UNIVERSITY OF TURKU

DIAGNOSTIC AND PROGNOSTIC ASPECTS OF CLINICAL BRAIN DOPAMINE TRANSPORTER IMAGING IN PARKINSONISM

Elina Mäkinen

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To my mother Anita

ABSTRACT

Elina Mäkinen
DIAGNOSTIC AND PROGNOSTIC ASPECTS OF CLINICAL BRAIN DOPAMINE TRANSPORTER IMAGING IN PARKINSONISM
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Idiopathic Parkinson's disease (PD) is the most frequent cause of clinical parkinsonism. However, the diagnostic accuracy of PD is suboptimal. One biomarker that assists with the parkinsonism differential diagnostics is functional dopamine transporter (DAT) imaging. The accuracy of DAT single photon emission computed tomography (SPECT) imaging is high for detecting striatal dopamine deficiency associated with neurodegenerative parkinsonism. However, its limitations and associations with clinical characteristics are not yet fully understood, particularly in patients with clinical parkinsonism of uncertain origin.

In this thesis, the retrospective studies investigated the associations between striatal dopamine deficiency and structural midbrain atrophy measurements and long-term survival in PD patients. As both visual and semi-quantitative analysis methods are broadly used for DAT SPECT scans, the concordance between these methods was investigated. The cross-sectional clinical and imaging study investigated which of the parkinsonian motor signs are associated with a higher likelihood of striatal DAT deficiency, and whether these signs are associated with DAT loss in certain striatal regions in patients with neurodegenerative parkinsonism.

The results showed that there were no associations between the midbrain-to-pons ratios, suggestive of midbrain atrophy, and striatal dopamine deficiency in PD. Expert visual DAT image analyses and the semi-quantitative analyses did not match in 10% of cases; however, none of these patients had neurodegenerative parkinsonism syndromes according to the clinical follow-ups. The level of DAT deficiency was not associated with survival in PD. Finally, both upper extremity rigidity and hypomimia were independently associated with a higher likelihood of striatal dopamine deficiency. Hypomimia was specifically associated with caudate nucleus dopamine loss in patients with abnormal striatal DAT binding.

The results indicate that midbrain-to-pons ratios cannot be used to estimate the level of striatal DAT deficiency in patients with PD. The scans that showed a discrepancy between the different analysis methods should likely be interpreted as normal. Dopamine deficiency levels cannot be used to predict patient survival in PD. Presence of upper extremity rigidity and hypomimia in clinical neurological examinations may be useful markers in the differential diagnosis of clinically uncertain parkinsonism and tremor as they point to striatal DAT deficiency.

Keywords: Parkinsonism, Parkinson's disease, dopamine, dopamine transporter, midbrain atrophy, SPECT, analysis, survival, mortality, differential diagnosis, rigidity, facial expression

TIIVISTELMÄ

Elina Mäkinen
DOPAMIINITRANSPORTTERIKUVANTAMINEN PARKINSONISMIN EROTUSDIAGNOSTIIKASSA JA ENNUSTEESSA
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Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Suomi, 2018

Idiopaattinen Parkinsonin tauti on yleisin parkinsonismin syy, mutta taudin diagnostinen tarkkuus on vaillinainen. Yksi parkinsonismin erotusdiagnostiikan apuväline on dopamiinitransportteri (DAT) -proteiinin yksifotoniemissiotomografia (SPECT), jolla pystytään melko tarkasti osoittamaan neurodegeneratiiviseen parkinsonismiin liittyvä striatumin dopamiinitoiminnan heikentyminen. Menetelmään liittyy myös rajoitteita, ja on osittain epäselvää, mihin kliinisiin ja kuvantamislöydöksiin heikentynyt DAT-sitoutuminen liittyy etenkin potilailla, joiden parkinsonismin syy on epäselvä.

Tämän väitöskirjan retrospektiivisissä tutkimuksissa tutkittiin heikentyneen DAT-sitoutumisen yhteyttä keskiaivoatrofiamittauksiin sekä elinajanennusteeseen Parkinsonin taudin potilailla. Lisäksi tarkasteltiin DAT SPECT -kuvien visuaalisen ja semikvantitatiivisen analyysimenetelmän yhtäpitävyyttä. Poikkileikkaustutkimuksessa tutkittiin, mitkä parkinsonismin motorisia oireista viittaavat suuremmalla todennäköisyydellä heikentyneeseen DAT-sitoutumiseen ja onko näiden oireiden vaikeusaste yhteydessä DAT-sitoutumiseen tietyillä striatumin alueilla neurodegeneratiivisessa parkinsonismissa.

Parkinsonin taudissa keskiaivoatrofiaan viittaava heikentynyt keskiaivojen ja aivosillan välinen suhdeluku ei ollut yhteydessä DAT-sitoutumiseen. Kokeneiden DAT-kuvien analysoijien visuaalinen analyysi poikkesi 10 %:ssa tapauksista semikvantitatiivisesta analyysista. Seurannassa kenellekään näistä potilaista ei kehittynyt neurodegeneratiivista parkinsonismia. DAT-sitoutumisen heikentymisen asteella ei ollut yhteyttä Parkinsonin tautia sairastavien elinajanennusteeseen. Yläraajarigiditeetti ja kasvojen ilmeettömyys olivat yhteydessä suurempaan DAT-sitoutumisen heikentymisen todennäköisyyteen. Kasvojen ilmeettömyys oli neurodegeneratiivisessa parkinsonismissa yhteydessä erityisesti häntätumakkeen dopamiinitoiminnan heikentymiseen.

Keskiaivojen ja aivosillan välisiä suhdelukuja ei voi käyttää Parkinsonin tautia sairastavien DAT-toiminnan arvioimiseksi. DAT SPECT -kuvauslöydös tulisi todennäköisesti tulkita normaaliksi silloin, kun eri analyysimenetelmillä on saatu erilainen tulos poikkeavasta DAT-sitoutumisesta. Dopamiinitoiminnan heikentymisen aste ei Parkinsonin taudissa vaikuta elinajanennusteeseen. Etenkin yläraajarigiditeetti ja kasvojen ilmeettömyys voivat auttaa parkinsonismin kliinisessä erotusdiagnostiikassa, sillä ne viittaavat suuremmalla todennäköisyydellä striatumin dopamiinitoiminnan heikentymiseen kuin normaaliin striatumin dopamiinitoimintaan.

Avainsanat: parkinsonismi, Parkinsonin tauti, dopamiini, dopamiinitransportteri, keskiaivoatrofia, SPECT, analysointi, elinajanennuste, kuolleisuus, erotusdiagnostiikka, rigiditeetti, kasvojen ilmeettömyys

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ABBREVIATIONS

[^{123}I]FP-CIT = [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane
AADC = aromatic amino acid decarboxylase
AD = Alzheimer's disease
CBD = corticobasal degeneration
CBS = corticobasal syndrome
CUPS = clinically uncertain parkinsonism syndrome
DAT = dopamine transporter
DIP = drug-induced parkinsonism
DLB = dementia with Lewy bodies
EANM = European Association of Nuclear Medicine
ET = essential tremor
FDA = The United States Food and Drug Administration
FDOPA = 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine
MRI = magnetic resonance imaging
H&Y = Hoehn & Yahr stage
HR = hazard ratio
LEDD = Levodopa equivalent daily dose
MDS = The International Parkinson and Movement Disorder Society
MDS-UPDRS = the revised Unified Parkinson's Disease Rating Scale by MDS
MMSE = Mini-mental stage examination
MSA = multiple system atrophy
MSA-C = multiple system atrophy with predominant cerebellar ataxia
MSA-P = multiple system atrophy with predominant parkinsonism
PD = Parkinson's disease
PIGD = postural instability and gait disorder
PET = positron emission tomography
PSP = progressive supranuclear palsy
PSP-RS = progressive supranuclear palsy - Richardson's syndrome
PSPS = progressive supranuclear palsy syndrome
ROI = region of interest
RBD = rapid eye movement sleep behavior disorder
SBR = specific biding ratio (of striatal DAT binding)
SNc = substantia nigra pars compacta
SPECT = single-photon emission computed tomography
SPM = Statistical parametric mapping
SWEDD = scan without evidence of dopaminergic deficit
TH = thyrosine hydroxylase

UPDRS = the Unified Parkinson's Disease Rating Scale
VMAT-2 = vesicular monoamine transporter 2
VP = vascular parkinsonism

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on four original publications, which have been reproduced with the permission of the copyright holders. The original publications, listed below, will be referred to by corresponding Roman numerals.

I Mäkinen E, Joutsa J, Isotalo J, Kaasinen V. No relevant midbrain atrophy in Parkinson's disease. *Acta Neurol Scand. 2016;134:378-381.*

II Mäkinen E, Joutsa J, Johansson J, Mäki M, Seppänen M, Kaasinen V. Visual versus automated analysis of [I-123]FP-CIT SPECT scans in parkinsonism. *J Neural Transm (Vienna). 2016;123:1309-1318.*

III Mäkinen E, Joutsa J, Vahlberg T, Kaasinen V. Survival in Parkinson's disease in relation to striatal dopamine transporter binding. *Parkinsonism Relat Disord. 2017;42:66-72.*

IV Mäkinen E, Joutsa J, Jaakkola E, Noponen T, Johansson J, Pitkonen M, Levo R, Mertsalmi T, Scheperjans F, Kaasinen V. Individual parkinsonian motor signs and striatal dopamine transporter deficiency: A study with [^{123}I]FP-CIT SPECT. *Submitted*.

1 INTRODUCTION

Parkinson's disease (PD) is one of the most common movement disorders (Dorsey *et al.*, 2007). However, many syndromes with different types of tremors, bradykinesia, rigidity or postural instability may clinically mimic PD and lead to diagnostic uncertainties, at least in the early stages of the disease. Current evidence shows that many patients who are presumed to have PD may have a false diagnosis, and many patients should instead be diagnosed with essential tremor (ET) or atypical parkinsonism (Rizzo *et al.*, 2016). Although there are consensus diagnostic criteria for PD, such as the UK Brain Bank Criteria (Hughes *et al.*, 1992) and the more recent International Parkinson and Movement Disorder Society (MDS) criteria (Postuma *et al.*, 2015), exact clinical diagnoses are difficult to determine. The clinical differential diagnostics is hard even by movement disorders specialists, as the different parkinsonism and tremor disorders have overlapping clinical features (Bajaj *et al.*, 2010), at least in the early stages. To achieve greater accuracy for differential diagnosis of parkinsonism and tremor syndromes, several biomarkers have been developed and studied (Obeso *et al.*, 2017).

Functional brain dopaminergic imaging may be a useful biomarker. Dopamine transporter (DAT) single photon emission computed tomography (SPECT) imaging, with the use of [^{123}I]N-ω-Fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{123}I]FP-CIT) as a tracer, is currently the most widely used dopaminergic imaging technique (Varrone *et al.*, 2013) and is used in both research and everyday clinical practice. As the tracer [^{123}I]FP-CIT binds to the DAT protein on the presynaptic nerve terminals of the nigrostriatal dopaminergic pathway, it can be used to distinguish neurodegenerative presynaptic parkinsonism syndromes from parkinsonism with normal striatal dopamine function. Even if it is considered to be well tolerated (Grosset *et al.*, 2014), DAT SPECT imaging has some limitations, as it is expensive and associated with radiation. In addition, DAT SPECT imaging does not have 100% accuracy (Bajaj *et al.*, 2013). One reason for its suboptimal accuracy could be the disagreement between different image analysis methods. Furthermore, DAT SPECT imaging is not available at every center in every country. Thus, the differential diagnosis of parkinsonism is still mainly based on clinical diagnostics, which are also highlighted in the Parkinson's disease diagnostic guidelines by Postuma *et al* (Postuma *et al.*, 2015). Finally, it is not completely clear what striatal dopamine deficiency reflects. Does the level of striatal dopamine deficiency have prognostic value for patients? Are there alternative imaging methods that could replace dopaminergic functional imaging? How should the scans be interpreted if they are neither clearly normal or abnormal? Are some of the parkinsonian motor signs more strongly associated with striatal dopaminergic deficiency? Moreover, few published studies have investigated these topics in patients representing the true clinical population, those with parkinsonism of an unknown origin.

The primary objective of this thesis was to understand the characteristics of striatal DAT deficiency in patients with parkinsonian motor symptoms. All patients were scanned with [^{123}I]FP-CIT SPECT, and the images were analyzed using visual, region of interest (ROI) and voxel-based analysis methods. Striatal [^{123}I]FP-CIT binding was studied in relation to the MRI imaging parameters, prognostic outcomes and clinical motor signs, and the effects of different image analysis methods on the imaging outcome were studied in detail.

2 REVIEW OF LITERATURE

2.1 Parkinsonism and Parkinson's disease

Parkinsonism is a clinical syndrome manifested with the cardinal feature bradykinesia, as well as a resting tremor, rigidity, or both (Catafau *et al.*, 2004). The most common etiology of parkinsonism is idiopathic PD (Christine & Aminoff, 2004; Horvath *et al.*, 2013); however, there are also numerous other neurodegenerative and symptomatic reasons for the syndrome (Marsden *et al.*, 2012) that must be considered in any diagnostic workup.

2.1.1 Parkinson's disease

PD is the second most frequent neurodegenerative disorder after Alzheimer's disease (AD) (Dorsey *et al.*, 2007). In most cases, the cause of PD is sporadic, and less than 10% of PD cases are monogenic (Michel *et al.*, 2016; Obeso *et al.*, 2017). In Europe, the prevalence of clinically diagnosed PD in the age group of 65 years or older is on average 1.6%, and the prevalence increases with age (de Rijk *et al.*, 1997).

The dopaminergic neurodegeneration in the substantia nigra pars compacta (SNc), particularly in its ventrolateral tier, is the most consistent neuropathological feature of idiopathic PD (Hall *et al.*, 2014; Kalia & Lang, 2015; Obeso *et al.*, 2017). Another classic neuropathological feature is the presence of misfolded protein aggregates, mainly consisting of alpha-synuclein, in nerve cell bodies (so-called Lewy bodies) and insoluble intraneuritic alpha-synuclein processes (so-called Lewy neurites) (Dickson *et al.*, 2009; Kalia & Lang, 2015). In addition to the central nervous system (CNS), the Lewy pathology also affects the peripheral nervous system (Beach *et al.*, 2010). Recently, in addition to Lewy pathology, other proteinopathic pathologies, e.g. neuropathology classically associated with AD, has also been found to be relevant in PD (Kalia & Lang, 2015).

The relationship between the two cardinal neuropathological processes, the dopaminergic cell death and the Lewy pathology, as well as the etiology of PD, remain unclear (Obeso *et al.*, 2017). According to the Braak hypothesis (Braak *et al.*, 2003), a pathogen makes contact with the olfactory and/or enteric neurons in the nasal cavity and gut, causing the alpha-synuclein to form aggregates. These aggregates then spread to the CNS, including the substantia nigra, leading to the dopaminergic cell degeneration (Rietdijk *et al.*, 2017). However, there is no clear consensus about the causal connection between intracellular alpha-synuclein aggregates and dopaminergic cell death (Schulz-Schaeffer, 2015). In

addition, it has been suggested that also other autonomous cellular mechanisms, e.g. lysosomal and mitochondrial dysfunction and changes in the calcium homeostasis, as well as non-autonomous neuroinflammatory processes, may lead to the dopaminergic neuron loss in the SNc in PD (Michel *et al.*, 2016). Furthermore, a recent review outlined that the cause of the Lewy pathology is still mainly unknown (Obeso *et al.*, 2017). There may be, at least partly, a genetic cause, as the monogenic mutations that cause PD seem to lead to a clinical syndrome that is indistinguishable from sporadic PD with nigral cell loss and Lewy pathology (Kasten & Klein, 2015; Obeso *et al.*, 2017). Monogenic mutations in *SNCA*, *LRRK2*, *parkin*, *PINK1* and *GBA* are examples of genes that are well known to be associated with the monogenic forms of PD (Kalia & Lang, 2015).

PD is a clinically heterogenous disorder, manifested with the parkinsonian motor signs but also with several non-motor problems (Jankovic, 2008). The progressive degeneration of the dopaminergic nigrostriatal neurons, which axons reach, above all, the putamen, but also the subthalamic nucleus, globus pallidus, thalamus and cortex, are suggested to primarily cause the motor signs of bradykinesia and rigidity in PD. However, the neuronal connections from the basal ganglia to the thalamus, cortex and especially the cerebellum are likely associated with the parkinsonian tremor, whereas the relationship between the dopaminergic nigrostriatal neurodegeneration and tremor is still unclear (Hallett, 2012; Hallett, 2014; Caligiore *et al.*, 2017; Obeso *et al.*, 2017). Furthermore, PD shows different clinical phenotypes, and the primary motor subtypes are classified to tremor-dominant (the main symptom is tremor and not bradykinesia/rigidity), postural instability and gait disorder (PIGD) and akinetic-rigid parkinsonism syndrome (Jankovic *et al.*, 1990; Marras & Lang, 2013; Kalia & Lang, 2015). Recently, the clinical subtypes and prognoses of *de novo* PD patients in the early stages of the disease were identified using cluster analysis, with clinical features and biomarkers. Three subtypes were identified (i.e., mild motor-predominant, intermediate and diffuse malignant), which clearly differed in terms of their clinical characteristics and disease progression. The diffuse malignant subtype demonstrated the most severe motor impairment, most severe postural instability and gait problems, most impaired cognition and more rapidly disease progression, whereas the mild motor-predominant subtype demonstrated the least impaired motor and cognitive function (Fereshtehnejad *et al.*, 2017).

2.1.2 Atypical parkinsonism

Atypical parkinsonism syndromes are not a well-defined clinical entity but are usually thought to include syndromes of multiple system atrophy (MSA), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), and corticobasal degeneration (CBD) (Wenning *et al.*, 2011). MSA and PSP are sporadic neurodegenerative disorders that have relatively rapid disease progressions compared to

PD. MSA is characterized by parkinsonian features, as well as features associated with the autonomic nervous system, cerebellar function, and pyramidal tract, and it is categorized as a synucleinopathy along with PD and DLB, as it manifests with oligodendroglial inclusion pathology, with the key constitute of alpha-synuclein (Spillantini *et al.*, 1998; Wenning *et al.*, 2004). MSA can be divided into syndromes with core clinical motor signs related to either parkinsonism (MSA-P) or cerebellar signs, such as ataxia (MSA-C). MSA-P is often characterized by severe bradykinesia and rigidity, as well as postural tremor and orofacial of craniocervical dystonia, whereas other common features of the disorder are the early onset postural instability and dysautonomic features such as disabling postural hypotension (Wenning *et al.*, 1994; Wenning *et al.*, 2003; Christine & Aminoff, 2004).

PSP is neuropathogically characterized as an intracerebral tauopathy (Höglinger *et al.*, 2017). The classical clinical subtype of PSP includes vertical supranuclear gaze palsy, falls and symmetric parkinsonian motor disorder named as Richardson's syndrome or PSP-RS (Whitwell *et al.*, 2013; Höglinger *et al.*, 2017). However, PSP is also a heterogenous disorder with several phenotypes, and approximately one-third of patients develop a parkinsonism predominant syndrome of PSP (PSP-P phenotype), with asymmetrical motor signs, that get less severe by the use of antiparkinsonian drugs (Williams & Lees, 2010; Wenning *et al.*, 2011). From a diagnostic perspective, the differentiation of PSP-P patients from PD patients may be challenging, as PSP-P may lack classical PSP features, such as the vertical gaze palsy. The other clinical phenotypes of patients with autopsy-confirmed PSP manifest with ocular dysfunction, postural defects, cognitive or behavioral impairment, freezing of gait, corticobasal syndrome (CBS), ataxia, or aphasia/apraxia of speech (Höglinger *et al.*, 2017).

Dementia of Lewy bodies, DLB is the second most common cause of neurodegenerative dementia, after AD. Patients have Lewy pathology extensively in the CNS, otherwise the neuropathology is similar to PD (Geser *et al.*, 2005; Jellinger, 2009). The primary clinical features include progressive cognitive decline with fluctuating cognition, optical illusions, and parkinsonism. Most of the patients present with a bilateral parkinsonism, mainly manifested with bradykinesia and rigidity (Geser *et al.*, 2005).

Corticobasal degeneration, CBD is a neuropathological diagnosis characterized, similar to PSP, as a tauopathy (Dickson *et al.*, 2002). Recently, four clinical phenotypes of pathologically confirmed CBD were identified; corticobasal syndrome (CBS), progressive supranuclear palsy syndrome (PSPS), frontal behavioral-spatial syndrome (FBS) and nonfluent/agrammatic variant of primary progressive aphasia (naPPA). The most common characteristics of the different CBD syndromes are limb rigidity and bradykinesia, postural instability, gait difficulties and dystonia, unresponsive to dopaminergic treatment (Armstrong *et al.*, 2013).

2.1.3 Secondary parkinsonism

Secondary parkinsonism indicates a parkinsonism syndrome that is caused by a specific etiology other than PD or atypical parkinsonism disorders. There are numerous causes of secondary parkinsonism, ranging from structural lesion, toxic, metabolic (such as Wilson disease) and infection-related to psychogenic parkinsonism (Pfeiffer, 2007; Wenning *et al.*, 2011; Sage & Mark, 2015), whereas drug-induced parkinsonism (DIP) and vascular parkinsonism (VP) may be the most common etiologies (Hughes *et al.*, 1993; Christine & Aminoff, 2004; Savica *et al.*, 2017). The drugs most frequently associated with DIP are the typical antipsychotic medications, which are most commonly used to treat schizophrenia and psychosis. DIP patients may show both tremor-dominant and akinetic-rigid motor parkinsonism syndromes, with both symmetrical and asymmetrical symptoms (Shin & Chung, 2012; Savica *et al.*, 2017). VP can be clinically characterized as a symmetric parkinsonism syndrome, mainly manifested with bradykinesia, rigidity and difficulties with gait. The pathophysiology of this so-called 'lower body parkinsonism' is suggested to be associated with subcortical and periventricular white matter lesions in the thalamocortical projections. Another phenotype of VP has a more acute onset, and manifests as parkinsonism on the contralateral body side and striatal infarcts affecting the putaminopallido-thalamic loops (FitzGerald & Jankovic, 1989; Wenning *et al.*, 2011).

2.1.4 Tremor disorders

There are several pathological tremors, including resting tremor, intention tremor and action or postural tremor (Louis, 2001). The most common disorder that classically manifests with action tremor is essential tremor (ET), which is also the most frequent movement disorder (Thanvi *et al.*, 2006). The etiology of ET has a clear familial component, as the disorder seems to be inherited autosomal dominantly, and close relatives of the ET patients have approximately a fivefold risk to get the disorder. ET is classically known to present with upper extremity action tremor, but there may also be tremor in head and voice, or in the lower extremities (Louis, 2001; Louis *et al.*, 2001; Louis & Ferreira, 2010). The tremor is typically only slightly asymmetrical; however, it can also be markedly asymmetrical or even purely unilateral in a minority of patients, and some ET patients may even present with an upper extremity resting tremor (Clark & Louis, 2018).

The differential diagnosis for essential tremor includes PD, enhanced physiologic tremor, psychogenic tremor, dystonic tremor, Holmes tremor and Wilson's disease (Louis, 2001; Crawford & Zimmerman, 2011). Most PD patients develop a tremor during the disease course; while resting tremor is the classical and most frequent form of tremor, some PD patients also have pure kinetic and postural tremor phenotypes (Deuschl, 1999; Baumann,

2012). Enhanced physiologic tremor comes and goes with the use of certain medications, metabolic conditions, anxiety or stress (Louis, 2001; Crawford & Zimmerman, 2011), whereas psychogenic tremor is characterized by a sudden start and remission of the symptom, fluctuation of the symptoms, and variation in the type of tremor. Dystonic tremor usually affects younger patients and is described as an irregular and jerky tremor that stops in certain hand or arm positions. In addition, patients with dystonic tremor present with other signs of dystonia in the same body region (Crawford & Zimmerman, 2011). Wilson's disease is a metabolic syndrome with a possible neurological dysfunction that may begin in childhood or adolescence. Tremor is often the initial neurological feature of Wilson's disease, classically characterized as "wing-beating" proximal tremor of the upper extremities; however, it may also exist distally and be small in amplitude, and may appear as both rest and action tremors (Louis, 2001; Pfeiffer, 2007). Finally, Holmes tremor is caused by a CNS insult, such as head trauma, stroke or demyelinating diseases, and is clinically characterized by slow frequency rest, intention or postural tremor, along with other neurologic manifestations, such as hemiparesis or ataxia (Raina *et al.*, 2016).

