. These studies were all very important and timely exploratory work and generated hypotheses for future studies. Now, in common effort, we all need to continue our search for MRI detectable pathology in a comparable manner using recommended guidelines, e.g. the recent VBM recommendations.
7. References


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8. Appendix

Papers I-IV