2.2 Parkinsonism diagnostics

2.2.1 Clinical differential diagnosis and clinically uncertain parkinsonism

2.2.1.1 Parkinson's disease

The PD diagnostics is primarily clinical; however, the absolute diagnosis can be obtained only at autopsy (Braak *et al.*, 2003; Rizzo *et al.*, 2016). The most frequently used criteria for PD clinical diagnosis has been the UK brain bank criteria from the Parkinson's Disease Society Brain Bank (Hughes *et al.*, 1992; Postuma *et al.*, 2015). In these criteria, PD is characterized by motor signs of bradykinesia, rigidity, a resting tremor or postural instability, along with supportive features such as unilateral motor symptom onset, or a progressive nature of the symptoms. History of stroke, neuroleptic treatment or sustained remission are examples of exclusion criteria for PD (Hughes *et al.*, 1992). The MDS reviewed the diagnostic criteria of PD in 2015. Even if there was increased knowledge of the non-motor manifestations, which can even dominate the clinical presentation of PD patients, the clinical diagnosis was still defined by the core parkinsonian motor features. Diagnosis of a clinically established PD, according to these criteria, requires the cardinal motor features of bradykinesia combined with at least a resting tremor or rigidity, as well as the absence of absolute exclusion criteria and red flag signs, such as a rapid progression of gait impairment, which is more suggestive of atypical or secondary parkinsonism, and

the presence of at least two supportive criteria for PD, including e.g. a positive response to dopaminergic therapy and resting tremor of a limb (Table 1a) (Postuma *et al.*, 2015).

Table 1a. PD diagnosis by MDS. The table was modified from the Executive Summary/Completion Form for the PD diagnosis by Postuma *et al.,* 2015.

Essential criterion: parkinsonism (bradykinesia + rest tremor and/or rigidity)

Clinically Established PD requires
- Absence of absolute exclusion criteria
- At least two supportive criteria, and
- No red flags

Clinically Probable PD requires
- Absence of absolute exclusion criteria
- Presence of red flags counterbalanced by supportive criteria

Supportive criteria
- Clear and dramatic beneficial response to dopaminergic therapy
- Presence of levodopa-induced dyskinesia
- Rest tremor of a limb
- Presence of olfactory loss or cardiac sympathetic denervation on MIBG scintigraphy

Absolute exclusion criteria
- Unequivocal cerebellar abnormalities
- Downward vertical supranuclear gaze palsy, or selective slowing of downward vertical saccades
- Diagnosis of probable behavioral variant frontotemporal dementia or primary progressive aphasia within the first five years of the disease
- Parkinsonian features restricted to the lower limbs for more than 3 years
- Treatment with a dopamine receptor blocker or a dopamine-depleting agent in a dose and time-course consistent with DIP
- Absence of observable response to high-dose levodopa despite at least moderate severity of disease
- Unequivocal cortical sensory loss, clear limb apraxia, or progressive aphasia
- *Normal functional neuroimaging of the presynaptic dopaminergic system*
- An alternative condition known to produce parkinsonism

Red flags
- Rapid progression of gait impairment within 5 years of motor onset
- A complete absence of progression of motor symptoms over 5 or more years unless stability is related to treatment
- Early bulbar dysfunction or severe dysphagia within first 5 years
- Inspiratory respiratory dysfunction
- Severe autonomic failure such as orthostatic hypotension or severe urinary retention or urinary incontinence in the first 5 years of disease
- Recurrent falls because of impaired balance within 3 years of onset
- Disproportionate anterocollis or contractures of hand or feet within first 10 years
- Absence of any of the common nonmotor features despite 5 years disease duration
- Otherwise-unexplained pyramidal tract signs, defined as pyramidal weakness or clear pathologic hyperreflexia
- Bilateral symmetric parkinsonism

2.2.1.2 Other parkinsonism syndromes

Multiple system atrophy, MSA. The MSA diagnosis is mainly based on patient information and a clinical neurological examination. A clinical diagnosis of a probable or possible MSA was based on signs of autonomic dysfunctions, parkinsonism, and cerebellar and corticospinal tract dysfunctions, and the consideration of exclusion criteria, such as early onset of symptoms at a young age or at least one relative having an equal syndrome. Poor response to levodopa, orofacial dystonia or REM-sleep behavior disorder (RBD) were considered to point to MSA (Wenning *et al.*, 2004). The criteria were simplified in 2007 by Gilman *et al.* (Gilman *et al.,* 2008). In the revised criteria, clinical MSA was defined as a sporadic disease, with an onset in the adulthood, manifested with autonomic failure (required in clinically probable MSA) and cerebellar dysfunction or parkinsonism, as well as (in clinically possible MSA) presence of at least one feature suggesting autonomic dysfunction and at least one additional feature (Table 1b).

Table 1b. MSA diagnosis based on the simplified criteria by Gilman and colleagues. The table was combined and modified from the tables in Gilman *et al.,* 2008.

Essential criterion for clinically probable and possible MSA: a sporadic and progressive disease with an adult (>30 years) onset

Probable MSA requires
- Autonomic failure involving urinary incontinence or an orthostatic decrease of blood pressure within 3 minutes of standing by at least 30 mm Hg systolic or 15 mm Hg diastolic and
- Poorly levodopa-responsive parkinsonism or
- A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction)

Possible MSA requires
- Parkinsonism or
- A cerebellar syndrome and
- At least one feature suggesting autonomic dysfunction (otherwise unexplained urinary urgency, frequency or incomplete bladder emptying, erectile dysfunction, or significant orthostatic blood pressure decline that does not meet the level required in probable MSA) and
- At least one of the additional features

Additional features for possible MSA (C suggestive for MSA-C and P suggestive for MSA-P)
- Babinski sign with hyperreflexia (C/P)
- Stridor (C/P)
- Rapidly progressive parkinsonism (P), or parkinsonism (C)
- Poor response to levodopa (P)
- Postural instability within 3 years of motor onset (P)
- Gait ataxia, cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction (P)
- Dysphagia within 5 years of motor onset (P)
- Atrophy on MRI of putamen, middle cerebellar peduncle, pons (P/C), or cerebellum (P)
- Hypometabolism on FDG-PET in putamen, brainstem, or cerebellum (P), or only in putamen (C)
- *Presynaptic nigrostriatal dopaminergic denervation on SPECT or PET (C)**

Supporting features

- Orofacial dystonia
- Disproportionate antecollis
- Camptocormia (severe anterior flexion of the spine) and/or Pisa syndrome (sever lateral flexion of the spine)
- Contractures of hands or feet
- Inspiratory sighs
- Severe dysphonia
- Severe dysarthria
- New or increased snoring
- Cold hands and feet
- Pathologic laughter or crying
- Jerky, myoclonic postural/action tremor

Features not supporting the diagnosis

- Classic pill-rolling rest tremor
- Clinically significant neuropathy
- Hallucinations not induced by drugs
- Onset after age 75 years
- Family history of ataxia or parkinsonism
- Dementia
- White matter lesions suggesting multiple sclerosis

*The presynaptic dopaminergic deficiency is also present in MSA-P, but particularly helpful in the differential diagnosis of patients with predominant cerebellar signs (and no parkinsonism).

Progressive supranuclear palsy, PSP. Like PD and the other atypical parkinsonism syndromes, the absolute PSP diagnosis needs autopsy. A proposal for clinical criteria of PSP was outlined by the National Institute of Neurological Disorders and Stroke and Society in 1996. These clinical criteria defined probable PSP as a disorder manifested with ocular motor dysfunction and early onset of postural problems; possible PSP is as syndrome with either one of these features (Litvan *et al.*, 1996; Höglinger *et al.*, 2017). The early accurate clinical diagnosis for PSP has remained challenging; in addition, the previous clinical criteria primarily focused on the PSP-RS phenotype diagnosis. Furthermore, it is possible that the cardinal features not occur until the disease is in more advance stages. In the revised criteria by MDS in 2017, the main PSP signs were outlined to be ocular dysfunction, cognitive impairment, postural instability and akinesia. The mandatory inclusion criteria include that the family history does not reveal similar syndromes and the symptoms start in the adulthood, whereas the mandatory exclusion criteria are features more suggestive of other neurodegenerative parkinsonism disorders (Table 1c) (Höglinger *et al.*, 2017).

Table 1c. PSP diagnosis by MDS. The table was combined and modified from the tables in Höglinger *et al.,* 2017.

Inclusion criteria
1. Sporadic occurrence
2. Age 40 or older at onset of first PSP-related symptoms
3. Gradual progression of PSP-related symptoms

Core clinical features
1. Ocular motor dysfunction: vertical supranuclear gaze palsy, slow velocity of vertical saccades, or "eyelid opening apraxia"
2. Postural instability: repeated unprovoked falls within 3 years, or tendency to fall/more than two steps on the pull-test within 3 years
3. Akinesia: progressive gait freezing within 3 years, predominantly axial akinetic-rigid and levodopa resistant parkinsonism, or parkinsonism with symmetric or asymmetric tremor and/or levodopa responsive
4. Cognitive dysfunction: speech/language disorders, frontal cognitive/behavioral presentation, or CBS

Supportive features
1. Predominant midbrain atrophy or hypometabolism
2. *Postsynaptic striatal dopaminergic degeneration e.g. in [^{123}I]IBZM-SPECT*

Exclusion criteria
1. Impairment of episodic memory suggestive of AD
2. Autonomic failure suggestive of MSA or DLB
3. Visual hallucinations or fluctuations in alertness suggestive of DLB
4. Multisegmental upper and lower motor neuron signs suggestive of motor neuron disease
5. Sudden onset, step-wise or rapid progression of symptoms, in conjunction with corresponding imaging or laboratory findings, suggestive of vascular etiology, autoimmune encephalitis, metabolic encephalopathies, or prion disease
6. History of encephalitis
7. Prominent appendicular ataxia
8. Identifiable cause of postural instability
9. Severe leukoencephalopathy, evidenced by cerebral imaging
10. Relevant structural abnormality, e.g., normal pressure or obstructive hydrocephalus; basal ganglia, diencephalic, mesencephalic, pontine or medullary infarctions, hemorrhages, hypoxic-ischemic lesions, tumors, or malformations

Corticobasal degeneration, CBD. The antemortem clinical diagnoses of the different CBD clinical phenotypes are extremely challenging (Armstrong *et al.*, 2013). The clinical criteria for possible and probable clinical CBD phenotypes require insidious onset and gradual progression of the symptoms. Features suggestive of idiopathic PD, hallucinations more suggestive of PD or DLB than CBD are the exclusion criteria for CBD, as well as distinct autonomic or cerebellar features, that point to MSA. It seems that PSPS is more likely to represent PSP than CBD; however, it may also be a possible CBD clinical syndrome (Table 1d) (Armstrong *et al.*, 2013). Alexander and colleagues assessed the new criteria by Armstrong *et al.* in a cohort study of patients with neuropathological CBD diagnoses. The accuracy of the clinical CBD diagnosis was found to be the same when compared to previous criteria, but, however, the descriptions of the different clinical CBD phenotypes was valued (Alexander *et al*., 2014).

Table 1d. Diagnoses of the different clinical CBD phenotypes. The table was combined and modified from the tables in Alexander *et al.,* 2014, based on work by Armstrong *et al.,* 2013.

Probable CBS requires asymmetric presentation of signs 1-3 plus two signs out of signs 4-6; possible CBS may be symmetric, and requires one sign out of signs 1-3 plus one sign out of signs 4-6 1. Limb rigidity or akinesia 2. Limb dystonia 3. Limb myoclonus 4. Orobuccal or limb apraxia 5. Cortical sensory deficit 6. Alien limb phenomena
Frontal behavioral-spatial syndrome requires two signs out of signs 1-3 1. Executive dysfuction 2. Behavioral or personality changes 3. Visuospatial deficits
Non-fluent/agrammatic variant of primary progressive aphasia requires effortful, agrammatic speech plus at least one sign out of signs 1-2 1. Impaired grammar/sentence comprehension with relatively preserved single word comprehension 2. Groping, distorted speech production (apraxia of speech)
Progressive supranuclear palsy syndrome requires three signs out of signs 1-5 1. Axial or symmetric limb rigidity or akinesia 2. Postural instability or falls 3. Urinary incontinence 4. Behavioral changes 5. Supranuclear vertical gaze palsy or decreased vertical saccade velocity
Exclusion criteria for probable sporadic CBD and possible CBD is based on evidence of MSA, AD, amyotrophic lateral sclerosis, or structural lesions suggestive for focal cause

Dementia with Lewy bodies, DLB. It has been suggested that DLB and Parkinson's disease dementia (PDD) are different manifestations of the same disease entity, as the patients with DLB and PDD have similar clinical and pathological features (Friedman, 2018). The clinical criteria for probable and possible DLB were revised by the DLB Consortium in 2017. In these guidelines, both clinical features and supportive biomarkers can be used in the antemortem diagnosis of DLB. The core clinical features were specified as fluctuations in cognitive features, dementia, RBD, parkinsonian motor symptoms and visual hallucinations. It was underlined that in the early stages of the disease, a persistent cognitive defect may not (yet) be present, but, however, problems with executive functions and attention may be particularly prominent (Table 1e) (McKeith *et al.*, 2017).

Table 1e. DLB diagnosis by the DLB Consortium. The table was modified from the table in McKeith *et al.,* 2017.

Essential criterion: dementia

Probable DLB requires at least two clinical features, with or without presence of indicative biomarkers, or one core clinical feature, but with at least one indicative biomarker

Possible DLB requires one core clinical feature, with no indicative biomarker evidence, or at least one indicative biomarker, but no core clinical features

Core clinical features
- Fluctuating cognition with pronounced variations in attention and alertness
- Recurrent visual hallucinations, typically well-formed and detailed
- RBD (may precede cognitive decline)
- At least one spontaneous cardinal motor feature of parkinsonism

Supportive clinical features
- Severe sensitivity to antipsychotic agents
- Postural instability, repeated falls, syncope or other transient episodes of unresponsiveness
- Severe autonomic dysfunction, e.g., constipation, orthostatic hypotension, urinary incontinence
- Hypersomnia
- Hyposmia
- Hallucinations in other modalities, systematized delusions
- Apathy, anxiety, depression

Indicative (the first three) and supportive biomarkers (the last three)
- *Reduced dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET*
- Abnormal (low uptake) MIBG myocardial scintigraphy
- Polysomnographic confirmation of REM sleep without atonia
- Relative preservation of medial temporal lobe structures
- Generalized low uptake on SPECT/PET perfusion/metabolism scan with reduced occipital activity 6 the cingulate island sign on FDG-PET imaging
- Prominent posterior slow-wave activity on EEG with periodic fluctuations in the pre-alpha / theta range

DLB is less likely
- In the presence of any other physical illness or brain disorder including cerebrovascular disease, sufficient to account in part or in total for the clinical picture, although these do not exclude a DLB diagnosis and may serve to indicate mixed or multiple pathologies contributing to the clinical presentation, or
- If parkinsonian features are the only core clinical feature and appear for the first time at a stage of severe dementia

Vascular parkinsonism, VP. A recent review (Rektor *et al.*, 2018) of the VP subtypes and diagnostic approaches presented three VP subtypes. The first is the (sub)acute post-stroke VP that is typically characterized by symmetric parkinsonism, which responds well to dopaminergic drugs. Another type is more common, the insidious onset VP with severe postural impairment, problems with walking, neurological focal signs, and minor benefit from antiparkinsonian medications. The third subtype is a clinical syndrome with mixed or overlapping features of VP and PD or other neurodegenerative parkinsonism.

Drug-induced parkinsonism, DIP may lead to a parkinsonism syndrome that is often indistinguishable from PD. The DIP patients are mostly women and typically older than patients with PD (Shin & Chung, 2012). DIP is classically clinically characterized as a parkinsonism with bilateral and symmetric motor symptoms, dominated by bradykinesia and rigidity. However, approximately half of patients show asymmetrical symptoms and resting tremor, usually linked to PD (Shin & Chung, 2012). The diagnosis may also be challenging because it can be hard to find out all the medications the patient has used, and, moreover, DIP can occur sooner or later after the usage, and by different doses, of the drug inducing the symptoms (Kägi *et al.*, 2010). The clinical course is also heterogenous. DIP usually resolves, in a varying time frame, after the use of the drug causing the parkinsonian symptoms ends; however, parkinsonism is also suggested to persist or even progress in some patients (Shin & Chung, 2012).

2.2.1.3 Clinically uncertain parkinsonism syndrome

Clinically uncertain parkinsonism syndrome, CUPS is a description that was initially used by Catafau and colleagues. Patients were defined to have a CUPS if only one of the parkinsonian motor signs was present in the clinical examination, there was lack of bradykinesia, the patients also showed some abnormal motor features, had mild parkinsonian motor symptoms, the motor signs did not respond to levodopa, or there was no severing in the motor symptoms by time (Catafau *et al.*, 2004).

Despite the detailed criteria that can used to aid with the clinical differential diagnosis of PD and other parkinsonism disorders, an accurate clinical diagnosis of parkinsonism remains obscure. In a European multicenter study, PD was initially erroneously diagnosed in 13 of 28 patients without PD (Marshall *et al.*, 2009). It was recently confirmed that the accuracy of the overall clinical diagnosis of PD is 73.8%, and the initial clinical diagnosis accuracy is 79.6% for movement disorders experts (Rizzo *et al.*, 2016). It particularly seems that PD is clinically overdiagnosed. Most often the diagnoses should have been some other tremor disorder, atypical parkinsonism, secondary parkinsonism, or other dementia disorders (Rizzo *et al.*, 2016; Obeso *et al.*, 2017). In Finland, the exactness of the PD clinical diagnostics appears to be comparable with these findings, as it was only 75% for general neurologists. Furthermore, while PD was found to be heavily overdiagnosed, atypical parkinsonism syndromes were underdiagnosed (Joutsa *et al.*, 2014). The different tremor disorders may be clinically indistinguishable from PD, even if the examinations are performed by movement disorder specialists. Patients with atypical tremor, dystonic tremor or monosymptomatic resting tremor were hard to clinically distinct from tremor dominant PD patients, as the patients without PD or neurodegenerative parkinsonism showed also other parkinsonian motor features besides tremor (Bajaj *et al.*, 2010). Thus, the review and meta-analysis by Rizzo *et al.* highlighted

the need for easily accessible biomarkers to support the clinical diagnosis. Particularly in the early stages, the clinical core features can be mild or atypical, and the different parkinsonism syndromes show overlapping clinical features (Rizzo *et al.*, 2016).

2.2.2 Structural measurements in magnetic resonance imaging

Conventional magnetic resonance imaging (MRI) is widely used for brain structural assessment in the differential diagnosis of parkinsonism to exclude other etiologies, such as vascular lesions, multiple sclerosis and brain tumors. While conventional MRI displays most often normal in PD, an MRI scan may show structural diagnostic clues referring to atypical parkinsonism. MSA-P was described to show atrophy and signal alterations in the putamen, cerebellum and pons, and the patients may demonstrate a 'hot-cross bun' sign on an axial T2-weigtened MRI. Midbrain atrophy, however, is considered to identify especially patients with PSP. The "hummingbird" sign or the so-called "penguin silhouette" on a midsagittal MRI lateral view represent atrophy of the midbrain (Seppi & Poewe, 2010). In addition, in 3D T1-weighted MRI, a ratio between pons and midbrain areas (pons/midbrain) seems to distinguish between patients with PSP and PD (Longoni *et al.*, 2011). However, it was further suggested that midbrain atrophy would solely be a feature of the PSP-RS phenotype, as there was no association detected between midbrain measurements and the general neuropathological changes of PSP (Whitwell *et al.*, 2013). Thus, midbrain atrophy might not be able to be used in the diagnostics of the clinicopathologically diverse PSP disorder, or for example PSP-P. Furthermore, midbrain atrophy was shown to be present not only in patients with probable PSP but also in vascular parkinsonism (Choi *et al.*, 2011).

Massey and colleagues developed a midbrain to pons ratio measurement to be used to aid the differential diagnostics of parkinsonism. They showed, that PSP patients had smaller midbrain measurements and the midbrain to pons ratios compared to controls and MSA patients. In MSA, the pons measurements were smaller when compared to controls (Massey *et al.*, 2013). However, abnormal midbrain to pons ratios were also associated with older age in healthy controls and patients with PD. Older PD patients, in particular, showed more decreased midbrain and pons measurements (Morelli *et al.*, 2014). In 2002, Arnold and colleagues compared D_2 receptor function in ^{123}I-iodobenzamide SPECT with midbrain atrophy in possible or probable PSP-RS patients. The dopaminergic function was associated with the midbrain measurement in the structural MRIs, and it was suggested that a possible or probable PSP diagnosis can be verified by a reduced midbrain diameter (Arnold *et al.*, 2002). Thus, it could be possible that simple structural midbrain measurements could reflect the grade of dopaminergic deficiency.

2.2.3 Functional dopaminergic imaging

Positron emission tomography (PET) and SPECT imaging can be used to study the striatal dopamine deficiency in PD and atypical parkinsonism (Brooks, 2012). In a recent meta-analysis of 142 PET and SPECT studies, striatal dopaminergic tracer binding was on average half of the normal already in the early stages of PD, and there was no overlap when compared to healthy individuals (Kaasinen & Vahlberg, 2017).

Striatal dopamine function can be studied *in vivo* through the different phases of the dopaminergic neurotransmission with a number of tracers. The presynaptic dopaminergic neurotransmission path starts when tyrosine hydroxylase (TH) hyrdoxylases tyrosine to L-dopa. Then, L-dopa is decarboxylated by aromatic L-amino-acid decarboxylase (AADC) to dopamine. Next, vesicular monoamine transporter 2 (VMAT-2), takes dopamine to vesicles and further to the synaptic cleft. Finally, dopamine transporter (DAT) removes it back to the axon terminal (reuptake) (Figure 1) (Meiser *et al.*, 2013; Kaasinen & Vahlberg, 2017).

The vesicular monoamine transporter 2 can be studied with [^{18}F]FP-DTBZ PET, and the decarboxylation of levodopa to dopamine, and further passing over to synaptic vesicles, with 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine (FDOPA) PET (Obeso *et al.*, 2017). FDOPA is a commonly used tracer for PET and, similar to endogenous dopamine, converted to [^{18}F]fluorodopamine by AADC and then further transported to the synaptic vesicles by VMAT-2 (Leenders *et al.*, 1990; Kaasinen & Vahlberg, 2017). Thus, FDOPA studies both the function of the AADC and the subsequent transportation process (Leenders *et al.*, 1990; Eshuis *et al.*, 2009). Dopamine transporter, DAT can be studied with several tracers in PET and SPECT. Currently, SPECT techniques are more widely available over PET for every-day clinical practice (Ba & Martin, 2015). The most commonly used SPECT ligands are [^{123}I]FP-CIT, [^{99m}Tc]TRODAT-1 and [^{123}I]β-CIT, which all bind to the presynaptic DAT (Cummings *et al.*, 2011), whereas PET radioligands for DAT are e.g. [^{11}C]PE2I (Appel *et al.,* 2015), [^{18}F]FE-PE2I (Schou *et al.,* 2009) and [^{18}F]FECNT (Nye *et al.,* 2014).

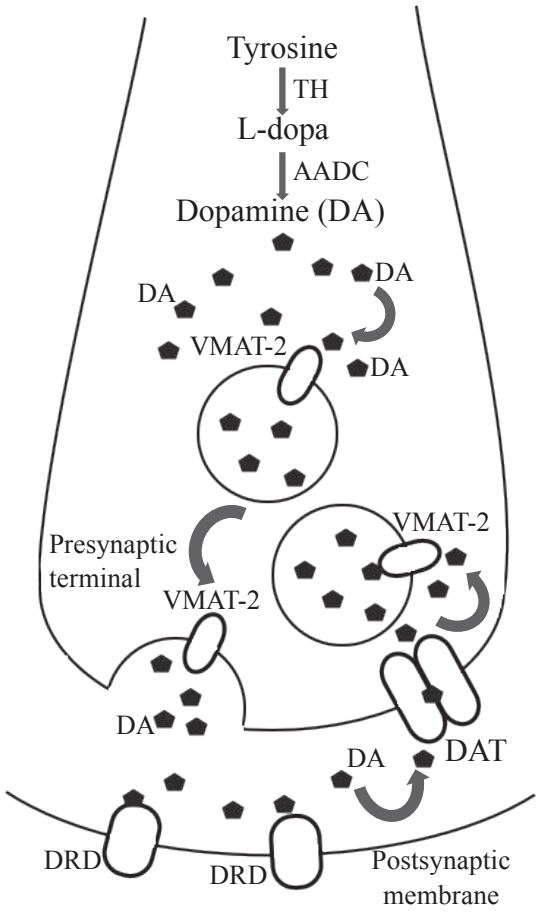

Figure 1. Striatal dopaminergic axon terminal, dopaminergic synapse and simplified presynaptic dopamine metabolism. DA = dopamine, TH = tyrosine hydroxylase, AADC = aromatic L-amino-acid decarboxylase, VMAT-2 = vesicular monoamine transporter 2, DAT = dopamine transporter, DRD = postsynaptic dopamine receptor.

2.3 DAT binding and [^{123}I]FP-CIT SPECT imaging

2.3.1 Dopamine transporter

Dopamine transporter (DAT) protein complex is present in the plasma membrane of dopamine-synthesizing neurons of the CNS, located predominantly in axon terminals (Ciliax et al., 1995; Nirenberg et al., 1996), but also in cell bodies and axons (Fazio, et al.

2018). The DATs are present mainly in the striatum, but there are lower levels of DATs also in amygdala, hypothalamus, hippocampus, thalamic nuclei and neocortex (Piccini, 2003).

As neurodegenerative parkinsonism is manifested with dopamine deficiency of the nigrostriatal dopaminergic cell bodies, axons and axon terminals, it occurs as a presynaptic parkinsonism, associated with reduced striatal DAT binding in the neuron terminals, both in PD (Kaufman & Madras, 1991; Niznik et al., 1991; Benamer et al., 2000b), as well as in atypical parkinsonism syndromes (Cummings et al., 2011). Striatal DAT binding was associated with the amount of substantia nigra dopaminergic neurons in mixed samples of patients with neurodegenerative parkinsonism (Colloby et al., 2012, Kraemmer et al., 2014). However, opposite findings were recently found in patients with PD, as the DAT uptake in putamen was not associated with the density of TH- or neuromelanin-containing dopamine producing neurons in the SNc, questioning whether striatal DAT imaging is able to reflect the nigral dopaminergic function (Saari et al., 2017). As a reaction to the synaptic dopamine loss, at least some of the DATs may lose, at least partly, their reuptake function in the axon terminals that are not yet degenerated (Ba & Martin, 2015). Thus, DAT imaging could reflect the dysfunction of DATs in addition to the deficiency of the dopaminergic nigrostriatal neurons and neuron terminals (Eshuis et al., 2009).

2.3.2 [^{123}I]FP-CIT SPECT imaging

The most commonly used DAT imaging tracer is [^{123}I]FP-CIT (Varrone et al., 2013; Albert et al., 2016). [^{123}I]FP-CIT SPECT was approved by the United States Food and Drug Administration (FDA) in 2011 for the differential diagnosis between ET and neurodegenerative parkinsonism, as DAT scans are usually normal in ET (Cummings et al., 2011). The European Association of Nuclear Medicine (EANM) further states, that [^{123}I]FP-CIT SPECT can be used in the differential diagnostics of DLB and other dementia disorders, in the establishment of early neurodegenerative parkinsonism diagnosis, in interpreting the disease severity of PD, and in distinguishing between neurodegenerative and symptomatic parkinsonism (Darcourt et al., 2010). DAT SPECT seems to be more accurate in differentiating DLB or PDD from AD in neuropathologically confirmed cases compared to the clinical diagnosis (Cummings et al., 2011; Walker et al., 2007). [^{123}I]FP-CIT SPECT can also be helpful in differentiating between PD and dystonic tremor or psychogenic parkinsonism. However, its ability to distinguish VP from PD is debatable, as some patients with VP may also have abnormal DAT scans (Cummings et al., 2011; Ba & Martin, 2015; Tolosa et al., 2003). A slightly and symmetrically lowered DAT binding, or a unilateral DAT deficiency in the same location as the CNS infarct, can be present in patients with VP (Kägi et al., 2010).

Functional dopaminergic imaging cannot aid in distinguishing between PD and atypical parkinsonism, even if the DAT binding loss may be more symmetrical in MSA and PSP compared to PD (Ba & Martin, 2015). In patients with CBS, the striatal DAT uptake seems to be reduced throughout the striatum in a more uniform way, and there may be greater hemispheric asymmetry in the DAT binding compared to PD, however, some CBS patients may even show bilaterally normal striatal DAT binding (Cilia et al., 2011). In patients with DLB, the striatal tracer uptake seems to be similar to PD (Kägi et al., 2010). It should be noted that there are some medications, e.g. cocaine, methylphenidate, bupropion and modafinil, that affect the presynaptic dopaminergic imaging outcome. Also some aminergic drugs, such as selective serotonin reuptake inhibitors, amantadine and ephedrine, may affect DAT binding (Ba & Martin, 2015). However, it seems that anti-parkinsonian medications cannot relevantly affect striatal DAT uptake (Cummings et al., 2011).

The [^{123}I]FP-CIT SPECT is well established in the parkinsonism diagnostics, and its accuracy to detect DAT deficiency is suggested to be almost perfect. Thus, a normal DAT scan is considered strongly to support evidence against PD or other neurodegenerative parkinsonism, whereas a pathological scan point to neurodegenerative presynaptic parkinsonism (Kägi *et al.*, 2010; Cummings *et al.*, 2011; Suwijn *et al.*, 2015). In a review of two multicenter [^{123}I]FP-CIT SPECT clinical trials, an abnormal baseline DAT scan supported a clinical diagnosis of neurodegenerative parkinsonism syndrome after three years of follow-up in 78-97% of cases, whereas the patients with normal initial DAT scans showed no evidence of neurodegenerative parkinsonism in 74-97% of cases after the same follow-up time interval (Hauser & Grosset, 2012). However, although DAT imaging is accurate, it is not absolute. In fact, a relatively high prevalence of scans without evidence of dopaminergic deficit (SWEDD) was reported in PD drug trials (Parkinson Study Group, 2002; Whone *et al.*, 2003). A SWEDD means a normal DAT scan in a patient with a clinical PD diagnosis (Erro *et al.*, 2016). As an example, one study reported sixteen PD patients with asymmetric arm rest tremor and with SWEDDs. These patients were clinically followed for 5 years or more, and during the follow-up, the striatal DAT uptake was reduced in two patients only (Batla *et al.*, 2014). Thus, there is a possibility that some of the patients with SWEDDs have PD, as some of these patients do benefit of L-dopa and the symptoms seem to get more severe by the disease course (Erro *et al.*, 2016). However, a longitudinal study (Marek *et al.,* 2014) with a large sample of patients with SWEDDs and patients with abnormal striatal DAT binding showed, that the patients with SWEDDs have only minor changes in DAT binding after a 22-months follow up. In addition, these patients showed no progression in the motor impairment, and only few of them received dopaminergic treatment at the end the follow-up, suggesting these patients to have other conditions that PD (Marek *et al.,* 2014).

2.3.3 Analysis of [^{123}I]FP-CIT SPECT scans

2.3.3.1 Visual analysis

The visual analysis of the striatal DAT binding depends on the expertise of the rater and may vary between observers (Scherfler & Nocker, 2009). In most of the neurodegenerative parkinsonism syndromes, DAT deficiency is initially detected in the posterior parts of the putamina, from which it spreads anteriorly to the caudate nuclei. In a visual inspection, this advancing striatal DAT deficiency can be characterized as 'from comma to a dot' by disease progression (Staff et al., 2009). Benamer *et al.* provided a predefined grading system with four grades of [^{123}I]FP-CIT SPECT images, which were presented to the visual scan observers. A normal scan was described to have a symmetrically normal DAT binding in both striata, whereas the abnormal images were categorized into three grades with a decreasing tracer uptake (Benamer et al., 2000b). A similar categorization approach was used later in similar research protocols (Staff et al., 2009; Kupsch et al., 2012).

2.3.3.2 Region of interest and voxel-based analyses

The ROI analysis is based on semi-quantitative measurements of the striatal DAT signal. The ROIs are outlined to the striatum and a reference region, lacking of DATs (Scherfler & Nocker, 2009). As it has been found that the [^{123}I]FP-CIT binding values are practically identical between the occipital cortex and cerebellum, choosing the reference region from these two regions seems to be irrelevant as to a clinical setting (Joutsa et al., 2015). However, some movement disorders such as MSA-C are associated with cerebellar atrophy, which could bias the estimate. Thus, in our studies, we chose the occipital cortex as the reference region. Before automated solutions, the ROIs were manually drawn by an investigator, leading to possible variability between the investigators. Currently, several operators carry independent automated approaches available to define ROIs. These techniques involve a registration process for the DAT SPECT images to a control template, and provide age-matched reference values for each ROI (Scherfler & Nocker, 2009).

Voxel-based approaches are not validated or recommended for routine scan analysis in the clinical practice, but they are valuable research tools. Several freely available software programs can be used for voxel-based analyses (refs/urls: FSL, AFNI, SPM, Freesurfer). These programs include tools for image registration and warping to a predefined image template space. The images are typically smoothed to reduce the effects of anatomic variability and to improve signal-to-noise ratio for the statistical analysis (Scherfler &

Nocker, 2009). The programs usually apply a general linear model that enables fairly complex statistical analyses. As a single brain image typically contains thousands of voxels, a statistical correction for multiple comparisons considering the spatial dependency of brain voxels must be applied to avoid inflation of the type I error, the so-called false positives.

2.3.3.3 The diagnostic accuracy of scan analyses

The initial interpretation of the DAT scan is based on visual analysis, but quantitative methods are often used to support the visual approach (Badiavas *et al.*, 2011). One of the validated automated methods is a ROI analysis used in the BRASS software by Hermes Medical Solutions, Stockholm. This method registers the image data to a template and applies ROIs over the striatum and reference region. The specific binding ratio (SBR) of DAT binding are calculated as SBR = ($ROI_{caudate\ or\ putamen}$ - $ROI_{occipital}$) / $ROI_{occipital}$ (Varrone *et al.*, 2013). A European Normal Control Database of DaTSCAN was generated in the ENC-DAT study, where the effects of age and gender on DAT uptake were also considered (Varrone *et al.*, 2013). In addition, Tossici-Bolt and colleagues further generated cross-calibration factors for different SPECT cameras (Tossici-Bolt *et al.*, 2011). Later, the age- and camera-corrected evaluation modes in the BRASS software were shown to provide a notable improvement in the diagnostic accuracy of [^{123}I]FP-CIT SPECT (Albert *et al.*, 2016). There are also other semi-quantitative commercial software programs used for the DAT scan analysis, such as the DaTView software program by Nihon Medi-Physics, Tokyo, Japan (Tossici-Bolt *et al.,* 2006), and DaTQUANT by GE Healthcare, Little Chalfont, UK, using the template developed in the ENC-DAT study (Varrone *et al.*, 2013, Yokoyama *et al.*, 2017).

One study suggested that less experienced observers tend to over-report normal scans as abnormal, but the use of both visual and semi-quantitative analysis methods result in more reproducible analyses of [^{123}I]FP-CIT SPECT scans (Söderlund *et al.*, 2013). The semi-quantitative analysis method was preferred to the visual analysis due to its greater accuracy in detecting the abnormal DAT binding (Filippi *et al.*, 2008), but it has also been suggested that the visual analysis is more suitable in everyday diagnostics (Benamer *et al.*, 2000b). In a multicenter [^{123}I]FP-CIT SPECT study of a large sample of patients with different diagnoses, the DAT images were analyzed visually in the different centers, blinded to the clinical patient information. In addition, a consensus analysis by a panel of five blinded readers was undertaken. The accuracy of both neurodegenerative parkinsonism diagnoses as well as the ET diagnoses were high in both the visual and the semi-quantitative analyses (Benamer *et al.*, 2000b). The visual analyses, tested on standardized semi-quantitative slab view displays of DAT SPECT images, showed an excellent inter-rater agreement between the observers and agreed with the semi-

quantitative analysis in 90% of cases. Moreover, there were no differences between the scan readers without experience and the more experienced readers (Buchert *et al.*, 2015). As there may be some discrepancy between the scan analysis outcomes by the different analysis methods, it is still uncertain how the scans exactly should be interpreted, and, moreover, how should the patients with discrepant scan analyses be diagnosed.

2.3.4 Clinical correlations and outcomes of DAT binding

2.3.4.1 Disease severity, motor signs and DAT binding

Motor rating scales. The Unified Parkinson's Disease Rating Scale (UPDRS) standardized the PD motor examination. Its revision, the MDS-UPDRS with four parts, was sponsored by the MDS and published in 2008. Part III of the MDS-UPDRS is the motor examination, which includes clinical assessments of speech, facial expression, posture, postural instability, rigidity, bradykinesia and tremor (Goetz *et al.*, 2008). The diagnostic criteria and definitions of the PD motor signs were recently reviewed by the MDS task force (Postuma *et al.*, 2015): bradykinesia means "slowness of movement and decreased amplitude or speed, or progressive hesitations/halts, as movement is continued", and can be evaluated in the MDS-UPDRS in sections of finger tapping, hand movements, pronation/supination movements, toe and foot tapping. Rigidity was referred to as "slow passive movement of major joints when the patient is in a relaxed position, with the examiner manipulating the limbs and neck". It was further outlined that an isolated 'cogwheel' rigidity without 'lead-pipe' rigidity cannot be evaluated as parkinsonian rigidity. Finally, a resting tremor was defined as a tremor of extremity or extremities, that is present in rest and attenuated in action (Postuma *et al.*, 2015). Another commonly used rating scale of parkinsonism, the Hoehn & Yahr (H&Y) stage (Hoehn & Yahr, 1967), was originally designed as a straightforward grading of the motor signs and symptoms in PD patients. In this scale, the stage of the motor status is graded into different categories, ranging from mild asymmetrical signs to symmetrical signs with postural defects, and further to a severing, bedridden motor condition. Subsequently, a modified H&Y scale with middle grades was created by MDS (Goetz *et al.*, 2004).

Disease severity. The motor parkinsonian signs appear when the DAT uptake in the posterior putamen is already half of the normal (Morrish *et al.*, 1998; Brooks & Tambasco, 2016). In PD, DAT binding was related both to the duration of the motor signs and the motor disease severity, assessed with the original UPDRS rating scale (Benamer *et al.*, 2000a). DAT binding in both the contralateral and ipsilateral striata were found to be associated with the body side of more severe motor impairment (Seibyl *et al.*, 1995). Furthermore, DAT binding in the contralateral striatum to the body side with worse

clinical signs correlated with the original UPDRS score at baseline but even more significantly with the UPDRS score at the 1-year time point of the follow-up, and also with the difference between the UPDRS scores of the 1-year interval (Djaldetti *et al.*, 2009). Out of 288 patients scanned with DAT SPECT, 51 had a clinical diagnosis of a probable-DLB after a clinical follow-up of 16 +/- 11.6 months. In these DLB patients, neither the H&Y stage nor the Mini-mental stage examination score (MMSE) (Folstein *et al.*, 1975) correlated with the striatal DAT uptake in ^{123}I-PE2I SPECT (Ziebell *et al.*, 2013).

Recently, *de novo* PD patients with the most severe motor impairment also showed a more severe striatal dopaminergic deficiency in SPECT, as well as more rapid disease progression, whereas patients with less severe motor impairment and less rapid progression showed a milder defect in both putamen and caudate DAT binding in the DAT scans (Fereshtehnejad *et al.*, 2017). In a longitudinal study with repeated DAT SPECT imaging and UPDRS motor examinations for PD patients, a correlation was seen between the reduced putamen DAT binding and original UPDRS score at baseline, and, at the end of the follow-up, both putamen and caudate DAT binding in the final DAT scans correlated with the final UPDRS scores. However, there were no correlations between the annual percentage change in the putamen, caudate or total striatal DAT binding and the clinical progression of the disease severity measured by the annual change in either the total UPDRS or motor UPDRS scores. Furthermore, patient age and the severity of the DAT deficiency in the initial scan were significant predictors of the rate of the annual percentage reduce in the tracer uptake, whereas the duration of the disease from diagnosis to the initial scan, initial motor symptoms with or without tremor or the initial UPDRS score did not influence the rate of the future DAT deficiency (Marek *et al.*, 2001). Finally, it was recently noticed, that the correlation between DAT deficiency and stage of the motor symptoms is linear in cross-sectional studies, but, however, most longitudinal studies suggest that the decline in dopamine loss is not linear but shows a negative exponential progression pattern (Kaasinen & Vahlberg, 2017).

Individual motor signs. In an earlier study (Pirker, 2003), the motor signs in the original UPDRS were categorized into subscores of speech, facial expression, tremor, rigidity, bradykinesia (finger taps, hand movements, pronation/supination, leg agility and body bradykinesia) and axial symptoms (arising from chair, gait, postural stability and posture). Striatal DAT binding was analyzed semi-quantitatively and normalized for age. Good correlations were reported between the general motor impairment and striatal DAT uptake. Of the individual motor signs, DAT binding was particularly associated with bradykinesia but less closely with rigidity. Good correlations were also observed between speech and facial expression and DAT uptake. It was verified that there are no correlations between any type of parkinsonian tremor and striatal DAT binding (Pirker, 2003). However, there is a lack of studies that have studied the associations between striatal DAT

binding and the individual motor signs of parkinsonism in patients with clinically uncertain parkinsonism or tremor.

2.3.4.2 Other demographical and clinical variables and DAT binding

Aging is independently related to DAT deficiency. In the European multicenter database of healthy controls, DAT binding in [^{123}I]FP-CIT SPECT was physiologically lowered by age, and the age effect was particularly observed in the caudate nucleus DAT uptake. Gender was also found to be associated with DAT binding (Varrone *et al.*, 2013; Kaasinen *et al.*, 2015a).

Current evidence shows that dementia may eventually affect up to eight PD patients out of ten (Hely *et al.*, 2008; Obeso *et al.*, 2017). In addition, many PD patients present with cognitive problems (impairment that does not fulfill the PDD diagnosis) (Litvan *et al.*, 2012), already in the early stages of the disease (Obeso *et al.*, 2017). Besides the cortical Lewy pathology, it seems that some of the PD patients also show features of AD neuropathology, and amyloid plaques have been found in most patients with DLB (Edison *et al.*, 2008; Obeso *et al.*, 2017). In fact, the cognitive decline and dementia in PD may actually be due to multidimensional neuropathological features (Obeso *et al.*, 2017). In a study of patients with DLB and AD, only the motor parkinsonism symptoms correlated with the DAT uptake in patients with DLB, whereas cognition, visual delusions or RBD did not (Shimizu *et al.*, 2017).

2.3.4.3 Survival in PD in relation to demographic and clinical characteristics and treatment

The ratio of mortality seems to be approximately 1.2 – 2.4 in patients with PD when compared to control subjects. The older age at onset and presence of dementia were the most consistent independent predictors of decreased survival (Macleod *et al.*, 2014). The incidence of dementia in particular, as well as the total UPDRS motor score, were associated with higher mortality in PD in one cohort study (Levy *et al.*, 2002). In another study of 414 PD patients, the predictors of mortality were older age, cognitive impairment, male gender, and a more severe PIGD (de Lau *et al.*, 2014). Male gender had also previously been linked to decreased survival in PD (Willis *et al.*, 2012). One study showed that the motor PIGD subtype as well as the symmetry of motor signs were associated with higher mortality risk in patients with early PD, whereas in this study gender had no effect on survival (Lo *et al.*, 2009). Finally, levodopa do not seem to be toxic or to increase the mortality rate in PD (Olanow, 2015). In addition, an earlier meta-analysis of five long-term prospective randomized trials demonstrated that there seems to

be no increased mortality in PD associated with selegiline treatment, regardless of whether patients were also receiving levodopa (Olanow *et al.*, 1998).

2.3.4.4 Survival in PD in relation to striatal dopamine function

In a longitudinal [^{123}I]β-CIT SPECT study with PD patients with abnormal DAT binding, a lower striatal DAT uptake independently pointed to an increased risk of a more severe motor and cognitive impairment, psychotic symptoms, and depression. Another DAT scan was taken after 22 months as part of the clinical follow-up, and the level of striatal DAT binding deficiency was again found to be independently associated with the motor, cognitive and behavioral outcomes (Ravina *et al.*, 2012). Even if a more severe presynaptic dopaminergic defect is associated with a higher risk of more severe outcomes in the clinical symptoms, it remains unclear whether the level of striatal dopaminergic neurodegeneration is associated with earlier or higher risk of mortality in PD. In an earlier PET study, the mortality of PD patients was not found to be related to the severity of the AADC dysfunction, reflecting the presynaptic dopamine synthesis defect. The study was first to investigate the possible associations between survival and striatal dopaminergic deficiency in PD, and included 88 unmedicated PD patients who were scanned with FDOPA PET. Nevertheless, older age and a more severe motor impairment were associated with decreased survival in these PD patients (Järvelä *et al.*, 2014), according to earlier results already described in *2.3.4.3*. It was considered that e.g. the variability in compensatory upregulation of FDOPA uptake could interfere the results. Namely, the FDOPA uptake can also depend on compensatory up-regulation of the AADC enzyme activity. Therefore, the analysis of the association with survival in PD patients might be influenced by adaptive metabolic changes in the pre-synaptic terminals. On the other hand, the DAT binding could be considered to be more directly related to the integrity of the pre-synaptic terminals, associated with the progression of the disease. The relationship between DAT availability and survival of PD patients remains to be investigated, and will be specifically examined in this thesis.

3 STUDY OBJECTIVES

The study objectives were to investigate the characteristics of striatal DAT binding and its associations with clinical signs and outcomes in parkinsonism patients scanned with [^{123}I]FP-CIT SPECT.

The retrospective studies investigated the effect of midbrain atrophy, measured with midbrain-to-pons ratios in conventional MRIs, on the level of striatal dopaminergic deficiency in PD (study I), the pitfalls and reliability of the DAT SPECT image analyses (study II), and the effects of the level of DAT deficiency and clinical factors on the future survival of PD patients (study III).

The cross-sectional study design included a clinical examination prior to the DAT imaging to investigate associations between the parkinsonian motor signs and striatal DAT deficiency in clinically uncertain parkinsonism and tremor. The possible associations
between the severity of these motor signs and dopamine loss in specific striatal subregions were further investigated in patients with abnormal DAT binding (study IV).

The following were the specific objectives:

Retrospective studies

I to investigate whether patients with PD show significantly lowered midbrain-to-pons ratios associated with the level of striatal DAT deficiency,

II to investigate [^{123}I]FP-CIT SPECT cases with discrepancies between visual and automated scan analyses, and the role of reader expertise in the visual analysis,

III to investigate whether the level of DAT deficiency can be used to predict survival in PD, and

Cross-sectional study

IV to identify the parkinsonian motor signs that point to striatal DAT deficiency in patients with clinically uncertain parkinsonism and tremor, and whether the severity of these signs is associated with DAT binding in specific parts of the striatum in neurodegenerative presynaptic parkinsonism.

4 PATIENTS AND METHODS

4.1 Overall study design

The retrospective studies (studies I – III) were based on the 'FKPAR' database (Kaasinen *et al.*, 2014), and the cross-sectional clinical and imaging study (study IV) was part of a larger prospective study ('NMDAT', ClinicalTrials.gov Identifier: NCT02650843). The studies were approved by the Ethics Committees of the Hospital District of the Southwest Finland (permission numbers 53/1802/2013 for FKPAR and 145/1801/2013 for NMDAT, respectively) and by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (NMDAT), and the studies were conducted according to the principles of Helsinki. In study IV, written informed consent was obtained from all participants prior to the study.

4.2 Patients

All patients included in this thesis work were scanned with [^{123}I]FP-CIT SPECT to assist with the differential diagnosis of clinically uncertain parkinsonism or tremor by their treating physician, most often a general neurologist. Note that the patients cannot be considered as fully comparable to CUPS patients (Catafau *et al.*, 2004), described in chapter *2.2.1.3*.

4.2.1 Retrospective studies (I-III)

Retrospective studies I, II and III are based on the FKPAR database of 559 patients scanned with [^{123}I]FP-CIT SPECT at the Department of Nuclear Medicine Turku University Hospital, Turku, Finland in 2007 – 2012 (Figure 2). The clinical information of the patients was collected, and the current clinical diagnoses were revised in March 2013, with a clinical follow-up 0,25 – 6,25 years after the [^{123}I]FP-CIT SPECT imaging (Kaasinen *et al.*, 2014).

Study I. A total of 150 PD patients with abnormal [^{123}I]FP-CIT SPECT scans and 155 patients with normal scans, who also had undergone a conventional brain 1.5T MRI, were selected in the study sample.

Study II. A total of 489 patients whose DAT SPECT imaging data were available for visualizations, had available semi-quantitative BRASS analysis scan results and sufficiently clinical and demographical patient information, including the age at scan,

gender, scanner, duration of motor symptoms prior to the SPECT imaging, predominant body side of the motor symptoms, presence of tremor, and clinical diagnosis in the follow-up after the DAT SPECT imaging, were initially selected for the study II.

The DAT SPECT scans of these patients were analyzed visually and categorized into different categories of striatal DAT binding deficiency, modified from the earlier research papers as described in chapters *4.3.3.1 – 4.3.3.2*. The patients in each of these categories were counted, and the data was sorted randomly using the sort randomly -tool in Microsoft Excel spreadsheet (Microsoft Corporation, USA). Ten different samples, each including 120 consecutive patients, were randomly extracted from the different parts of the previous randomly sorted list of patients. One of the extracted samples with 120 patients and with the most representative distribution of striatal DAT binding categories in the DAT SPECT scans, when compared to the initial sample of 489 patients, was selected as the final study sample for study II. The selection of the final study sample was blinded to patients' clinical information.

Study III. A total of 162 patients with a diagnosis of idiopathic PD, sufficient clinical information available to verify the clinical PD diagnosis and abnormal [^{123}I]FP-CIT SPECT scans were selected as the study sample of study III. Sixty-nine PD patients with abnormal DAT binding were excluded from the analyses due to insufficient clinical information (e.g. unknown levodopa response, unknown progression after DAT scan).

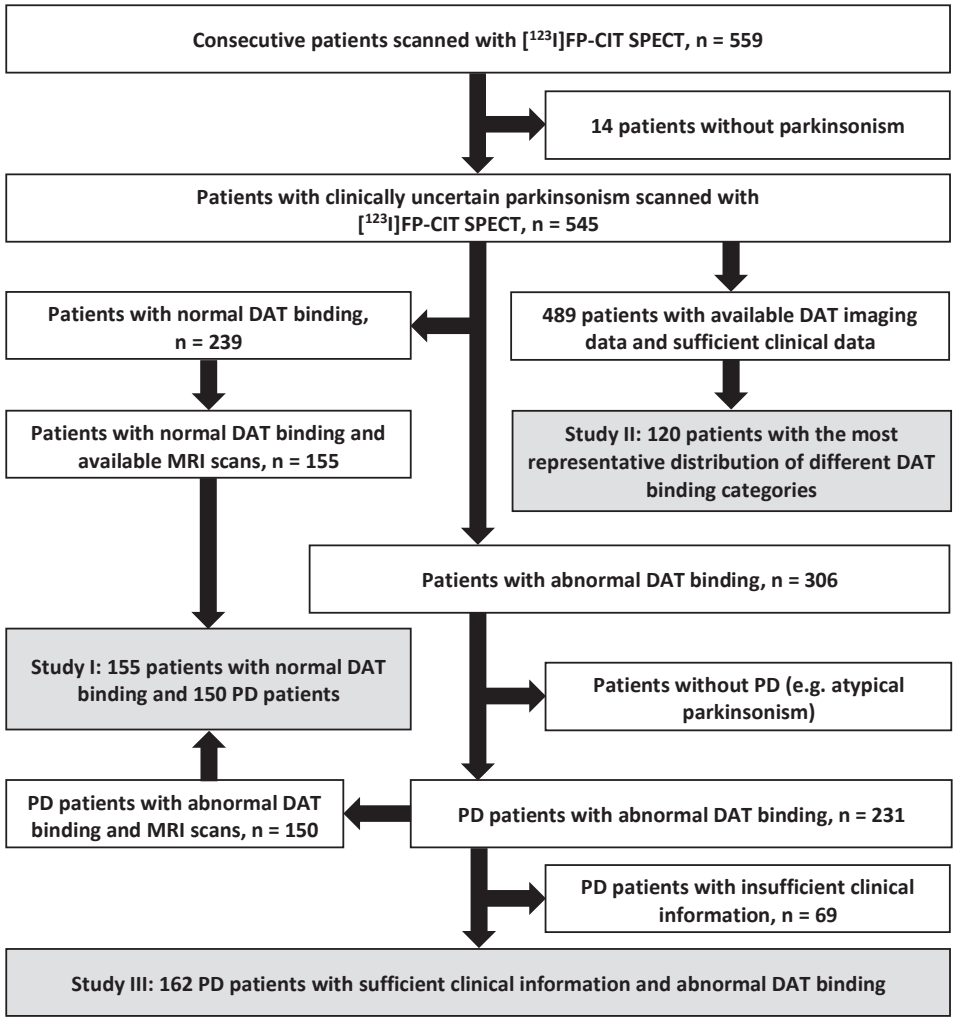

Figure 2. A study flow chart of the retrospective studies I, II and III.

4.2.2 Cross-sectional clinical and imaging study (study IV)

The study sample of study IV consisted of 221 consecutive patients who participated in the NMDAT study in 2014 – 2017 at the Department of Nuclear Medicine at Turku University Hospital or at Helsinki University Hospital, Finland. The clinical examinations were performed prior to the [^{123}I]FP-CIT SPECT imaging. The investigators had passed the MDS-UPDRS Training Program and Exercise (Goetz *et al.*, 2010). Of these 221 patients, 178 (80.5%) were scanned and examined in Turku, and 43 (19.5%) were scanned and examined in Helsinki. Most often, the DAT SPECT scan was ordered to confirm or exclude an atypical or incomplete clinical idiopathic PD diagnosis (n = 167), or to exclude

or confirm a probable or possible clinical MSA, CBD or PSP diagnosis (n = 27). Other reasons for scanning were the differential diagnosis between PD and ET (n = 8), PD and DIP (n = 9), PD and VP (n = 3), DLB and other dementias (n = 6), and PD or secondary parkinsonism due to a brain tumor (n = 1). According to the DAT SPECT imaging study protocol, the medications that bind to DAT and that might interfere the imaging outcome and scan analysis, were paused prior to imaging. However, the antiparkinsonian medications were not paused, as they do not seem to affect the DAT binding (Cummings *et al.*, 2011). Five patients with incomplete motor examinations and/or unsuccessful automated semi-quantitative analyses of the DAT scans were excluded from the analyses.

4.3 Methods

4.3.1 DAT SPECT imaging, image reconstructions and BRASS analyses

4.3.1.1 [^{123}I]FP-CIT SPECT imaging

To prevent accumulation of radiation to the thyroid gland, an oral dose of 250-300 mg of 1% potassium perchlorate was given 30-60 min before the intravenous [^{123}I]FP-CIT tracer injection, with an activity of 185 MBq. The scanning was performed approximately 4 hours after the tracer injection.

In retrospective studies I-III, either a GE Infinia II Hawkeye SPECT/CT scanner with 3/8-inch thick crystals (GE Medical Systems, Milwaukee, WI, USA) or a Picker Irix gamma camera with 3/4-inch thick crystals (Picker International, Uniontown, OH, USA) were used for the [^{123}I]FP-CIT SPECT imaging in the Department of Nuclear Medicine, Turku University Hospital, Turku, Finland (Kaasinen *et al.*, 2014).

In study IV, patients who were scanned and examined in Turku, were scanned with two GE Infinia II Hawkeye SPECT/CT scanners (GE Healthcare, Tirat Hacarmel, Israel), and of the patients who were scanned and examined in Helsinki, 41 were scanned using Philips Brightview XCT (Philips Healthcare, Eindhoven, The Netherlands) and two patients were scanned using a Siemens Symbia T2 (Siemens Healthineers, Erlangen, Germany) SPECT/CT scanner.

All SPECT scanners were dual-head scanners with low-energy high-resolution collimators. In studies I-III, a circular orbit with 120 projections and 25 s scanning time per view was acquired during 180° of rotation. A 64 x 64 matrix size, a zoom of 2.0 and a photopeak energy window at 159 keV ± 10% were used (Kaasinen *et al.*, 2014). In study IV, the energy window was 159 keV ± 10% for the GE Infinia and the Brightview and

159 keV ± 7.5 % for the Symbia, the acquisition matrix size was 128x128 for all scanners, and the rotation arc per each scanner head was 180° in the step-and-shoot mode, and the angular step was 3°, resulting in 60 projections for each scanner head and a total of 120 projections. For the Brightview and Symbia, the acquisition zoom was 1.46, and the time per projection was 30 s, whereas the corresponding values for the GE Infinia were 1.25 and 30 s, respectively.

4.3.1.2 Image reconstructions

The SPECT scans were reconstructed using the three-dimensional (3D) ordered-subset expectation algorithm in the Hybrid Recon Neurology software program by Hermes Medical Solutions AB, Stockholm, Sweden. In studies I-III, version 1.0.15 (Kaasinen *et al.*, 2014) was used, and in study IV, version 1.3, was used; both versions included 16 iterations, four subsets, uniform attenuation correction with the attenuation coefficient of $\mu = 0.146$ 1/cm, collimator response correction using Gaussian diffusion model, Monte-Carlo-based scatter correction for the ^{123}I isotope and 3D Gaussian postfiltering, with a full-width-at-half-maximum of 0.7 cm (Kaasinen *et al.*, 2014).

4.3.1.3 BRASS analyses

In all four studies, automated semi-quantitative analyses of the scans were performed with the BRASS software by Hermes Medical Solutions AB Stockholm, Sweden. In studies I-III, version 3.6 was used, whereas version 2.6H was used in study IV. Co-registration of the images was visually verified by the investigators. Before scanning, the SPECT scanners were calibrated using a striatal phantom to minimize the effect of sensitivity variations between the different scanners (Tossici-Bolt *et al.*, 2011). Scanner-specific corrections, and in study IV, the patient age corrections, were used to calculate the striatal SBRs for six ROIs (right caudate, left caudate, right and left anterior putamen, and right and left posterior putamen) using the occipital cortex as the reference region: $SBR = (ROI_{caudate\ or\ putamen} - ROI_{occipital}) / ROI_{occipital}$ (Varrone *et al.*, 2013; Kaasinen *et al.*, 2014; Joutsa *et al.*, 2015). In studies I-III, the age-matched reference SBRs and SDs were available for every SBR value.

4.3.1.4 Patients with normal and abnormal striatal DAT binding

The patients in studies I-III were divided into groups of normal and abnormal DAT SPECT scans, according to the original reviews of the scans by the nuclear medicine physicians, whose interpretations were based on visual analysis, with or without an

additional automated analysis method (Kaasinen *et al.*, 2014). In study IV, the nuclear medicine physicians' initial interpretations of the scans were based on visual and automated semi-quantitative BRASS analyses, with age-matched reference SDs. Patients were categorized into groups of normal and abnormal striatal DAT binding, primarily based on these initial semi-quantitative BRASS analyses. DAT binding was considered abnormal if the SBR was more than two standard deviations below the age-matched reference mean in at least one of the striatal ROIs as described in Albert *et al* (Albert *et al.*, 2016). In the original scan analysis statements, there were eleven cases with uncertainty of the possible DAT abnormality due to discrepancies between the visual and semi-quantitative analysis methods. These cases were carefully re-evaluated, together with the most recent clinical diagnoses made by the treating physicians, and a consensus interpretation was performed by the investigators.

4.3.2 Midbrain-to-pons ratios (study I)

The midbrain-to-pons ratios were calculated, as described in Kaasinen *et al.* (Kaasinen *et al.*, 2015b), from sagittal conventional T1 and T2 MRIs, using a simplified method modified from the earlier approach by Massey and colleagues (Massey *et al.*, 2013). The MRI scanner and MRI protocol were not the same for all patients. Three line measurements were drawn over the pons (P) and the midbrain (M), blinded to the clinical patient information and perpendicular to the oblique superior–inferior axis (Figure 3) that was visually evaluated for each image. The mean values of the three measurements over the pons and the midbrain were used to calculate the midbrain width, pons width and the midbrain-to-pons ratios (midbrain width divided by the pons width).

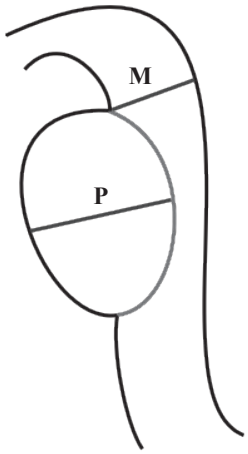

Figure 3. Designs of measurements over the pons (P) and the midbrain (M).

4.3.3 Visual analyses of SPECT scans (study II)

4.3.3.1 Visualizations of the DAT SPECT images

Horizontal visualizations of DAT SPECT scans of the 489 patients, from whom the study sample for study II was later selected, were created by using version 3.0 of the Vinci software program (Max Planck Institute, Cologne, Germany). Twelve subsequent horizontal slices in the neurological convention, with a thickness of 4.7 mm and a Speckle rainbow color scale with a region-to-occipital cortex DAT binding ratio (SBR + 1 ratio) from 0.0 to 5.5, were used to generate these visualizations. Each DAT SPECT image was visualized in four larger and eight smaller slices, and an assortment of these certain visualized slices were later named as simply an image. Four images representative for the different categories of striatal DAT binding were selected from patients outside the final study sample of 120 scans to be used as example images of the different DAT binding categories (Figure 4).

4.3.3.2 Visual and automated semi-quantitative classifications of SPECT images

The 489 images were visually categorized into one of the four categories of striatal DAT binding (normal, slightly abnormal, abnormal and clearly abnormal, Figure 4). Four visual categories of DAT binding with example images and written simple category descriptions were modified from the earlier classifications of different visual DAT binding uptakes (Benamer *et al.*, 2000b; Staff *et al.*, 2009; Kupsch *et al.*, 2012). A normal striatal DAT binding was defined as a symmetrical DAT uptake in all striatal nuclei in both hemispheres. A slightly abnormal striatal DAT binding was defined as a visually detectable reduction in putamen uptake in one or both hemisphere(s) in association with (nearly) normal caudate DAT binding in both hemispheres. An abnormal striatal DAT binding was defined as a clear bilateral reduction in putamen uptake, with mostly preserved DAT uptake in the caudate nuclei in both hemispheres. Finally, the uptake was categorized as clearly abnormal when the striatal DAT binding showed a clear bilateral reduction in the putamen uptake in association with the DAT deficiency in one or both of the caudate nuclei.

The scans were also classified as normal or abnormal in the striatal DAT uptake, according to the automated semi-quantitative BRASS analyses (Kaasinen *et al.*, 2014). In these analyses, the scan was defined as abnormal if at least one of the six SBRs was more than two standard deviations (-2 SD) below the age-matched reference mean (Albert *et al.*, 2016).

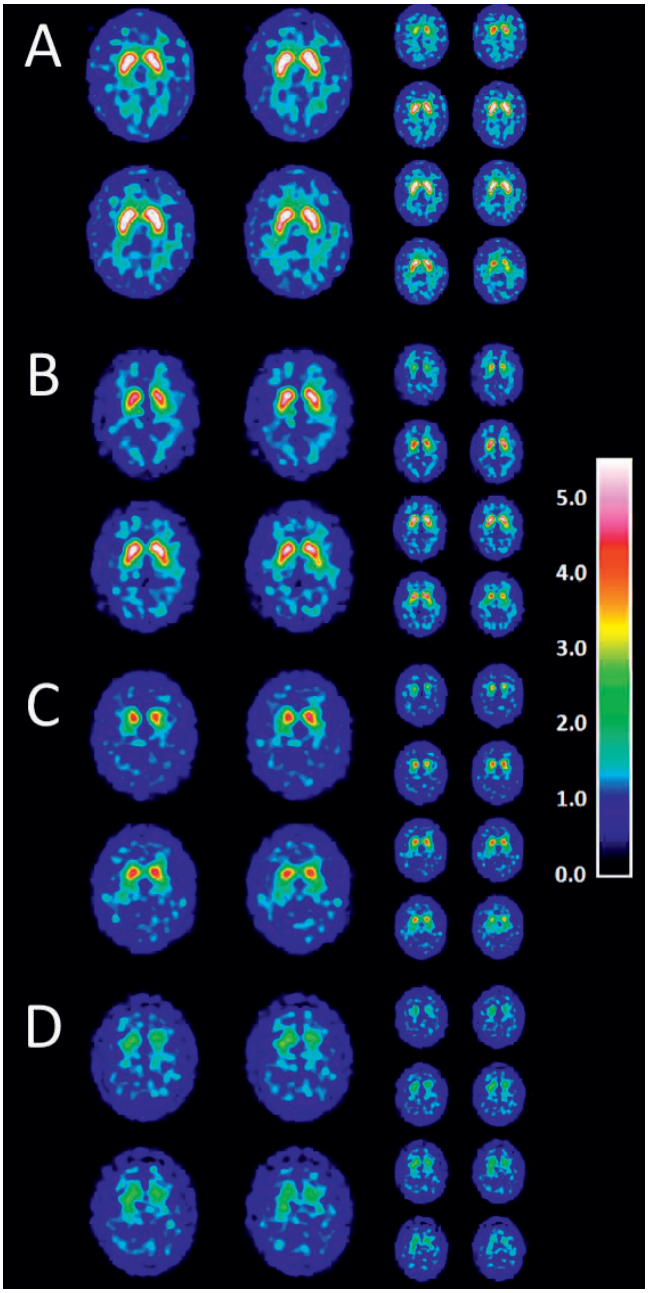

Figure 4. Four example categories of striatal dopamine transporter binging. The examples are images of patients who were not included in the final study sample of study II to avoid bias in the visual evaluation. A = normal, B = slightly abnormal, C = abnormal, D = clearly abnormal striatal DAT binding. The color scale bar indicates the region-to-occipital cortex DAT binding ratios of the color scale used in the scan visualizations.

4.3.3.3 Visual analyses by experts and nonexperts

Six raters were recruited to visually and independently analyze the 120 DAT SPECT images blinded to the BRASS analysis results and one another's ratings. The raters were asked to visually evaluate the images and to categorize each image into one of the four categories of striatal DAT binding. In addition, the raters were asked to interpret possible interhemispheric differences for every patient. The 120 images, based on the visualizations of the certain twelve scan slices of every patient, were downloaded in a random order to a single PDF file, and the four example images (Figure 4) were given to the raters in another PDF file. In addition, the raters were given the written category descriptions, brief written and oral guidance of what they were asked to do, and a concise patient information of each patient including the age at the time of imaging, gender, duration of motor symptoms at the time of DAT SPECT imaging and the predominant side of the motor symptoms. Two of the raters were nuclear medicine physicians with over ten years of experience in visual DAT SPECT scan analysis. The other raters were nonexperts. Two of them (nonexperts 3 and 4) were registered nurses who had some knowledge of DAT SPECT imaging but no knowledge of the visual analysis of the scans, and two were nonmedical laymen (nonexperts 5 and 6), with no previous knowledge of functional brain imaging.

4.3.4 Clinical follow-up and the level of dopamine transporter loss (study III)

4.3.4.1 Clinical follow-up and outcomes

The patients in study III were retrospectively followed until death or to the end of the follow-up, which was set at October 4, 2016. The beginning of the follow-up was the date of DAT SPECT imaging (years 2007 – 2012). Thus, the follow-up ranged from 1 to 115 months (0,1 – 9,6 years), with a median of 69 months (5,8 years), and the survivors were followed for at least 3,8 years. At the end of the follow-up, complementary clinical and demographical patient information was collected from the patients' files by carefully interpreting the medical records. The use of anti-parkinsonian medications was assessed by defining the levodopa daily dose and the levodopa-equivalent daily dose (LEDD). LEDD is calculated by adding together the daily doses of different antiparkinsonian medications, that are separately converted by the use of drug-specific levodopa equivalent conversion factors (Tomlinson *et al.*, 2010). The presence of cognitive impairment and dementia were defined by interpreting the patient diagnoses, MMSE scores and descriptions of the clinical conditions of the patients, assessed by their treating physicians. Thus, congruent diagnostic criteria for cognitive impairment and dementia was not used for all patients. The available MMSE scores at baseline or at the end of the follow-up

ranged from 16 to 24. The descriptions of the motor signs by the treating physicians were used to define the motor impairment by using the modified H&Y stage (Goetz *et al.*, 2004). For some patients, the H&Y stage had already been defined by the treating physician. Finally, the motor symptom severity was divided into three clinical categories. Patients without impairment of balance (patients with modified H&Y stages of 1, 1.5 and) were included in category 1, patients with bilateral motor signs and some postural impairment (modified H&Y stages 2.5 and 3) were included in category 2, and patients with the most severe motor impairment and postural impairment (H&Y 4 and 5) were included in category 3.

4.3.4.2 Level of dopamine transporter deficiency

In study III, the level of striatal dopaminergic neurodegeneration was characterized by the SBR of DAT binding in the more severely affected posterior putamen of each PD patients, as this region is classically suggested to be initially affected. The median, bottom and upper quartiles (the 25, 50 and 75 percentiles, respectively) of the worse side posterior putamen SBRs were calculated over the whole sample, and the level of dopaminergic deficiency was categorized using these calculations as category dividers. Patients with SBR < 1.37 in the more severely affected posterior putamen were classified in category 1, with the most severe DAT deficiency. Patients with SBR = 1.37 – 2.23 in the more severely affected posterior putamen were classified in category 2, with the average dopaminergic deficiency, and patients with SBR > 2.23 in the more severely affected posterior putamen were classified in category 3, with the less severe dopaminergic deficiency.

4.3.5 Clinical examination (study IV)

Of the NMDAT study protocol, the clinical interview, Hoehn & Yahr stage, MDS-UPDRS part III motor examination (Goetz *et al.*, 2008), and the Mini-Mental State Examination (MMSE) (Folstein *et al.*, 1975) were included in the analyses of study IV. Of the study sample of 221 patents, 40 (18.1%) were receiving anti-parkinsonian medications (e.g. levodopa, dopamine receptor agonists, MAO-B inhibitors) at the time of imaging, and 26 of these 40 patients were receiving levodopa. The reason for scanning for most of these patients was an uncertain clinical PD diagnosis (n = 32). For ten patients who had a maximum of five randomly missing items in the motor MDS-UPDRS examination, MDS-UPDRS part III total scores were adjusted, according to the number of missing items, as described earlier (Goetz *et al.*, 2015).

Table 2. Motor signs, motor asymmetry and [^{123}I]FP-CIT uptake variables in study IV.

Bradykinesia total score	Finger tapping, hand movements, pronation-supination, toe tapping, leg agility and global bradykinesia in MDS-UPDRS-III
Rigidity total score	Rigidity in neck, right and left upper extremity, right and left lower extremity in MDS-UPDRS-III
Tremor total score	Postural, kinetic and rest tremor of upper extremities, rest tremor of lower extremities, lip/jaw rest tremor and constancy of rest tremor in MDS-UPDRS-III
Axial signs	Arising from chair, gait, freezing of gait, postural instability and posture in MDS-UPDRS-III
Predominant side of all bilateral motor signs in MDS-UPDRS-III	Right = all bilateral motor signs asymmetry index > 0.15, Left = all bilateral motor signs asymmetry index < -0.15, Symmetric = -0.15 ≤ all bilateral motor signs asymmetry index ≤ 0.15
Asymmetry index*	(Score of all bilateral motor signs in MDS-UPDRS-III right side – Score of all bilateral motor signs left side) / (Right side + Left side)
Asymmetry index, bradykinesia*	(Score of bradykinesia right side – Score of bradykinesia left side) / (Right side + Left side)
Asymmetry index, rigidity*	(Score of rigidity right side – score of rigidity left side) / (right side + left side)
Asymmetry index, tremor*	(Score of tremor right side – Score of tremor left side) / (Right side + Left side)
Putamen DAT binding	[(Posterior putamen SBR right + anterior putamen SBR right) / 2 + (Posterior putamen SBR left + anterior putamen SBR left) / 2] /2
Caudate DAT binding	(Caudate SBR right + caudate SBR left) / 2
Putamen asymmetry index*	(SBR of putamen DAT binding rigt hemisphere – SBR of putamen DAT binding left hemisphere) / (right + left hemisphere putamen SBR)

*Absolute values were used in the analyses, except in the Spearman's correlations (see *5.4.2.5*)

4.3.5.1 Motor signs of MDS-UPDRS part III

The MDS-UPDRS part III ratings were distributed to six main subscores of axial signs, bradykinesia, facial expression, rigidity, speech and tremor. The classifications were modified from the previously created classifications (Pirker, 2003) of the original motor UPDRS rating scale. In addition, the current information of the definitions of parkinsonian motor signs (Postuma *et al.*, 2015) were considered when defining the classifications. The new items of toe tapping and freezing of gait, which are not part of the original UPDRS rating scale (Goetz *et al.*, 2008), were decided to include in the subscores of bradykinesia and axial signs, respectively. Furthermore, the tremor subscore was subdivided into categories of resting tremor, postural tremor and kinetic tremor. Finally, the bilateral motor signs of bradykinesia, rigidity and tremor were separately divided into more and less affected body sides (worse and better sides, respectively). The

main subscores and subdivisions for MDS-UPDRS part III and the SPECT variables used in study IV are presented and described in Table 2.

4.4 STATISTICAL ANALYSES

The statistical analyses were performed using version 23 (studies I and II) and version 24 (studies III and IV) of the IBM SPSS Statistics (IBM Corp., New York, USA). The assumption of normality was evaluated visually from histograms, together with Shapiro–Wilk tests. In all statistical analyses, *P*-values less than 0.05 were considered to be significant. In studies I, II and IV, voxel-based analyses were also performed using Statistical Parametric Mapping software, version SPM8 (Wellcome Trust Center for Neuroimaging, London, UK, www.fil.ion.ucl.ac.uk/spm/software/spm8/) running in MATLAB R2015a (Mathworks, Inc., Natick, MA, USA) in studies I and II, and version SPM12 (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in study IV. Cluster- or voxel-level family-wise error corrected *P*-values < 0.05 were considered to be significant.

4.4.1 Demographic data (studies I, II, IV)

Group differences in continuous demographic variables were tested using independent samples t-test, Mann-Whitney U-tests and one-way ANOVA, with post hoc Bonferroni corrections in study I, and the group differences in categorical demographic variables were tested using Pearson's Chi-squared test, or the Fisher exact test, as appropriate, in study I. Correlation analyses were performed with Pearson's correlation coefficient. In study IV, the Benjamini-Hochberg procedure was used to control for the probability of type I errors due to multiple group comparisons in the continuous and categorical motor signs, with a false discovery rate of 5%.

4.4.2 [^{123}I]FP-CIT and midbrain-to-pons ratios (study I)

Pearson's partial correlation coefficients were used to investigate correlations between SBRs and midbrain-to-pons ratios. Patients with PD were compared with the control patients using ANCOVA. Age, gender, SPECT scanner, time interval between SPECT and MRI scans, duration of motor symptoms and motor symptom subtype (presence of tremor or no tremor), predominant side of motor symptoms, and medications were used as covariates in both analyses.

4.4.3 Kappa statistics (study II)

Cohen's unweighted κ (Cohen J, 1960) was used to study the interrater agreement in the visual analyses and the agreement between the visual and automated semi-quantitative BRASS analyses in separating the scans either as normal or abnormal. A higher κ value was considered to indicate a higher strength of agreement; agreement of $\kappa < 0.20$ was considered to indicate poor agreement, $\kappa = 0.21 - 0.40$ was mild agreement, $\kappa = 0.41–0.60$ was moderate agreement, $\kappa = 0.61 - 0.80$ was good agreement, and $\kappa = 0.81 - 1.00$ was excellent agreement (Altman, 1991). *Z* tests were used to study the agreement differences between experts and nonexperts.

4.4.4 Survival analyses (study III)

4.4.4.1 Kaplan-Meier analyses and Cox regression analyses

Kaplan-Meier analyses and the corresponding Kaplan-Meier curves were initially interpreted to outline the influence of gender and different clinical and demographical factors at the time of DAT SPECT imaging on patient survival in PD. These factors included the modified H&Y stage categories, presence of cognitive impairment, use of antiparkinsonian medications (not including levodopa), levodopa daily dose (divided in three categories of no levodopa, levodopa daily dose below 300 mg and levodopa daily dose of 300 mg or more), and LEDD (Tomlinson *et al.* 2010) (divided in three categories of no medication, LEDD below 200 mg, or LEDD of 200 mg or more). Then, univariate Cox regression analyses were performed to study the hazard ratios (HR) of these above-mentioned factors on survival. Finally, factors that showed associations with mortality in the univariate models and due to clinical interest were selected in the final multivariate Cox regression analysis. A multivariate Cox regression analysis including these factors was also performed in the subgroup of patients with less advanced motor disability; 120 patients with modified H&Y stages of 1, 1.5 or 2.

4.4.4.2 Voxel-based analyses

The voxel-based analyses were used to study the associations between striatal and extrastriatal DAT binding and mortality. The analyses were available for 148 patients, due to 14 inadequate DAT SPECT images to run the SPM analysis. The [^{123}I]FP-CIT SPECT images were warped to the Montreal Neurological Institute (MNI) space using an inhouse template (Ashburner, 2007; Joutsa *et al.*, 2015). Then, SBR+1 images were generated by dividing the original image voxel values by the extracted average DAT

binding in the occipital cortex reference region. To improve the signal-to-noise ratio, the SBR+1 images were smoothed using an 8 mm isotropic Gaussian kernel, and the images were analyzed using a Gaussian linear model (GLM). An analysis mask was used to limit the search volume to regions where the tracer uptake exceeded the reference region DAT uptake (midbrain, pons, cerebral cortex) (Joutsa *et al.*, 2015). Non-survivors (i.e., patients who died during the follow-up) and survivors (patients alive at the end of the follow-up) were compared, and the associations between DAT binding and time intervals in between DAT SPECT imaging and death were studied in the group of non-survivors. The analyses were also conducted with age, modified H&Y categories and presence of cognitive impairment as covariates.

4.4.5 Associations of motor signs with striatal DAT deficiency (study IV)

4.4.5.1 One-way ANOVA and Spearman's correlations

One-way ANOVA was used to investigate the associations between SBRs and facial expression and between SBRs and upper extremity rigidity. First, the upper extremity rigidity was modified to five grades of mean rigidity for both upper extremities: 0 - 0.5, 1 - 1.5, 2 - 2.5, 3 - 3.5, and 4 mean points). Due to a low number of patients who had a mean score of 4 for both hypomimia and rigidity, patients with the most severe hypomimia (facial expression scores of 4) were combined into the same category as patients with facial expression scores of 3, and patients with the mean upper extremity rigidity grades of 3 – 3.5 points and 4 points were all combined to represent the most severe state of mean upper extremity rigidity. One-way ANOVA was also used to study differences in SBRs between different SPECT scanners. Levene's test was used to check the equality of variances, and Tukey HSD corrections were used to correct for multiple comparisons. In addition, Spearman's rank correlation coefficients were used to investigate the correlations between SBRs and individual motor signs and between asymmetry of SBRs and asymmetry of motor signs.

4.4.5.2 Logistic regression

Binary logistic regression was used to study whether some of the parkinsonian motor signs are associated with striatal DAT deficiency in the DAT SPECT. Initially, the odds ratios (ORs) of several motor factors were studied in univariate logistic regression models. The motor signs and clinical factors that showed differences between patients with normal and abnormal striatal DAT binding were added in a multivariate logistic regression model at once. Upper extremity rigidity was chosen from the rigidity variables

as there were no significant differences in lower extremity rigidity or neck rigidity between patients with and without DAT deficiency (Table 8). ORs of the motor signs were calculated for the presence of any stage of the sign vs. no detection of the sign due to clinical interest. Receiver operating characteristic curves (ROC curves) for the continuous upper extremity rigidity total score of both extremities and the facial expression total score were plotted, and the areas under the curves (AUC) were analyzed. In a ROC curve, the true positive rate (sensitivity) is plotted against the false positive rate (100-Specificity) for different cut-off points of a parameter, and the AUCs demonstrates how well a parameter can distinguish between two groups (https://www.medcalc.org/manual/roc-curves.php).

4.4.5.3 Voxel-based analyses

The [^{123}I]FP CIT SPECT images were co-registered to the standard template implemented in BRASS. The average co-registered SBR image was then used to calculate nonlinear registration for the in-house [^{123}I]FP-CIT template in the MNI152 standard space (Kaasinen *et al.*, 2015a). To study the associations between motor signs and SBRs in patients with abnormal striatal DAT binding (voxel by voxel within the striatum), all individual SBR images of these patients were warped to the MNI152 space to perform the SPM analysis. Of the 110 patients with abnormal DAT SPECT scans, one patient lacked [^{123}I]FP CIT SPECT imaging data. A previously published striatal mask (Choi *et al.*, 2012) was used to restrict the analyses within the striatum. Facial expression and mean upper extremity rigidity were divided into two grades: hypomimia scores of 0 – 1 (n = 59) and scores of 2 - 4 (n = 50), and rigidity scores of 0 - 1.5 (n = 54) and 2 – 4 (n = 56). Univariate regression models and a multivariate regression model were used to investigate the effects of more severe hypomimia (scores of 2 – 4 versus scores of 0 – 1) and more severe upper extremity rigidity (scores of 2 – 4 versus 0 – 1.5), separately and together, on more severe DAT deficiency in specific striatal subregions. The cluster coordinates were presented with spatial extents of more than 5 voxels for all clusters.

5 RESULTS

5.1 Midbrain atrophy and striatal dopamine deficiency (study I)

5.1.1 Demographical and clinical characteristics

Patients with PD did not differ from patients with a normal striatal DAT function, in terms of age, gender, time interval between MRI and SPECT scans, or the motor symptom subtype. There were no significant differences in the pons or midbrain widths between patients with normal DAT binding and patients with PD. PD patients had shorter motor symptom durations, and their mean striatal DAT binding was 43.9% lower compared to patients with a normal DAT function.

Table 3. Demographical and clinical characteristics in Study I. The means (SD) are presented for continuous variables. Striatal DAT binding and the structural MRI measurements are covariate-adjusted means from the ANOVA (with 95% confidence intervals).

	Parkinson's disease	Normal DAT	*P* value
n	150	155	
Age (years)	64.9 (10.6)	62.1 (11.7)	0.16[1]
Sex (M / F)	91/59	78/77	0.08[2]
Interval between MRI and SPECT (months)	6.5 (8.3)	6.4 (9.5)	0.97[1]
SPECT scanner (I / II / III)[5]	54/60/36	60/57/36	0.84[2]
Motor symptom duration at the time of SPECT imaging (years)	2.0 (2.3)	4.2 (7.3)	< 0.001[1]
Motor symptom type (no tremor / tremor)	51/99	64/91	0.20[2]
Predominant side of motor symptoms (right / left / symmetric)	70/63/17	66/46/43	< 0.001[2]
Medication (D / AD / no medication)[4]	30/5/115	20/26/109	< 0.001[2]
Mean striatal DAT binding	1.51 (1.40 – 1.62)	2.69 (2.60 – 2.79)	< 0.001[3]
Midbrain-to-pons ratio	0.59 (0.58 – 0.60)	0.61 (0.59 – 0.62)	0.04[3]
Pons width (mm)	17.4 (17.1 – 17.8)	17.1 (16.9 – 17.4)	0.10[3]
Midbrain width (mm)	10.3 (10.0 – 10.5)	10.4 (10.2 – 10.5)	0.57[3]

[1]Independent samples t-test, [2] the Fisher exact test or Chi-squared test, [3]and one-way ANOVA, with age, sex, interval, scanner, symptom duration, symptom type, predominant side and medication as covariates, [4]Dopaminergic / antidopaminergic (neuroleptics) / no medication, [5]Scanner I = Picker Irix gamma camera with ¾-inch crystals, Scanners II and III = GE Infinia II Hawkeye SPECT/CT with 3/8-inch crystals.

5.1.2 Midbrain atrophy in Parkinson's disease

Patients with PD had 3.3% lower midbrain-to-pons ratios compared to patients with normal DAT binding (Table 3). Eighteen (12%) of the 150 PD patients had abnormal (< 0.52) midbrain-to-pons ratios. The mean (95% confidence interval) striatal DAT binding was 1.44 (1.17 – 1.72) for the PD patients with abnormal ratios and 1.47 (1.26 – 1.68) for the PD patients with normal ratios, and there were no differences in the mean striatal DAT binding between the PD patients with normal and abnormal midbrain-to-pons ratios (P = 0.83). In addition, there were no statistically significant correlations between the midbrain-to-pons ratios and striatal DAT binding in patients with PD (r = -0.04–0.00, P > 0.65), patients with normal DAT function (r = 0.02–0.13, P > 0.10) (Figure 5), or PD patients with abnormal midbrain-to-pons ratios combined with abnormal striatal DAT binding in the DAT SPECT scans [r = -0.47– (-0.08), P > 0.16)].

In the voxel-based analysis, when analyzed separately for the whole study sample, there were no associations between the midbrain-to-pons ratios and DAT binding in any striatal or extrastriatal regions in patients with PD or patients with normal striatal DAT binding.

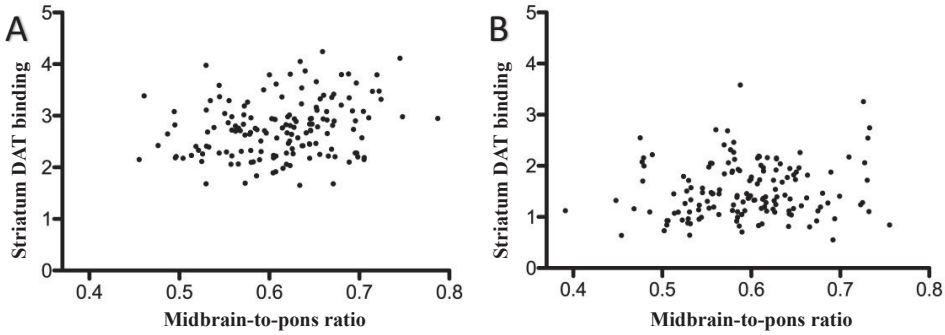

Figure 5. Midbrain-to-pons ratios and striatal SBRs in patients with normal striatal DAT uptake (A) and patients with PD (B).

5.2 Visual vs. automated analysis of [^{123}I]FP-CIT SPECT (study II)

5.2.1 Visual analysis by experts versus automated analysis

Of the 120 scans, 34 (28.3%) were categorized as non-discrepant normal scans, as the BRASS analysis agreed with the visual analysis of at least one of the experts in analyzing the scan as normal. Twenty-six (21.7%) of these scans were analyzed as normal, both in the visual analysis by both experts and in the BRASS analysis, whereas eight of these scans (6.7%) were analyzed as normal in the visual analysis by either one of the experts and in the BRASS analysis. A total of 74 (61.7%) scans were categorized as non-discrepant abnormal scans; in these cases, the BRASS analysis agreed with the visual analysis of at least one of the experts in analyzing the scan as abnormal. Seventy-three of these scans (60.8%) were analyzed as abnormal, both in the visual analysis by both experts and in the BRASS analysis, whereas one scan (0.8%) was analyzed as abnormal in the visual analysis by either one of the experts and in the BRASS analysis.

5.2.2 Scans with discrepant visual and automated analyses

In twelve (10%) of the 120 cases, there was a discrepancy between the visual analysis by both experts and the BRASS analysis, and these cases were categorized as discrepant scans. Nine (7.5%) of these scans were analyzed as normal by the BRASS analysis and visually abnormal by both experts, and three (2.5%) were analyzed as abnormal by the BRASS analysis and visually normal by both experts.

5.2.2.1 Demographics of patients with discrepant and non-discrepant scans

The mean striatum DAT binding was 17.6% lower in patients with discrepant scans compared to patients with non-discrepant normal scans ($P = 0.003$), whereas it was 62.7% higher compared to patients with non-discrepant abnormal scans ($P < 0.001$) (Figure 6A). Patients with discrepant scans were older (mean age 72.6 years) compared to patients with non-discrepant normal scans (mean age 62.4 years) ($P = 0.023$), but there were no differences in age between patients with discrepant scans and patients with non-discrepant abnormal scans ($P = 0.33$) (Figure 6B). There were also no differences in gender ($P = 0.38$), motor symptom duration ($P = 0.082$) or in the predominant side of the motor symptoms at the time of SPECT imaging ($P = 0.19$) between patients with discrepant and non-discrepant scans. The prevalence of discrepant cases did not differ between the different SPECT cameras ($P = 0.80$).

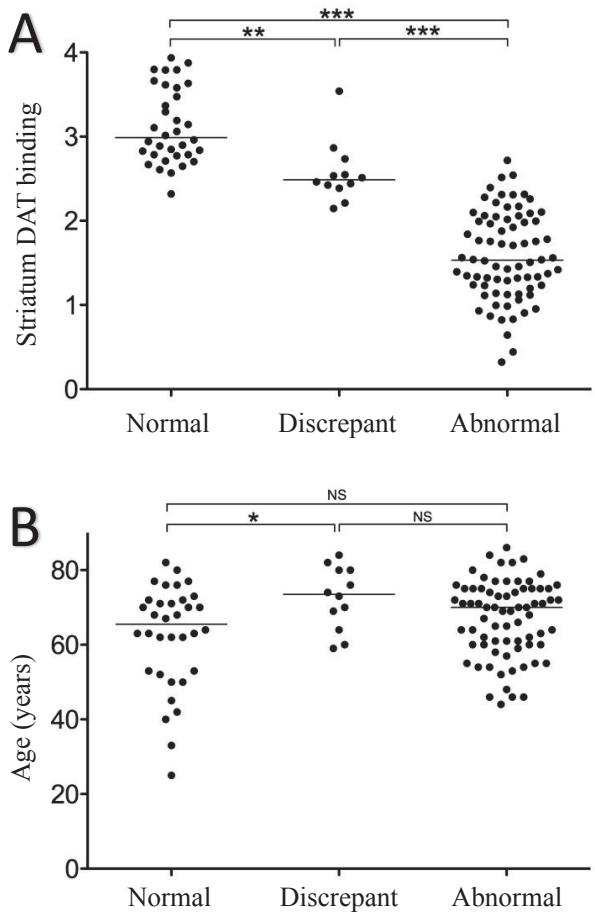

Figure 6. Differences in the mean SBRs of striatal DAT binding (A) and differences in the ages (B) of patients with non-discrepant normal, non-discrepant abnormal and discrepant [^{123}I]FP-CIT SPECT scans in the expert visual and automated semi-quantitative BRASS analyses. N (normal) = 26, n (discrepant) = 12, and n (abnormal) = 73. Statistical significances represent post hoc Bonferroni-corrected *P* values after one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, NS = non-significant.

5.2.2.2 Clinical details of patients with discrepant scan analyses

Of the nine patients whose scans were abnormal in the visual analyses by both experts but normal in the BRASS analyses, eight were 70 years of age and older at the time of the SPECT imaging, six presented with some type of cognitive impairment at the time of the SPECT imaging or developed dementia within five years of the post-scan follow-up, and the final clinical diagnosis for four patients was DIP (Table 4a, Figure 7). Of the three

patients whose scans were analyzed as abnormal by the BRASS analysis but normal in the visual analyses by both experts, the final diagnosis for two patients was ET. In the BRASS analyses, the standard deviations from the age-matched reference SBR means were -2.26 in the left anterior putamen for patient number 10 (cervical degenerative disease was the final diagnosis), -2.36 in the right anterior putamen, -2.34 in the left anterior putamen, -2.46 in the left posterior putamen and -2.21 in the left caudate for patient number 11 (a final diagnosis of ET), and -2.01 in the right anterior putamen, -2.31 in the left anterior putamen, -2.08 in the left posterior putamen, -2.03 in the right caudate and -2.13 in the left caudate for patient number 12 (a final diagnosis of ET) (Table 4b, Figure 8).

Table 4a. Study II: Clinical characteristics of the nine patients with discrepant [^{123}I]FP-CIT SPECT scan analyses. The scans were analyzed abnormal in the visual analyses by both experts but normal in the BRASS analyses. The corresponding DAT scans of these patients are presented in Figure 7.

No	Age	Sex	Reason[1]	Symptom duration	Predominant[2]	Current diagnosis[3]	CD[4]
1	59	M	Atypical parkinsonism susp.	2	Left	AD	Yes
2	70	F	Re-evaluation of PD	11	Right	Undetermined[6]	No
3	73	F	PD / DIP[5]	0,5	Right	DIP[7]	Yes
4	80	F	PD / DIP[5]	5	Right	DIP[8]	Yes
5	84	F	PD susp.	0,5	Right	DIP[9] + AD	Yes
6	82	F	PD / DIP[5]	1,5	Symmetrical	DIP[10] + AD	Yes
7	74	M	Unclear parkinsonism	5	Symmetrical	ET	No
8	76	F	Re-evaluation of PD	5	Symmetrical	Unknown	No
9	80	M	Unclear parkinsonism	1	Right	VP + vascular dementia	Yes

[1]Reason = a clinical reason for the DAT SPECT imaging (based on the patient referral), [2]Predominant side of the motor parkinsonism symptoms, [3]Minimum of 4,5 years of follow-up, [4]CD = cognitive defect at the time of imaging or within 5 years after the DAT SPECT imaging, [5]Differential diagnosis between these two disorders, [6]Parkinsonism for 20 years prior to imaging, no progression, no levodopa response, [7]risperidone 2 mg/day, [8]prochlorperazine, dose unknown, [9]perfenazine 8 mg/day, [10]risperidone, dose unknown.

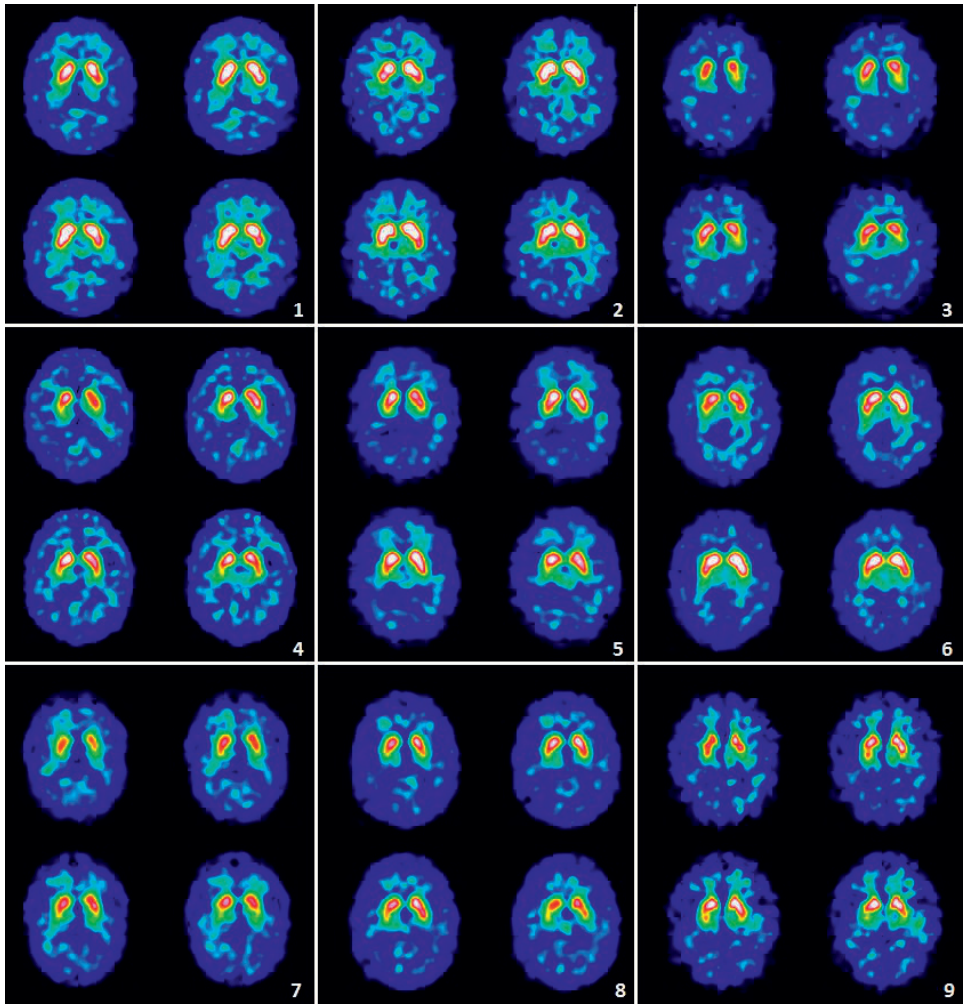

Figure 7. Nine [^{123}I]FP-CIT SPECT scans of patients with abnormal scan analyses in the expert visual analysis but normal scans in the automated semi-quantitative BRASS analysis. The color scale indicates SBRs of striatal DAT binding and the scale bar previously presented in Figure 4.

Table 4b. Study II: Clinical characteristics of the three patients with discrepant [^{123}I]FP-CIT SPECT scan analyses. The scans were analyzed as abnormal by the BRASS analysis but normal in the visual analyses by both experts. The corresponding DAT scans of these patients are presented in Figure 8.

No	Age	Sex	Reason[1]	Symptom duration	Predominant[2]	Current diagnosis[3]	CD[4]
10	60	M	Unclear parkinsonism	1	Symmetrical	Cervical degenerative disease	No
11	64	M	Unclear parkinsonism	30	Right	ET	No
12	69	M	PD / ET[5]	10	Right	ET	No

[1-5] Please see the footnotes in Table 4a.

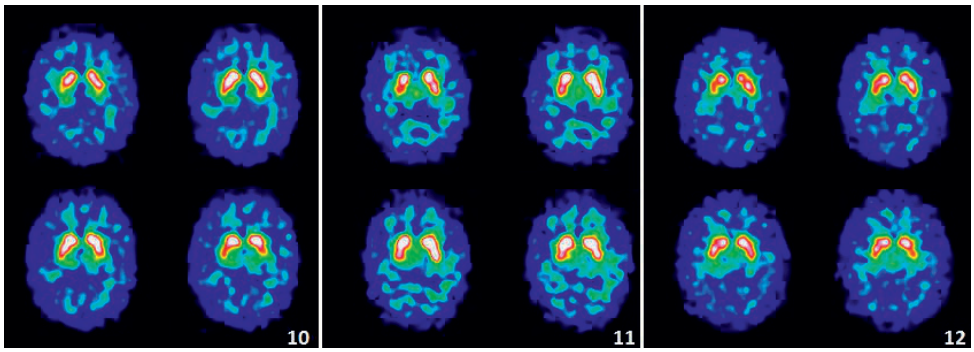

Figure 8. Three [^{123}I]FP-CIT SPECT scans of patients with normal scan analyses in the expert visual analysis but abnormal scans in the automated semi-quantitative BRASS analysis. The color scale indicates SBRs of striatal DAT binding and the scale bar previously presented in Figure 4.

5.2.3 Effect of expertise in the visual analysis

There was a good agreement between the BRASS analyses and the visual analyses by the experts. The agreement between the visual and BRASS analyses ranged from mild to moderate among the non-experts (Table 5, agreement with BRASS). The inter-rater agreement in the dichotomous visual analysis, categorizing the scan either as normal or as some grade of abnormal, was excellent in the experts, whereas it ranged from moderate to good in non-experts (Table 5). The inter-rater agreement for visually interpreting the

inter-hemispheric differences showed the poorest agreement and was only moderate among the experts (Table 5). The visual analyses required 60 – 90 seconds per scan for the experts and 30 – 45 seconds per scan for the non-experts.

Table 5 Effect of expertise in visual DAT SPECT scan analyses in Study II. κ-values with 95% confidence intervals on standard error are presented to demonstrate the inter-rater agreement and agreement between visual and automated semi-quantitative BRASS analyses. The *P* values demonstrate whether the differences between visual ratings were statistically significant (* $P < 0.05$).

Visual analyses	Agreement with BRASS	Comparison to Expert 1	Comparison to Expert 2	Agreement for normal vs. abnormal[1]	Agreement for four DAT binding categories[2]	Agreement for inter-hemispheric differences[3]
Expert 1	***0.66*** (0.51 – 0.80)			***0.81*** (0.70 – 0.92) between experts	***0.75*** (0.65 – 0.84) between experts	***0.60*** (0.43 – 0.69) between experts
Expert 2	***0.72*** (0.58-0.85)					
Non-expert 1	***0.54*** (0.38 – 0.69)	$P = 0.27$	$P = 0.09$	***0.44*** (0.23 – 0.66) between non-experts 1 and 2	***0.61*** (0.50 – 0.72) between non-experts 1 and 2	***0.39*** (0.26 – 0.52) between non-experts 1 and 2
Non-expert 2	***0.23*** (0.09 – 0.36)	$P < 0.001$	$P < 0.001$			
Non-expert 3	***0.47*** (0.30 – 0.64)	$P = 0.005$	$P < 0.001$	***0.63*** (0.45 – 0.82) between non-experts 3 and 4	***0.79*** (0.71 – 0.88) between non-experts 3 and 4	***0.42*** (0.28 – 0.55) between non-experts 3 and 4
Non-expert 4	***0.52*** (0.36 – 0.68)	$P = 0.22$	$P = 0.07$			

[1]Inter-rater agreement for normal versus abnormal scans.
[2]Inter-rater agreement for four categories of striatal DAT binding (normal, slightly abnormal, abnormal, clearly abnormal).
[3]Inter-rater agreement for categorizing the inter-hemispheric differences of whether the striatal DAT deficiency was more dominant in the right or left striatum or whether there was (nearly) symmetrical DAT uptake.

5.3 Survival in PD in relation to DAT and clinical factors (study III)

5.3.1 Demographical and clinical characteristics

At the end of the follow-up, there were 42 non-survivors (patients who had died during the follow-up time interval) and 120 survivors. Thus, the overall mortality was 25.9%. The Kaplan-Meier estimate for mortality was 36%. The demographical and clinical characteristics of the study sample at the time of the SPECT imaging are presented in Table 6, in which the characteristics of survivors and non-survivors are also separately presented.

Table 6. Demographical and clinical characteristics of the Study III sample at the time of [^{123}I]FP-CIT SPECT imaging, which was the onset of the clinical follow-up. Survivors = patients alive at the end of the follow-up.

	All	Survivors	Non-Survivors
N (%)	162	120 (74.1)	42 (25.9)
Age at the time of SPECT (years), mean (SD)	66.4 (10.4)	63.1 (9.3)	75.9 (7.1)
Gender (male / female)	96/66	68/52	28/14
Cognitive impairment, n (%)	33 (20.4)	19 (15.8)	14 (33.3)
Modified H&Y stages, median	1.5	1.5	2.8
1, 1.5 and 2, n (%)	120 (74.1)	104 (86.7)	16 (38.1)
2.5 and 3, n (%)	25 (15.4)	11 (9.2)	14 (33.3)
4 and 5, n (%)	17 (10.5)	5 (4.2)	12 (28.6)
Levodopa daily dose[1] (mg), median	300	300	300
No levodopa, n (%)	130 (80.2)	105 (87.5)	25 (59.5)
Levodopa daily dose < 300 mg, n (%)	10 (6.2)	4 (3.3)	6 (14.3)
Levodopa daily dose ≥ 300 mg, n (%)	22 (13.6)	11 (9.2)	11 (26.2)
LEDD[2] (mg), median	200	142	300
No antiparkinsonian medication, n (%)	105 (64.8)	82 (68.3)	23 (54.8)
LEDD < 200 mg, n (%)	27 (16.7)	21 (17.5)	6 (14.3)
LEDD ≥ 200 mg, n (%)	30 (18.5)	16 (11.3)	13 (31.0)
Posterior putamen DAT binding[3], median	1.76	1.80	1.67
SBR < 1.37, n (%)	39 (24.1)	25 (20.8)	14 (33.3)
SBR = 1.37-2.23, n (%)	82 (50.6)	61 (50.8)	21 (50.0)
SBR > 2.23, n (%)	41 (25.3)	34 (28.3)	7 (16.7)

[1]Median levodopa daily dose in patients who were receiving levodopa at the time of the DAT imaging.
[2]Median levodopa equivalent daily dose (LEDD) of all antiparkinsonian medications in patients who were

receiving levodopa and/or other antiparkinsonian medications at the time of DAT imaging. [3] Specific binding ratio of DAT binding in the posterior putamen of the most severely affected hemisphere in the automated semi-quantitative BRASS analysis.

5.3.2 Cox regression and voxel-based analyses

5.3.2.1 Univariate Cox regression analyses

The results of the Cox regression analyses are presented with hazard ratios (HR), with 95% confidence intervals (CI). In the univariate regression analyses, age *(*$P < 0.001$), levodopa daily dose ($P < 0.001$), LEDD ($P = 0.01$), cognitive impairment ($P < 0.001$), modified H&Y categories ($P < 0.001$), and the level of the more severely affected posterior putamen DAT binding ($P = 0.03$) were associated with survival. There were no significant associations between the use of antiparkinsonian mediations other than levodopa and survival ($P = 0.11$) or between gender and survival ($P = 0.17$). There was a high correlation between LEDD and levodopa daily dose, and therefore, only LEDD was chosen for the multivariate Cox regression model due to the clinical interest.

5.3.2.2 Multivariate Cox regression analysis

In the multivariate Cox regression model (Table 7), there were no associations between the level of the more severely affected posterior putamen DAT binding and survival (Figure 9). In addition, there were no associations between gender and survival or between LEDD categories and survival. The factors significantly associated with survival in PD were age, stage of motor impairment and the presence of cognitive impairment at the time of DAT imaging. As assumed, the mortality significantly increased by every one-year increase of age. The mortality risk was most clearly increased if the patient suffered from cognitive problems at the time of the DAT SPECT imaging. The modified H&Y stage categories were associated with survival, and the mortality risk was increased in patients in motor symptom categories 2 (H&Y 2.5 and 3) and 3 (H&Y 4 and 5) compared to patients in the category 1 (H&Y 1, 2.5 and 2) (Figure 10). Neither the significant or the non-significant associations of factors with survival changed when the duration of motor symptoms was added into the multivariate model, and the symptom duration had no associations with survival in PD ($P = 0.61$) patients. In a separate multivariate regression analysis of 120 patients with less severe motor impairment (H&Y 1, 1.5 and 2), there were still no associations between the level of the more severely affected posterior putamen DAT binding and survival ($P = 0.76$).

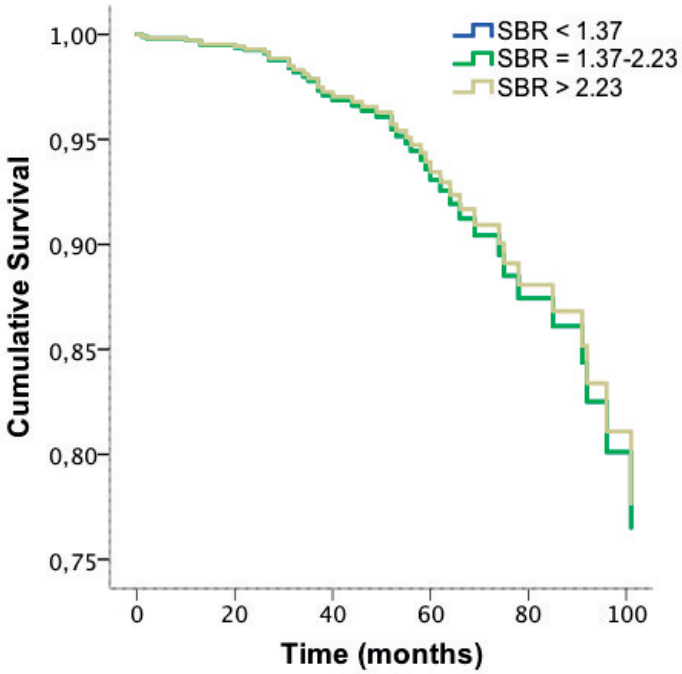

Figure 9. Independent impact of the level of semi-quantitative DAT binding deficiency in the more severely affected posterior putamen of each patient with PD, after adjusting for other variables in the multivariate Cox regression model. The survival function graph is plotted against the follow-up time.

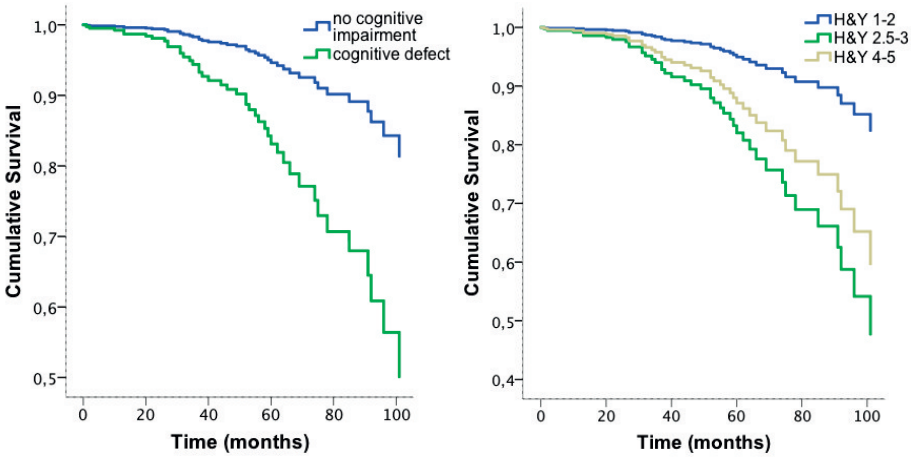

Figure 10. Independent impact of the presence of cognitive impairment and more severe motor impairment on survival in patients with PD. The survival function graphs are plotted against the follow-up time.

Table 7. Effects of demographical and clinical factors on survival in PD in Study III.

	Adjusted HR (95% CI)	***P* value**
Age at the time of SPECT (years)	1.14[1] (1.09 – 1.20)	< 0.001
Male gender	1.84 (0.90 – 3.84)	0.10
Presence of cognitive impairment	3.35 (1.67 – 6.72)	0.001
Modified Hoehn & Yahr stage categories[2]		0.002
H&Y category 2 vs. 1	3.83 (1.75 – 8.36)	0.001
H&Y category 3 vs. 1	2.67 (1.11 – 6.39)	0.03
Levodopa equivalent daily dose (LEDD)		0.10
LEDD <200 vs. no medication	0.60 (0.22 – 1.64)	0.32
LEDD ≥200 vs. no medication	1.79 (0.83 – 3.89)	0.14
Posterior putamen DAT binding[3, 4]		0.99
SBR category 2 vs. 1	1.00 (0.49 – 2.05)	1.00
SBR category 3 vs. 1	0.95 (0.35 – 2.58)	0.91

[1]HR for every one-year increase in age. [2]H&Y category 1 = modified H&Y stages of 1, 1.5 and 2; H&Y category 2 = modified H&Y stages of 2.5 and 3; H&Y category 3 = modified H&Y stages of 4 and 5. [3]DAT binding in the posterior putamen of the most severely affected hemisphere in the automated semi-quantitative BRASS analysis. [4]SBR category 1: SBR < 1.37; SBR category 2: SBR = 1.37 – 2.23; and SBR category 3: SBR > 2.23.

5.3.2.3 Voxel-based analysis

No striatal or extrastriatal regions showed differences in DAT binding between the survivors and non-survivors at the 5-year follow up. In addition, there were no significant associations between any regional DAT binding and the time interval between DAT SPECT scan and death among non-survivors. The results remained the same even after adjusting for age, modified H&Y stage categories and the presence of cognitive impairment.

5.4 Parkinsonian motor signs in relation to DAT deficiency (study IV)

5.4.1 Characteristics of patients with normal and abnormal [^{123}I]FP-CIT SPECT

There were no differences in the demographical characteristics or in general motor and cognitive status between patients with normal (n = 111) and abnormal (n = 110) DAT SPECT scans, as the patients did not differ in terms of age, gender distribution, years of education, MMSE score, MDS-UPDRS part III total score or Hoehn & Yahr stage (Table 8, Figure 11AB).

In the group comparisons of MDS-UPDRS-III subscores, there were no differences in axial signs, bradykinesia, speech or overall tremor between patients with and without DAT deficiency (Table 8). However, patients with abnormal DAT scans had shorter motor symptom durations, more severe overall rigidity, more severe upper extremity rigidity and more severely reduced facial expressions compared to patients with normal scans (Table 8, Figure 11CD). Lower extremity rigidity (P = 0.27) or neck rigidity (P = 0.09) did not differ between the two patient groups.

The asymmetry index of all bilateral motor signs in MDS-UPDRS-III (P = 0.015), overall rigidity asymmetry index (P = 0.14), upper extremity rigidity asymmetry index (P = 0.033) and bradykinesia asymmetry index (P = 0.027), as well as unilateral upper extremity kinetic tremor (P = 0.048) and kinetic tremor of both upper extremities (P = 0.043) tended to differ between the two patient groups, but, however, none of these differences remained significant after the Benjamini-Hochberg procedure. There were no differences in other tremor categories (P > 0.052) or overall tremor asymmetry index (P = 0.20) between patients with and without striatal DAT deficiency.

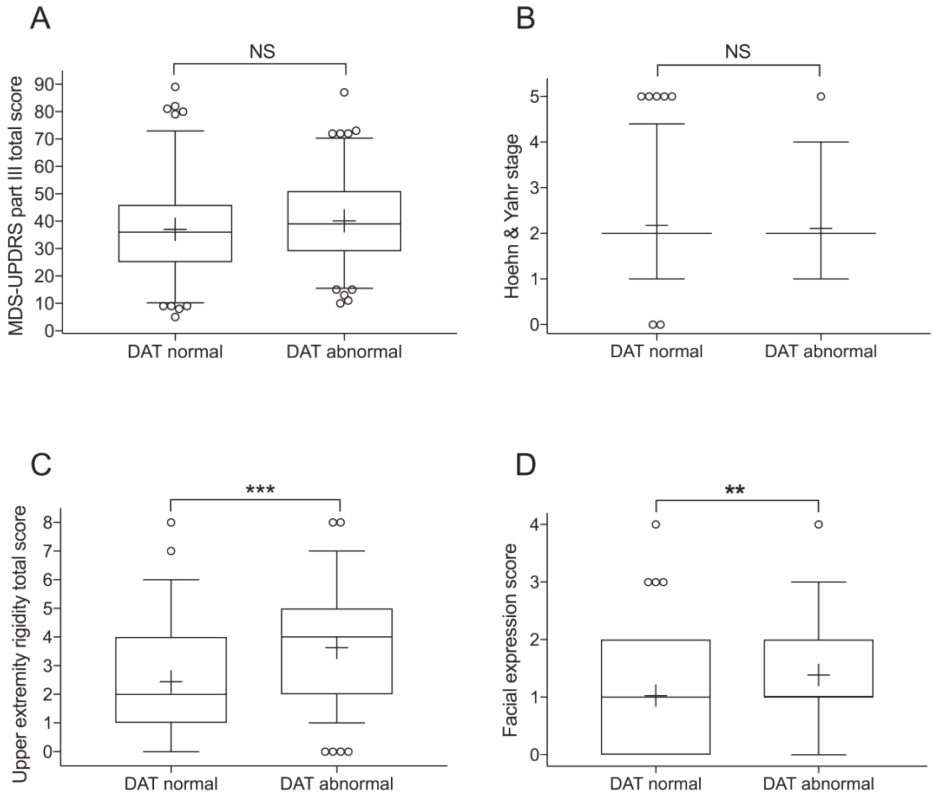

Figure 11. Clinical differences in the motor status in clinically uncertain parkinsonism patients with normal (n = 111) and abnormal (n = 110) DAT SPECT imaging outcomes. There were no differences in the MDS-UPDRS part III total scores (A) or in the original H&Y stages (B) between patients with and without striatal DAT deficiency. Upper extremity rigidity (C) and hypomimia (D) showed differences between these two patient groups. *** $P < 0.001$, ** $P < 0.01$, NS = non-significant.

Table 8. Demographic and clinical characteristics, as well as a portion of the motor MDS-UPDRS examinations of 221 patients with clinically uncertain parkinsonism with normal and abnormal striatal DAT binding in [^{123}I]FP-CIT SPECT in Study IV.

	Normal DAT, n = 111	**Abnormal DAT, n = 110**	***P value***
Demographic and clinical characteristics			
Age (years)	64.2 (12.2)	65.5 (9.4)	0.88
Sex (F / M)	54/57	55/55	0.84
Formal education (years)	12.6 (4.7)	13.2 (4.1)	0.19
Mini-mental stage examination score	26.2 (3.1)	26.6 (2.7)	0.27
Motor symptom duration at SPECT scan (years)	4.3 (5.8)	2.6 (3.7)	*0.013*
MDS-UPDRS part III examinations			
Original Hoehn & Yahr stage	2.2 (0.9)	2.1 (0.9)	0.42
MDS-UPDRS part III total score	37.0 (17.4)	40.2 (15.9)	0.13
Facial expression	1.03 (0.89)	1.39 (0.92)	*0.004*
Speech	0.81 (0.84)	0.96 (0.91)	0.22
Axial signs	4.19 (3.51)	4.46 (3.83)	0.70
Bradykinesia total score	16.60 (9.04)	18.10 (9.24)	0.27
Worse side bradykinesia	8.70 (4.33)	9.92 (4.38)	0.067
Better side bradykinesia	6.54 (4.37)	6.48 (4.64)	0.88
Rigidity total score	7.02 (4.53)	8.88 (4.62)	*0.002*
Worse side rigidity	3.14 (1.89)	4.13 (1.88)	*< 0.001*
Better side rigidity	2.44 (1.92)	3.00 (2.04)	0.039
Upper extremity rigidity total score	2.44 (1.81)	3.63 (1.79)	*< 0.001*
Worse side upper extremity rigidity	1.42 (0.96)	2.16 (0.92)	*< 0.001*
Better side upper extremity rigidity	1.02 (0.95)	1.46 (0.99)	*0.001*
Tremor total score	6.84 (5.28)	6.15 (4.39)	0.48
Worse side tremor	3.33 (2.45)	3.15 (2.34)	0.48
Better side tremor	1.90 (1.93)	1.30 (1.29)	0.050

Non-parametric Mann-Whitney U-tests were used to investigate the continuous variables, and Chi-squared tests were used to investigate categorical variables (values are presented as n or mean and SD for demonstrative purposes). **Significant* after Benjamini-Hochberg procedure. Missing values in education (n = 3), MMSE (n = 2), motor symptom duration (n = 22), facial expression (n = 1), axial signs (n = 10), bradykinesia total score (n = 3).

5.4.2 Upper extremity rigidity, facial expression and striatal DAT binding

5.4.2.1 One-way ANOVA

Hypomimia was associated with caudate DAT binding (F = 7.51, $P < 0.001$, Figure 12E) and putamen DAT binding (F = 4.52, $P = 0.005$, multiple comparisons: 0 vs. 3, $P = 0.005$, 1 vs. 3, $P = 0.054$, 0 vs. 2, $P = 0.060$, the rest comparisons, $P > 0.32$), and upper extremity rigidity was associated with caudate DAT binding (F = 3.88, $P = 0.011$, Figure 11F) and putamen DAT binding (F = 3.17, $P = 0.027$, multiple comparisons: 1-1.5 vs. 3-4, $P = 0.024$, the rest comparisons, $P > 0.27$) in patients with abnormal DAT binding. There were no associations between hypomimia and caudate DAT binding (F = 1.21, $P = 0.31$) or putamen DAT binding (F = 1.24, $P = 0.30$), or between upper extremity rigidity and caudate DAT binding (F = 1.08, $P = 0.36$) and putamen DAT binding (F = 1.49, $P = 0.22$) in patients with normal DAT binding. The associations between the motor signs and caudate nucleus are shown in Figure 12, as these signs showed more significant pairwise post hoc comparisons.

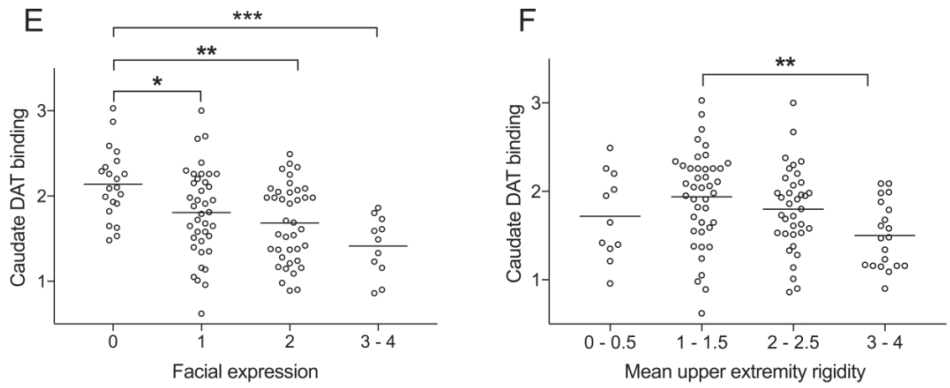

Figure 12. In patients with abnormal DAT SPECT imaging, in particular, increasing hypomimia was associated with a decline in the caudate DAT uptake. The means are presented with lines. Caudate mean DAT binding and hypomimia (facial expression) (E): F = 7.51, $P < 0.001$, the separate comparisons 0 vs. 1, $P = 0.029$; 0 vs. 2, $P = 0.001$; 0 vs. 3-4, $P < 0.001$; 1 vs. 3-4, $P = 0.068$; 1 vs. 2, $P = 0.64$; 2 vs. 3, $P = 0.33$. Caudate DAT binding and mean upper extremity rigidity score (F): F = 3.88, $P = 0.011$. 1-1.5 vs. 3-4, $P = 0.006$; 2-2.5 vs. 3-4, $P = 0.12$; rest pairwise post hoc comparisons, $P > 0.43$. *** P < 0.001, ** $P < 0.01$, * $P < 0.05$, NS = non-significant.

5.4.2.2 Logistic regression analyses

The OR (95% CI) was 4.79 (1.56 – 14.75) for the presence of upper extremity rigidity versus no detection of rigidity ($P = 0.006$) and 2.14 (1.14 – 4.00) for the presence of hypomimia versus no detection of hypomimia ($P = 0.018$), for the likelihood of striatal DAT deficiency (Table 3). The area under the curves (AUCs, with 95% CI) were 0.68 (0.61 – 0.75), $P < 0.001$ and 0.61 (0.53 – 0.68), $P = 0.006$ for the increasing upper extremity rigidity total score for both upper extremities and the increasing facial expression score, respectively, in the ROC curves that were analyzed for these factors (Figure 13). In the multivariate regression analysis, both upper extremity rigidity and hypomimia independently pointed to higher likelihood of striatal DAT deficiency with ORs (95% CI) of 3.34 (1.03 – 10.86), $P = 0.045$ and 2.15 (1.10 – 4.20), $P = 0.025$, respectively. A longer duration of the motor symptoms prior to the DAT SPECT imaging was independently associated with a lower likelihood of abnormal striatal DAT binding (Table 9).

Table 9. Study IV: Logistic regression models and analyses.

	OR	***P* value**	***95% CI***
Univariate analyses			
Bradykinesia total score	1.02	0.23	0.99 – 1.05
Tremor total score	0.97	0.30	0.92 – 1.03
Axial signs	1.02	0.60	0.95 – 1.10
Speech	1.22	0.20	0.90 – 1.66
Rigidity total score	1.09	*0.003*	1.03 – 1.16
Facial expression score	1.55	*0.004*	1.15 – 2.10
Upper extremity rigidity score	1.44	*<0.001*	1.23 – 1.69
Hypomimia yes vs. no	2.14	*0.018*	1.14 – 4.00
Upper extremity rigidity yes vs. no	4.79	*0.006*	1.56 – 14.75
Motor symptom duration (years)	0.92	*0.023*	0.86 – 0.99
Multivariate model			
Hypomimia yes vs. no	2.15	*0.025*	1.10 – 4.20
Upper extremity rigidity yes vs. no	3.34	*0.045*	1.03 – 10.86
Motor symptom duration (years)	0.92	*0.026*	0.86 – 0.99

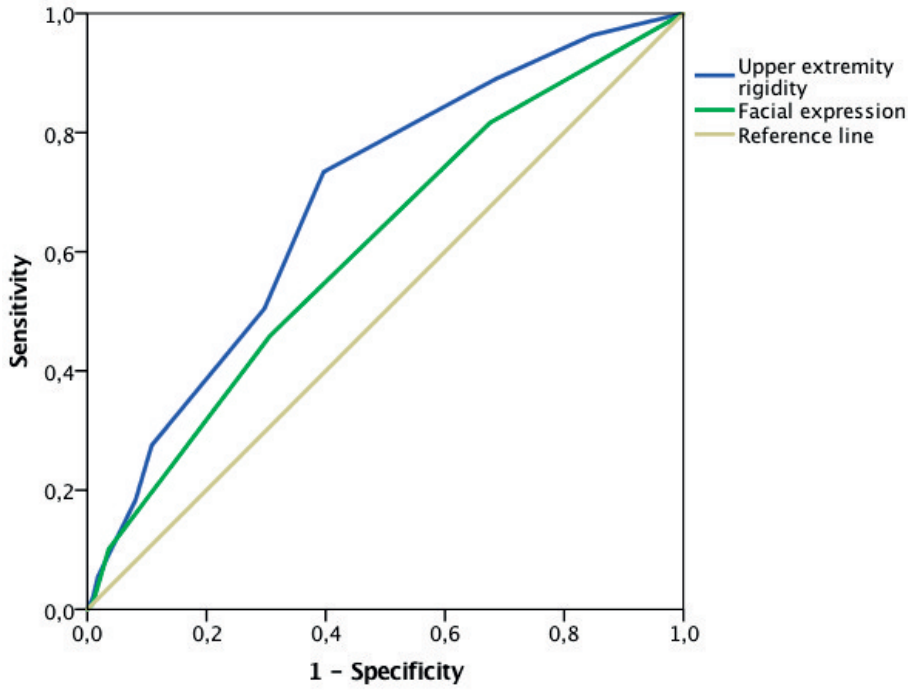

Figure 13. ROC curves for the increasing upper extremity rigidity total score and increasing facial expression score in the MDS-UPDRS part III motor examination in 221 patients with clinically uncertain parkinsonism and tremor with normal (n = 111) and abnormal (n = 110) striatal DAT binding in [^{123}I]FP-CIT SPECT imaging.

5.4.2.3 Voxel-based analyses

A more severe hypomimia was associated with a bilateral reduction of DAT binding in the caudate nucleus in patients with striatal DAT deficiency (cluster 1 extent 459 voxels, peak at -14, -3, 24 with $T_{max} = 4.51$, $P_{FWE} = 0.003$; cluster 2 extent 456 voxels, peak at 9, 20, 0 with $T_{max} = 4.31$, $P_{FWE} = 0.006$) (Figure 14A). A more severe upper extremity rigidity was associated with a reduction of DAT binding in the left putamen in patients with striatal DAT deficiency (cluster extent 74 voxels, peak at -22, -8, 12 with $T_{max} = 4.24$, $P_{FWE} = 0.007$) (Figure 14B). The independent associations of these signs with DAT loss in the specific striatal subregions remained significant when both of these signs were added to a multivariate regression model (cluster 1 extent 234 voxels, peak at -14, -3, 24 with $T_{max} = 4.26$, $P_{FWE} = 0.007$; cluster 2 extent 262 voxels, peak at 10, 20, 0 with $T_{max} = 3.93$, $P_{FWE} = 0.01$ for hypomimia and caudate nuclei, and cluster extent 53 voxels, peak at -26, -8, -12 with $T_{max} = 4.10$, $P_{FWE} = 0.01$ for upper extremity rigidity).

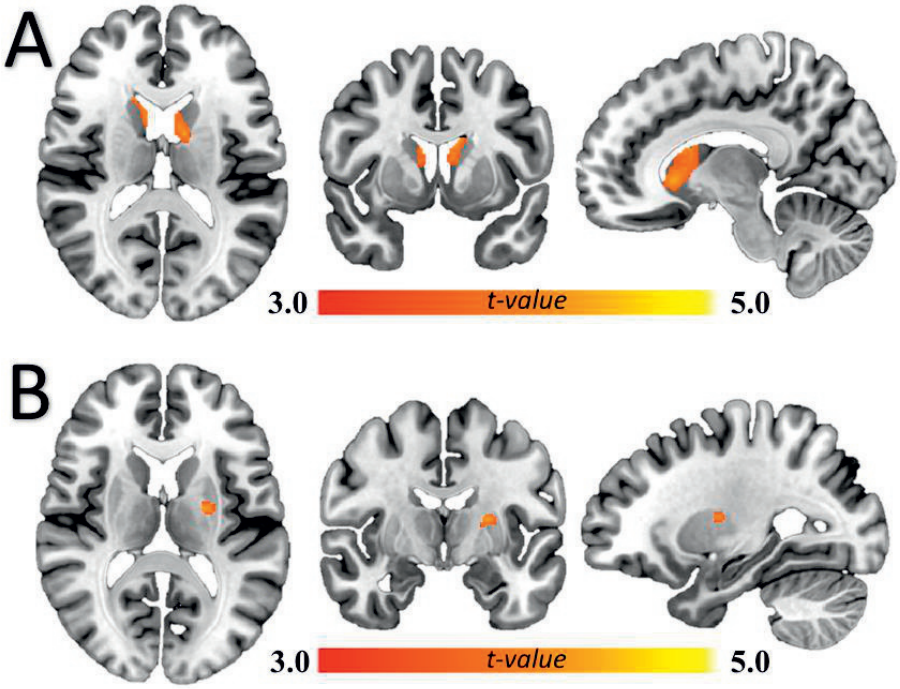

Figure 14. Associations between reduced striatal DAT binding and hypomimia (A) and upper extremity rigidity (B). Statistical t-maps including only voxels with voxel-level FWE-corrected P < 0.05 are shown and overlaid on the MNI152 T1-weighted template.

5.4.2.4 Effects of scanners and medications on the previous results

The mean DAT binding in the more severely affected posterior putamen did not differ between the different SPECT cameras ($P = 0.37$) or between patients who were and were not receiving antiparkinsonian medications in the group of abnormal DAT binding ($P = 0.23$) and normal DAT binding ($P = 0.42$).

All the previous results, except the association between a reduced facial expressiveness and a higher likelihood of abnormal DAT binding ($P = 0.21$), remained the same in the subsample analyses of patients who were not receiving any antiparkinsonian medications (n = 181). In the univariate logistic regression analyses, the OR of the presence of any stage of upper extremity rigidity versus no rigidity for striatal DAT deficiency was even higher, OR = 7.33, $P = 0.009$. Patients with normal (n = 104) and abnormal (n = 77) DAT SPECT scans differed in terms of the duration of motor symptoms, overall rigidity,

rigidity on the more affected body side, upper extremity rigidity, upper extremity rigidity on the more and less affected extremities ($P < 0.01$) and facial expression ($P = 0.041$). However, the differences in facial expression were not significant after the Benjamini-Hochberg procedure. Similarly, unilateral upper extremity kinetic tremor ($P = 0.039$), asymmetry index of all bilateral motor signs in MDS-UPDRS part III ($P = 0.022$) and asymmetry of overall rigidity ($P = 0.029$) also tended to differ between patients with and without DAT deficiency but did not remain significant after the Benjamini-Hochberg procedure.

5.4.2.5 Correlations between motor signs and DAT binding (unpublished data)

In patients with abnormal DAT binding, putamen asymmetry index correlated with the asymmetry index of all bilateral motor signs in MDS-UPDRS-III (r = 0.62, Figure 14), bradykinesia asymmetry index (r = 0.58), rigidity asymmetry index (r = 0.49) and tremor asymmetry index (r = 0.56) (all P values < 0.001), but these significant correlations were not observed in patients with normal DAT binding (r = -0.059, $P = 0.54$, Figure 14; r = -0.14, $P = 0.16$; r = 0.063, $P = 0.51$; r = -0.093, $P = 0.33$, respectively).

The MDS-UPDRS part III total score (r = -0.37, $P < 0.001$), bradykinesia total score (r = -0.29, $P = 0.002$), rigidity total score (r = - 0.33, $P < 0.001$), facial expression (r = -0.34, $P < 0.001$) and axial signs (r = -0.26, $P = 0.006$) showed an inverse correlation with putamen DAT binding (definition in Table 2) in patients with abnormal DAT binding but not in patients with normal DAT binding (r = -0.091, $P = 0.34$; r = -0.073, $P = 0.45$; r = -0.18, $P = 0.056$; r = -0.11, $P = 0.26$ and r = 0.074, $P = 0.56$, respectively). Facial expression showed a better correlation with caudate DAT binding (definition in Table 2) in patients with abnormal DAT binding (r = -0.39, $P < 0.001$); however, no correlation was observed in patients with normal DAT binding (r = -0.16, $P = 0.089$). Tremor total score or speech did not correlate with putamen DAT binding in patients with abnormal DAT binding (r = -0.046, $P = 0.63$; r = -14, $P = 0.15$) nor in patients with normal DAT binding (r = -0.13, $P = 0.18$; r = -0.03, $P = 0.75$, respectively).

Worse side bradykinesia showed an inverse correlation with its contralateral (r = -0.22, $P = 0.028$) and ipsilateral (r = -0.30, $P = 0.002$) putamen DAT binding in patients with abnormal DAT binding but not in patients with normal binding (r = 0.063, $P = 0.60$ and r = 0.013, $P = 0.91$, respectively). However, worse side rigidity did not correlate with its contralateral (r = -0.062, $P = 0.60$) or ipsilateral (r = -0.20, $P = 0.091$) putamen DAT binding in patients with abnormal DAT binding or in patients with normal DAT binding (r = -0.09, $P = 0.52$ and r = -0.007, $P = 0.96$, respectively). The worse side tremor score was not correlated with its contralateral (r = 0.067, $P = 0.59$) or ipsilateral (r = 0.12, $P = 0.32$) putamen DAT binding in patients with abnormal DAT binding or patients with normal DAT binding (r = -0.049, $P = 0.67$ and r = -0.008, $P = 0.94$, respectively).

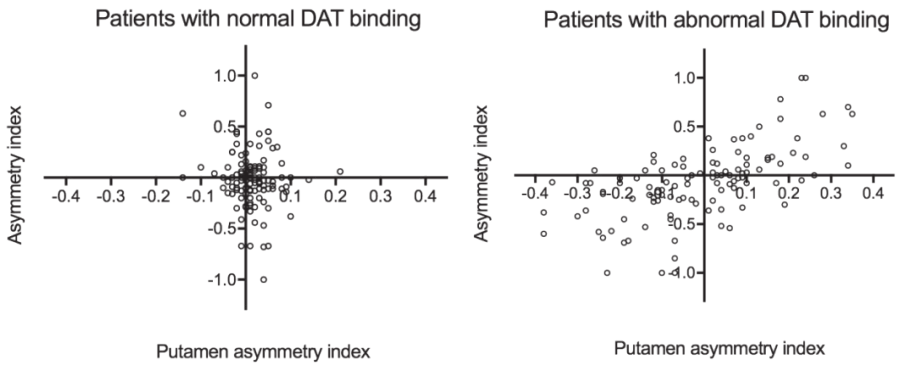

Figure 14. Correlations between the asymmetry index of all bilateral motor signs in MDS-UPDRS part III and the asymmetry index in putamen mean DAT binding in 111 patients with normal DAT binding (the graph on the left) and 110 patients with abnormal DAT binding (the graph on the right).

6 DISCUSSION

6.1 Brainstem measurements and striatal dopamine deficiency

6.1.1 Midbrain atrophy in PD

The results of study I suggested that there are slightly lowered midbrain-to-pons ratios in PD compared to patients with normal striatal DAT function, according to [^{123}I]FP-CIT SPECT. However, the clinical significance of this finding remains uncertain. Note that neither the pons width nor the midbrain width differed significantly between the PD patients and patients with normal striatal DAT binding. Thus, only the ratio of midbrain and pons showed slight differences between normal and abnormal striatal DAT binding patient groups, displaying slight midbrain atrophy in PD patients compared to control patients with normal DAT function. In this study, the average midbrain-to-pons ratios were higher in PD patients (ratio of 0.59) and patients with normal DAT binding (ratio of 0.61), but midbrain-to-pons ratios in PSP patients were reported by Massey *et* colleagues as low as 0.52 or even lower. Only 12% of the PD patients showed midbrain-to-pons ratios below 0.52 in study I.

The slight decrease in the midbrain-to-pons ratios in PD patients could reflect midbrain atrophy but also a relatively preserved pons width in PD patients compared to patients without neurodegenerative presynaptic parkinsonism. However, in the study by Massey *et al*., the midbrain width and midbrain-to-pons ratios, as well as the pons width, seemed to be smaller in PD versus controls in the pathologically proven cases, even if the differences were not statistically significant. It should be noted that there were no healthy controls in study I, as patients with a normal striatal DAT function were symptomatic parkinsonism patients scanned with DAT SPECT due to diagnostic uncertainties. However, the control patients had normal DAT imaging outcomes and no evidence of PD or other parkinsonism with presynaptic dopamine deficiency. In addition, although the diagnoses of patients in study I were not pathologically proven, the size of the study sample was considerably larger than that in the study by Massey *et al.* (n = 29 for the pathologically proven study group and n = 62 for the clinical study group).

Age, gender and disease duration were used as covariates in the analyses. Previously, higher age was found to be associated with midbrain measurements (Morelli *et al.*, 2014). In a longitudinal MRI study, patients with PSP-P showed some changes in the midbrain MRI indices suggesting for midbrain atrophy after disease progression, with no MRI measurement differences among PD patients in the early stages of the disease. However, patients with PSP-RS showed a higher ratio of the midsagittal pontine to midbrain

tegmental areas already in the early stages of the disease, with the most profound changes after two years of follow-up (Hwang *et al.*, 2017). Thus, it cannot be excluded that some of the PD patients were misdiagnosed early PSP, particularly PSP-P, patients, in study I.

Midbrain atrophy measurements seem to be valuable particularly in the PSP diagnosis. Massey *et al.* showed that a midbrain measurement of 9.35 mm or below, and a midbrain-to-pons ratio of 0.52 or below, strongly pointed to PSP when the cases were pathologically proven PSP patients (Massey *et al.*, 2013). In another study of six pathologically proven PSP patients and twenty-three patients without pathologically proven PSP, a midbrain-to-pons ratio below 0.50 was clearly associated with PSP (Kaasinen *et al.*, 2015b). In a study with a large sample of patients with neurodegenerative parkinsonism and healthy controls, the presence of the 'hummingbird' sign had a very high specificity and positive predictive value for a PSP diagnosis. However, only 2 of 289 PD patients and none of the healthy controls featured this sign (Mueller *et al.*, 2018). There are also results from another structural index, the Magnetic Resonance Parkinsonism Index ("MRPI, the ratio of the midsagittal areas of the pons and the midbrain multiplied by the ratio of the middle cerebellar peduncle and superior cerebellar peduncle widths" in Quattrone *et al.* 2018) in distinguishing possible PSP from PD. It was suggested that this index is not influenced by aging, like the ratio of the midsagittal midbrain and pons (Morelli *et al.*, 2014), and a recent study suggested that this index should be preferred to the measurements of the midbrain to pons area ratio in the differential diagnosis of PD and PSP (Nigro *et al.*, 2017).

It could be suggested that the differences in midbrain-to-pons ratios between PD and PSP are related to different pathophysiological changes in the two diseases outside of striatal dopaminergic deficiency. While PD is categorized as an alpha-synucleinopathy, PSP is a progressive tauopathy (Long *et al.*, 2015). A study with eight autopsy PSP cases showed that the midbrain atrophy of tegmentum, tectum and dilatation of the aqueduct in T1-weighted MRI images was connected with the periaqueductal and tegmentum atrophy, and the density of the tau-positive structures was clearly associated with the stage of the atrophy (Aiba *et al.*, 1997).

6.1.2 Midbrain atrophy in relation to striatal dopamine function

The study I showed that there was no association between midbrain-to-pons ratios and striatal dopamine deficiency in patients with PD or in patients with normal striatal DAT binding. Therefore, midbrain-to-pons measurements cannot be used to predict striatal dopamine loss in PD. In the study by Arnold *et al.,* both midbrain atrophy and reduced striatal dopamine function, detected with IBZM SPECT, were found in patients with PSP, and the midbrain atrophy correlated with the reduced striatal D2 receptor binding (Arnold *et al.*, 2002). Thus, the connection between midbrain atrophy and the level of striatal

dopamine deficiency is likely to be related to PSP only. If study I had included PSP patients, it could be possible that an association between midbrain atrophy and the striatal DAT binding would have been detected also between the midbrain-to-pons ratios and [^{123}I]FP-CIT uptake measurements. However, the neuropathology behind these two imaging outcomes of midbrain atrophy and striatal dopamine deficiency may represent separate but concurrently progressive pathophysiological mechanisms of the disease.

Finally, if the MRI midbrain measurements are related to striatal dopaminergic deficiency only in patients with PSP, it is debatable whether the association between midbrain atrophy and striatal dopamine function provides any new relevant information in the diagnostics of parkinsonism, as the midbrain atrophy measurements solely has shown high accuracy in the PSP diagnosis (Möller *et al.*, 2017; Mueller *et al.*, 2018). Future studies could confirm whether the midbrain atrophy measurements are significantly associated with the dopaminergic neurodegeneration in PSP and whether this association is clinically relevant in the diagnosis, prognosis or treatment of PSP patients.

6.2 Variability in the [^{123}I]FP-CIT SPECT image analyses

6.2.1 Visual versus automated analysis

6.2.1.1 The potential of visual and automated analyses

In the study II, 10% of the DAT SPECT scans (n = 12) showed discrepancy between visual analysis by both experts and the automated semi-quantitative analysis. The problem is that both of the analysis methods could have been accurate compared to the clinical diagnosis after a follow up. However, most of these twelve cases (nine of twelve) were visually interpreted to show a slightly abnormal striatal tracer binding, whereas the BRASS analysis indicated normal binding. None of these patients revealed a neurodegenerative parkinsonism at the end of the follow-up. Thus, it is suggested that the scans on the borderline of abnormality with discrepant analyses should probably not be interpreted as abnormal. This suggestion is strengthened by the studies of SWEDDs, where most patients with clinically suspected PD but normal FDOPA PET or DAT SPECT scans do not show progression of the symptoms and the scan is still normal when taken after a clinical follow-up. It seems that these SWEDD patients reveal diagnoses such as benign dystonic tremor, VP or DIP (Brooks & Tambasco, 2016). Note that the overall accuracy of the analysis methods cannot be estimated in study II, as the clinical outcomes and diagnoses were only compared to the imaging interpretations in the twelve discrepant cases.

The use of ROI techniques, together with the experts visual assessment, is recommended by the European Association of Nuclear Medicine (EANM) for scan analysis to minimize the sources of error that might arise from the sole visual rating (Scherfler & Nocker, 2009). In the study by Albert *et al.,* 101 [^{123}I]FP-CIT SPECT scans were visually and semi-quantitatively re-evaluated, and a clinical follow-up was obtained in 62 of these patients. Of these 62 patients, 11 showed a neurodegenerative parkinsonism, and 14 patients had discordant visual and semi-quantitative analyses. The semi-quantitative analysis discovered all five patients without neurodegenerative parkinsonism from nine patients with uncertainty in the visual evaluations. However, in the remaining four patients with visually normal scans, the semi-quantification incorrectly defined the cases as abnormal, even if no neurodegenerative parkinsonism were diagnosed later on (Albert *et al.*, 2016). The results of the study II similarly showed that both of the two analysis methods are needed, as both have their own diagnostic uncertainties, even if it seemed that more often the visual analysis tended to evaluate a scan incorrectly as abnormal. Furthermore, the results were in concordance with earlier findings where, in particular, elderly patients with scans that appeared to be abnormal in the visual interpretation, were correctly categorized as normal after the additional semi-quantitative analysis (Albert *et al.*, 2016). However, even if the semi-quantitative analysis is many times able to correctly interpret the scan as normal, it cannot be used alone, as sometimes it do fails in this evaluation. In cases where the semi-quantitative analysis incorrectly interpreted the scan as abnormal, the semi-quantitative analysis showed reduced DAT binding particular in the posterior putamen in the Albert study (Albert *et al.*, 2016), whereas in study II, these cases tended to show uniform slight reductions in the striatal DAT uptake.

6.2.1.2 Clinical characteristics of patients with discrepant scan analyses

A careful clinical interpretation is also needed for the differential diagnosis of patients with discrepant DAT scan analyses, as none of these patients should have received a neurodegenerative parkinsonism diagnosis. The discrepant scans belonged to patients who were older than patients with normal scans, many of whom suffered from cognitive problems. Four of these patients received a final diagnosis of DIP, whereas three received a final diagnosis of AD.

Note that two of the DIP patients were receiving atypical antipsychotic drug risperidone, which is generally thought to have a lower risk in developing extrapyramidal side effects. However, as risperidone binds to D_2 receptors in a dose-dependent manner, it may actually induce parkinsonism and extrapyramidal side effects to a similar extent as high doses of typical antipsychotics (Shin & Chung, 2012). DIP develops when enough striatal dopamine postsynaptic receptors are blocked (Tolosa *et al.*, 2003) and cannot be interpreted in presynaptic DAT SPECT imaging; therefore, patients with DIP should

belong to the group of normal DAT SPECT imaging outcomes. However, there are also opposite results. In a study of 20 parkinsonism patients who were taking neuroleptic medications, up to 11 patients had abnormal [^{123}I]FP-CIT SPECT scans, and these patients were suggested to have exacerbation a subclinical PD (Lorberboym *et al.*, 2006). This finding could however be questioned, as DIP cases are much more common than the prevalence of PD in the clinical practice (Tolosa *et al.*, 2003).

Two patients whose scans were categorized as abnormal in the BRASS analysis received a final diagnosis of ET in the clinical follow-up. While the BRASS analysis showed SBRs of 2.0 – 2.5 standard deviations below the age-matched reference mean in at least one striatal region, the visual analysis performed by experts was more reliable than the automated analysis in these cases. One reason for this could be the borderline set at -2SD and not lower between a normal and abnormal scan. Furthermore, it could be suggested that the cases with slight but atypical DAT binding reduction pattern, suggesting a neurodegenerative disorder, were more accurately detected visually. Even if the visual analysis is subjective, it can evaluate whether the DAT reduction pattern fits the clinical description of the patient.

ET is suggested to be a heterogenous disorder, and some patients are speculated to have a combination of long-standing ET with subsequent PD (Thenganatt & Jankovic, 2016). Also an earlier study found ET to be associated with quantitatively reduced DAT uptake in all parts of the striatum, whereas the qualitative analysis revealed normal imaging outcomes (Gerasimou *et al.*, 2012). Semi-quantitative analysis revealed striatal dopaminergic deficits also in some ET patients with concomitant PD (Waln *et al.*, 2015). However, the proper diagnoses of these patients is doubtful, and could highly likely be also tremor-dominant PD.

6.2.2 Effect of expertise in the visual analysis

In study II, the visual analyses were more equivalent with the automated analysis in the experts, and the experts performed better than nonexperts in the inter-rater agreement. This seems to be the case at least when the visual analyses are conducted with the help of example categories of normal and different stages of abnormal scans, and concise patient information is given to the visual scan readers. The readers with several years of functional neuroimaging experience showed good inter-observer agreement also in a review of five multicenter clinical trials (Seibyl *et al.*, 2014).

Nonexperts showed more divergence in the visual analyses. While only 7 – 21% of the scans were interpreted as normal by the four nonexperts, the corresponding percentages were 28% for experts and 36% for the BRASS analysis. Thus, it seems that, in particular, the less experienced readers tend to visually categorize the cases as abnormal, even if

patients do not have neurodegenerative parkinsonism. In an earlier study, DAT SPECT images were visually analyzed individually by three experienced nuclear medicine physicians, followed by a visual interpretation with additional quantitative information of the striatal DAT binding (Söderlund *et al.*, 2013). For the two raters with less experience, the number of striata interpreted visually as abnormal decreased as semi-quantitative analysis information was given. In addition, these less experienced readers changed the reads to a larger extent when semi-quantitative data were provided. Thus, even if the less experienced readers were trained nuclear medicine physicians with some experience of [^{123}I]FP-CIT SPECT scan analysis, they tended to overreport their readings as abnormal when only a visual interpretation of the scans was performed (Söderlund *et al.*, 2013).

These findings indicate that the visual analysis of DAT scans should be performed by trained, ideally experienced physicians. It is evident that the visual analysis is not easy, as also the experts showed some discrepancy especially when interpreting the inter-hemispheric differences or the stage of abnormality of the striatal DAT biding. These are important findings, as the DAT reduction pattern or differences in DAT binding between hemispheres, as well as the stage of DAT deficiency, may be important factors in the differential diagnosis of parkinsonism. Quantification of the DAT binding seems to help in these issues. Finally, note that the access to the clinical patient information could be considered to be a confounding factor, as it may be valuable for experts but unnecessary for nonexperts. However, without this information, the study would not have as closely simulated a real clinical setting in the differential diagnosis of uncertain parkinsonism. The experts may have taken more advantage of the clinical patient information as they used more time in the analyses when compared to nonexperts.

6.3 Clinical associations and outcomes of DAT deficiency

6.3.1 Survival in Parkinson's disease in relation to dopamine deficiency

The study III show that there is no association between striatal or extrastriatal DAT binding and survival in PD. The results remained the same in the subgroup of patients with less advanced motor disability (H&Y stage < 2.5). These findings verify the earlier results of striatal dopaminergic function related to FDOPA PET and survival in a different sample of PD patients, which also suggests that the degree of hypodopaminergic defect does not predict mortality (Järvelä *et al.*, 2014). Thus, it seems that the level of presynaptic dopaminergic deficiency in general has no value in predicting survival in PD. Note that the PD diagnoses were not neuropathologically proven, but the clinical diagnoses were confirmed in a follow-up. In addition, all of these patients had a pathological DAT scan that increased the confidence of their diagnoses.

Although the correlations between the clinical disease severity and DAT deficiency seem to be linear, at least when studied in cross-sectional studies (Kaasinen & Vahlberg, 2017), and the more severe motor signs seem to be associated with a more rapid disease progression and a more severe DAT loss in the early stages of PD (Fereshtehnejad *et al.*, 2017), there was a lack of association between the level of striatal presynaptic dopamine deficiency and mortality in PD. This finding could either indicate that the nigrostriatal dopaminergic neurodegeneration is simply not associated with the mortality in PD, or that the effect is somehow hidden by the compensatory mechanisms or the characteristics of the progressive dopamine deficiency in different parts of the nigrostriatal dopaminergic neural pathway. The exact mechanism of the striatal DAT deficiency detected with DAT SPECT imaging is not completely clear, as the DAT deficiency may represent loss in endogenous dopamine, down-regulated DAT expression or function, or the pure loss of the nigrostriatal axon terminals (Eshuis *et al.*, 2009).

Longitudinal studies suggest that there is a negative exponential progression of striatal DAT uptake deficiency in PD, which could indicate that some type of compensatory mechanisms are involved in the process (Kaasinen & Vahlberg, 2017). While neuropathological evidence shows that dopaminergic fibers in the dorsal striatum are virtually absent by 4-5 years and thereafter, the neuroimaging evidence point to a relevant presynaptic dopamine function in the striatum of PD even after several years of disease onset (Djaldetti *et al.*, 2011; Kordower *et al.*, 2013; Kaasinen & Vahlberg, 2017). It could also be suggested that the downregulation in the DAT function (Eshuis *et al.*, 2009) result in a relatively more decreased striatal DAT binding of the [^{123}I]FP-CIT ligand compared to the true current nigrostriatal dopamine deficiency or the amount of nigrostriatal dopaminergic neurons. Furthermore, there is upregulation of the AADC function measured with FDOPA PET (Kaasinen & Vahlberg, 2017), and the upregulated AADC function may have underestimated the true striatal dopaminergic deficiency that could lead to no significant associations between the striatal dopamine function and survival in the FDOPA PET study (Järvelä *et al.*, 2014).

The nonsignificant associations between the level of DAT deficiency and survival in PD could also be explained by the 'dying back' hypothesis of the nigrostriatal dopaminergic cell death, in which cell degeneration starts from the axons containing the DATs, while the neuron somas are still well-preserved (Burke & O'Malley, 2013). Recently, a correlation between striatal DAT deficiency and dopamine producing cell death in the substantia nigra was found to be lacking (Saari *et al.*, 2017). In addition, the DAT availability within the entire nigrostriatal pathway was reported for the first time by Fazio and colleagues, who used a novel [^{18}F]FE-PE2I high resolution PET in early PD patients. This technique allows the examination of the DAT in the whole nigrostriatal system from the cell bodies in substantia nigra to the striatal presynaptic nerve endings. The study showed that the DAT function was reduced from 36% to 70% in the striatal nerve

terminals but only by 30% in cell bodies, suggesting a relative perpetuation of dopaminergic neuron somas in the early stages of PD (Fazio *et al.*, 2018).

It could also be suggested that non-dopaminergic mechanisms are more relevant in the decreased survival of PD patients. In patients with autopsy-confirmed alpha-synucleinopathy, an independent negative association was found between cerebral tau neurofibrillary tangles and survival in a multivariate regression model that also included the cerebral alpha-synuclein scores (Irwin *et al.*, 2017). Thus, the accumulation of cerebral neurofibrillary tangles burden seemed to be the most obvious pathological predictor of a shorter timespan between the start of the motor and cognitive symptoms and future mortality in patients with clinical PDD and DLB. However, patients with increasing AD neuropathology also showed increasing alpha-synuclein pathology, and there were strong correlations between the global tau-related and alpha-synuclein pathology. Thus, both AD and alpha-synuclein neuropathology was further suggested to be related to earlier mortality, in addition to the fact that also the AD neuropathology seemed to be a key feature in the pathogenesis of most patients with synucleinopathy (Irwin *et al.*, 2017). In an earlier study of 140 patients with a clinical PD diagnosis and either normal cognition or the onset of PDD two or more years after motor symptoms, in particular the cerebral Lewy pathology was associated with dementia (Irwin *et al.*, 2012), that is the most consistent independent predictor of mortality in PD (Macleod *et al.*, 2014). Thus, it could be suggested that biomarkers of cognitive decline and dementia could also predict patient survival. The cortical and striatal beta-amyloid scores, tau burden and cortical Lewy pathology were all significantly greater in pathologically confirmed PD patients with dementia, and again these pathologies were associated with each other (Compta *et al.*, 2011). Studying this wide range of proteinopathic burden could forecast patient survival in PD more accurately than the radionuclide imaging of presynaptic dopaminergic function. Biomarkers, such as alpha-synuclein and tau-sensitive functional neuroimaging ligands, could be more useful for predicting advancing disability and increased mortality in patients with PD and other alpha-synucleinopathies and tauopathies. However, currently it is not possible to directly image the aggregated alpha-synuclein burden in the synucleinopathies, as the peptides and antibodies that bind to alpha-synuclein aggregates cannot enter the CNS when given intravenously. On the other hand, functional radiotracer-based molecular imaging approaches can detect abnormal aggregations of tau and beta-amyloid in synucleinopathies, and some PET tracer are able to study cortical cholinergic terminal function, which has found to be reduced in demented PD patients (Brooks & Tambasco, 2016).

6.3.1.1 Survival in Parkinson's disease in relation to other clinical and demographical factors

In line with earlier results on survival in PD (Levy *et al.*, 2002; Lo *et al.*, 2009; Posada *et al.*, 2011; de Lau *et al.*, 2014; Macleod *et al.*, 2014), a higher risk of earlier mortality was associated with older age, presence of cognitive impairment and more severe motor signs. These findings verify that the data can be viewed as reliable in the interpretation of the associations between DAT binding and survival. Gender was not associated with survival, nor was the lack or amount of dopaminergic medications measured with LEDD categories.

The mortality risk seemed to be higher in patients with H&Y stages 2.5 and 3 than in patients with the higher stages, but this finding was likely due to the small number of patients (n = 5) in the highest category. However, the differences between patients with a severe motor disability (H&Y > 2) and patients with milder motor severity stages (H&Y 1, 1.5 and 2) were clearly significant. As there is no impaired balance in the lowest H&Y stages of 1, 1.5 and 2, the results are in line with previous evidence that the postural impairment and gait problems are associated with higher mortality in PD (de Lau *et al.*, 2014). Furthermore, previous findings have suggested that the motor and cognitive progression of tremor-dominant PD is slower than in PIGD or akinetic-rigid PD subtypes (Zetusky *et al.*, 1985; Williams-Gray *et al.*, 2007; Kaasinen *et al.*, 2014)

Note that cognitive status particularly influenced the future survival. However, the definition of the presence of cognitive impairment was based on information provided by the treating physician in the medical records and not on neuropsychological cognitive testing or congruent diagnostic criteria for cognitive impairment and dementia. In addition, cognitive impairment was not separated from dementia in the analyses. In a Parkinson's Progression Markers Initiative (PPMI) cohort study, the predictive values of several clinical and demographical factors were studied in relation to upcoming cognitive defects in PD patients. In addition to older age, hyposmia, RBD, depression and a more severe motor impairment pointed to a higher risk of future cognitive defects. In addition, reduced striatal DAT uptake pointed to cognitive problems at the two-year time point (Schrag *et al.*, 2017).

6.3.2 Parkinsonian motor handicap in relation to DAT binding

6.3.2.1 The differences in patients with and without striatal DAT deficiency

Study IV, a cross-sectional clinical and imaging study with a large group of patients with clinically uncertain parkinsonism or tremor, was the first study to investigate likelihoods of individual parkinsonian motor signs for striatal dopaminergic neurodegeneration. In addition, the associations and correlations of individual motor signs with DAT were studied in this unique sample, representing the clinical diagnostic reality much better than the previous studies with patients with established PD diagnoses. However, as the study sample consisted of patients with clinically uncertain parkinsonism and tremor who needed to undergo DAT SPECT, the results cannot be considered to be fully representative of all patients with clinically uncertain parkinsonism syndromes. In addition, the results are not straightforward applicable in distinguishing between clinical parkinsonism patients with two certain diagnoses with and without DAT deficiency.

The results showed that rigidity, particularly upper extremity rigidity, and hypomimia are the only motor parkinsonian signs that show differences between clinically uncertain parkinsonism patients with and without striatal DAT deficiency. It is equally interesting that the overall motor symptom severity, as measured with the MDS-UPDRS part III, and original H&Y staging, and the most cardinal motor sign bradykinesia, did not differ between patients with a normal and abnormal striatal DAT function. Surprisingly, not even unilateral rest tremor differed between the two patient groups. These findings highlight the significance of upper extremity rigidity and hypomimia in the clinical examination but also underline clinical motor similarities between patients with different causes of parkinsonism, leading to diagnostic uncertainties.

Previous studies support the notion that patients with and without evidence of striatal DAT deficiency show overlapping motor features. As demonstrated by a blinded video study, the clinical symptom based separation of parkinsonism patients with and without DAT deficiency appears to be difficult even for movement disorder specialists, particularly when patients have tremor-dominant symptoms (Bajaj *et al.*, 2010). Furthermore, ET may be associated with resting tremor or asymmetric symptoms, and PD is often associated with kinetic and postural tremor (Kwon *et al.*, 2016). In one study, there were no differences in tremor, bradykinesia or rigidity between DIP and PD patients (Lorberboym *et al.*, 2006). Even if patients with VP may classically present with more severe motor features of the lower limbs, more symmetrical gait difficulties, postural instability, postural tremor and cognitive problems when compared to PD patients, no specific clinical features have been able to reliably differentiate VP from PD (Kalra *et al.*, 2010).

Even if the bradykinesia total score, as well as the axial signs, showed a weak but significant inverse correlation with putamen DAT binding in patients with abnormal DAT binding, these signs did not point to hypodopaminergic parkinsonism. It is somewhat sensible that bradykinesia, the core feature of any parkinsonism, despite the etiology of it, did not distinguish patients with presynaptic dopaminergic deficiency from other causes of parkinsonism and tremor. There are several causes of slowness and muscle weakness that might mimic global bradykinesia, such as pain, musculoskeletal diseases or even depression (Berardelli *et al.*, 2001). In addition, note that bradykinesia, a sign characterized by "slowness of movement and decreased amplitude or speed, or progressive hesitations/halts" (Postuma *et al.*, 2015) is a heterogenous motor sign with multiple dimensions, and this complexity may have confounded the results. The amplitude and speed impairments were previously reported to respond differently to dopaminergic medication, and they may be unrelated, suggesting that the pathophysiology behind these different dimensions of bradykinesia is somewhat separate (Berardelli *et al.*, 2001; Espay *et al.*, 2009).

6.3.2.2 Associations and correlations of motor signs with DAT binding

Rigidity. Upper extremity rigidity showed the highest independent likelihood of striatal DAT deficiency in patients with clinically uncertain parkinsonism and tremor. Both the increasing upper extremity rigidity total score and the presence of rigidity versus no detection of rigidity significantly pointed to an abnormal DAT scan in these patients. In the voxel-based analyses, more severe upper extremity rigidity was associated with dorsal putamen DAT loss in patients with neurodegenerative presynaptic parkinsonism.

The reason why upper extremity rigidity particularly stood out with the strongest effect in pointing to DAT deficiency may be due to more similar lower extremity motor problems between parkinsonism patients with and without presynaptic dopaminergic deficiency. It is interesting that rigidity is the only sign of MDS-UPDRS part III that is solely based on hands-on examination by the investigator. However, as the associations and correlations between upper extremity rigidity and DAT binding were only modest in the ROI analyses, it seems that the severity of rigidity cannot be used to predict the level of striatal DAT deficiency in patients with abnormal DAT function. This may have been due to inter-rater variability in defining the severity of rigidity in the clinical examinations. However, contrary to bradykinesia, the body side with more severe rigidity showed no significant correlations with its contralateral or ipsilateral putamen DAT binding in patients with abnormal DAT binding, suggesting stronger associations e.g., between bradykinesia and striatal DAT binding than between rigidity and DAT.

Facial expression. The presence of hypomimia also pointed to striatal DAT deficiency and an association was further detected between the increasing hypomimia and increasing

DAT deficiency in patients with neurodegenerative presynaptic parkinsonism. Both in the ROI analyses and the voxel-based analyses, hypomimia was particularly associated with caudate nucleus DAT loss in patients with abnormal striatal DAT uptake. This was detected also in the subsample analysis of non-medicated patients, but, however, note that the presence of hypomimia was not associated with a higher likelihood of DAT abnormality in the subsample analysis. Thus, the medication status may have interfered with hypomimia. In the correlation analyses of patients with abnormal DAT binding, facial expression and decreasing DAT binding showed correlations in both the putamen and the nucleus caudate, with a slightly greater r-value with the caudate compared to the putamen. Patients with normal striatal DAT function showed no associations or correlations between hypomimia and striatal DAT deficiency. Therefore, hypomimia in presynaptic neurodegenerative parkinsonism seems to be associated with striatal DAT binding deficit, particularly in the caudate nucleus.

Earlier results have demonstrated that apathy is also associated with caudate nucleus DAT loss in PD in the early stages of the disease (Santangelo *et al.*, 2015). Thus, it could be speculated that hypomimia in neurodegenerative parkinsonism may not only be a motor sign but also, and possibly even more so, a non-motor sign. In line with this idea, hypomimia has been defined as a sign with both reduced spontaneous facial movements (such as a reduced blinking rate) and loss of emotional facial expressions (such as less spontaneous smiling) (Bologna *et al.*, 2013). In addition, voluntary facial movements may also be affected in neurodegenerative parkinsonism, leading to slower and smaller orofacial movements and problems in voluntary blinking in PD and PSP (Espay *et al.*, 2009). The reduction in spontaneous facial expressions is suggested to be associated with dopaminergic deficiency and to respond positively to dopaminergic medications (Karson, 1983). Reduced emotional facial expressiveness may be linked to other emotional changes in neurodegenerative parkinsonism (Ricciardi *et al.*, 2015), as some PD patients may also have problems in the imagery or recognition of facial emotions (Gray & Tickle-Degnen, 2010). PD patients have also showed impairments in posing facial emotions, which could point to a motor impairment of the facial muscles. However, there seemed to be no correlations between posing of facial emotions and the degree of other motor signs, whereas a positive correlation was found between the reduced facial expressiveness and the defective recognition of facial emotions. Especially expressing disgust was found to be associated with facial emotion recognition (Ricciardi *et al.*, 2015). Finally, a review of emotional processing in PD concluded that deficient facial expressions may result from cell death in the substantia nigra, striatum, as well as the pathophysiological changes in a complex neural network affecting e.g. the amygdala and ventral striatum (Péron *et al.*, 2012). Thus, it seems that the reduced facial expressiveness in neurodegenerative parkinsonism is more than a motor problem.

Other motor signs. In patients with abnormal DAT binding, the bradykinesia score on the more affected body side seemed to correlate slightly better with the ipsilateral than the

contralateral putamen DAT binding. On the contrary, tremor in general or tremor in the more or less severely affected body sides did not correlate with DAT binding in patients with normal or abnormal DAT uptake. This finding is in line with earlier results showing of no correlation between striatal DAT uptake and any type of parkinsonian tremor (Pirker, 2003; Helmich *et al.*, 2012). Earlier results in PD patients have indicated a good correlation between bradykinesia and axial symptoms and striatal DAT binding (Pirker, 2003). Furthermore, the UPDRS motor score on the body side with less severe motor symptoms has shown a better correlation with its contralateral striatum DAT uptake in PD, whereas the correlation between the body side with more severe symptoms and its contralateral striatum DAT binding have been reported to be poor. This result was explained by the greater range of DAT binding in the less affected striatum and in the motor UPDRS score on the body side with less severe motor signs, leading to a better correlation (Pirker, 2003).

6.4 Summary

In summary, this thesis is based on four studies of patients who were scanned with [^{123}I]FP-CIT SPECT due to clinically uncertain parkinsonism or tremor. The retrospective studies are based on a retrospective database of patients scanned during the years 2007 to 2012 in Turku, Finland. The cross-sectional clinical and imaging study is part of a larger prospective study, in which the patient clinical examinations were carried out on the day of DAT SPECT imaging during the years 2014 to 2017 in Turku, Finland and Helsinki, Finland.

Study I showed that there were no associations between midbrain atrophy, measured with the midbrain-to-pons ratios in sagittal conventional brain MRIs, and striatal dopamine deficiency in the DAT SPECT scans in patients with PD or patients with normal DAT uptake. Thus, midbrain-to-pons ratio measurements cannot be used as a predictor of the level of striatal DAT deficiency in these patients.

Study II showed that in 10% of cases scanned with [^{123}I]FP-CIT SPECT there is still uncertainty as to whether a patient has neurodegenerative presynaptic parkinsonism with striatal dopaminergic deficiency, due to disagreement between the visual and automated analysis methods. As none of these patients with discrepant scans proved to have neurodegenerative parkinsonism in the follow-up, physicians should be cautious in interpreting marginally or uncertainly abnormal DAT SPECT scans as abnormal. It also seems that the visual analysis, particularly when performed by non-experienced raters, more often tends to incorrectly interpret a scan as being abnormal, whereas visual analyses performed by experts seems to be more consistent and best align with the automated scan analysis.

Study III demonstrated that the level of dopaminergic deficiency had no effect on survival in PD, but mortality was associated with older age, presence of cognitive impairment and more severe motor signs at the time of DAT SPECT imaging. Future functional neuroimaging studies could investigate whether, for example, the biomarkers of the CNS proteinopathic burden are able to forecast patient survival in PD more accurately than the radionuclide imaging of presynaptic dopaminergic function.

Study IV showed that rigidity, particularly in the upper extremities, and hypomimia were the only motor signs that showed differences between parkinsonism patients with and without striatal DAT deficiency. As both of these signs were independently associated with a higher likelihood of an abnormal striatal DAT function, the value of these signs in the clinical differential diagnosis of uncertain parkinsonism and tremor was highlighted. Furthermore, hypomimia in patients with neurodegenerative presynaptic parkinsonism was especially associated with caudate nucleus DAT loss. Future studies should verify the findings of rigidity and hypomimia in pointing to DAT deficiency. In addition, it should be clarified whether the association between hypomimia and DAT deficiency in hypodopaminergic parkinsonism purely reflects a motor problem or rather reduced emotional facial expressions, which may be part of a broader spectrum of emotional processing problems in neurodegenerative parkinsonism.

7 CONCLUSIONS

I Parkinson' disease is associated with slightly lower midbrain-to-pons ratios compared to a matched clinical population with normal striatal DAT binding. However, there is no association between the midbrain-to-pons ratios and striatal DAT binding in patients with PD. Therefore, midbrain-to-pons measurements cannot be used as a marker of striatal dopamine deficiency in PD.

II Expert visual and automated semi-quantitative analyses of [^{123}I]FP-CIT SPECT scans disagree in 10% of cases. Patients with discrepant findings do not seem to develop neurodegenerative parkinsonism, encouraging a conservative interpretation of the scans and the clinical patient information in these cases. The diagnostic accuracy is likely to be improved by an automated method in borderline abnormal cases.

III The level of presynaptic dopamine deficiency in [^{123}I]FP-CIT SPECT scans is not associated with mortality in PD. However, older age, greater clinical motor symptom severity and the presence of cognitive impairment at the time of DAT imaging do influence long-term patient survival in PD.

IV The detection of upper extremity rigidity or hypomimia in the clinical motor examination point to neurodegenerative parkinsonism with striatal presynaptic dopamine loss. Hypomimia in patients with neurodegenerative presynaptic parkinsonism is particularly associated with the loss of dopamine function in the caudate nucleus.

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APPENDICES

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