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The L-dopa response in Parkinson's disease

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Abstract

L-dopa is the most commonly prescribed drug for the treatment of Parkinson's disease (PD). Most patients benefit from this treatment as it can restore motor function but over time, a large proportion of patients report the manifestation of side effects. The L-dopa response is also a supportive criterion for the diagnosis of PD.

This thesis aimed to explore the variation of responsiveness to L-dopa and to identify predictor variables for responsiveness. This was achieved by systematically reviewing pathological studies and case reports; analysing two large and longitudinal clinical cohort studies with focus on short- and long-term indicators of responsiveness; and the analysis of brain imaging data indicative of the degree of dopaminergic loss at different stages of the disease.

The systematic review established a great variation in responsiveness to L-dopa, analysing pathologically confirmed cases where there is little to no doubt about diagnostic accuracy: 10% of definite Parkinson's are unresponsive to L-dopa and 12% show a modest response. The clinical cohort analysis showed that current treatment management approaches lead to an overall lower prevalence of motor complications compared to earlier studies, even when L-dopa is introduced early-on. Motor fluctuations have the greatest impact on motor function but also on the patients' abilities in everyday life situations. Investigating the short-term response showed an association of better motor function with the development of dyskinesia, and dyskinetic patients with a better response to challenge testing. Finally, SPECT imaging data showed a high residual activity of dopamine in early PD, and an association of lower putaminal uptake with higher medication doses at later stages of the disease.

In conclusion, a lesser response to L-dopa should be considered as a definite phenomenon in a large proportion of PD patients. Assessing L-dopa responsiveness more widely, in clinical practice and clinical research would enhance both our understanding of patients and our interpretation of the effects of new drug treatments.

List of Publications

Pitz V, Malek N, Grosset KA, Grosset DG, THUR 117 The L-DOPA response in pathologically confirmed Parkinson's disease (2018). *J Neurol Neurosurg Psychiatry*; 86; A12-A13. [abstract, conference article], doi: *10.1136/jnnp-2018-ABN.46*

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Pitz V, Malek N, Tobias ES, Grosset KA, Gentleman S, Grosset DG (2020), The Levodopa response varies in pathologically confirmed Parkinson's disease: a systematic review. *Mov Disord Clin Pract*, doi: 10.1002/mdc3.12885

Kanavou S, **Pitz V**, Lawton MA, Malek N, Grosset KA, Ben-Shlomo Y, Grosset DG (2020), Defining impaired olfaction in Parkinson's disease: assessing agreement between four published methods. *Parkinsonism Relat Disorders,* submitted

List of Conference Presentations

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Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

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Vanessa Pitz

____14.04.2021_____

Date

Abbreviations

AD	Alzheimer's disease
ADL	Activity of Daily Living Scale
ADORA2A	Adenosine A2a receptor
ANKK1	Ankyrin repeat and kinase domain containing 1
ATP13A2	ATPase cation transporting 13A2
BDNF	Brain-derived neurotrophic factor
BFI	Big five index of personality traits
BMI	Body mass index
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CDR	Clinical Dementia Rating
CDS	Continuous dopaminergic stimulation
CERAD	Consortium to establish a registry for Alzheimer's disease
COMT	Catechol-O-methyltransferase
CT	Computed tomography
CUPS	Clinically uncertain parkinsonian syndrome
CYP2D6	Cytochrome P450 family 2 subfamily D member 6
DAT	Dopamine transporter
DaTSCAN	Dopamine transporter scan, tradename for 123-I-ioflupane
DBS	Deep brain stimulation
DDC	Dopa decarboxylase, alias Aromatic L-amino acid decarboxylase (AADC or AAAD)
DDI	Dopa decarboxylase inhibitor
DJ-1	alias PARK7, Parkinsonism associated deglycase

DLB	Dementia with Lewy bodies
DLBD	Diffuse Lewy body disease
DRD2	Dopamine receptor D2
DRD3	Dopamine receptor D3
DSM	Diagnostic and Statistical Manual of Mental Disorders
EQ5D	EuroQuol – 5 Dimension
ESS	Epworth Sleepiness Scale
FDA	US Food and Drug Administration
F-dopa	Fluorodopa, 6-fluoro-L-DOPA
FP-CIT	123-I-ioflupane, [I-123] N-ω-fluoropropyl- 2β-carbomethoxy- 3β-(4-iodophenyl) nortropane
FTD	Frontotemporal dementia
GABA	Gamma-aminobutyric acid
GBA	Glucosylceramidase beta
GDS	Geriatric depression score
GEE	Generalised estimating equations
GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A
H&Y	Hoehn & Yahr scale
HADS	Hospital anxiety and depression scale
HAM-D	Hamilton Depression Rating Scales
HC	Healthy controls
HOMER1	Homer scaffold protein 1
IQR	Interquartile range
LD	L-dopa dose
LDR	Long-duration response

LEDD	Levodopa equivalent daily dose
LID	L-dopa induced dyskinesia
LILACS	Latin American and Caribbean Health Sciences Literature
LRRK2	Leucine-rich repeat kinase 2
MAO-B	Monoamine oxidase B
MDS	Movement Disorder Society
MDS-PD Criteria	Movement Disorder Society revised Clinical Diagnostic Criteria for Parkinson's disease
M-EDL	Motor Aspects of Experiences of Daily Living
MERQ-PD	Mini environmental risk questionnaire for PD patients
MIBG	123I cardiac meta-iodiobenzylguanidine
MJFF	Michael J. Fox Foundation
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
NMDA	N-methyl D-aspartate
nM-EDL	Non-Motor Aspects of Experiences of Daily Living
NMSS	Non-motor symptoms scale
NSAIDs	Non-steroidal anti-inflammatory drugs
PARK2	Parkin
PARK7	alias DJ-1, Parkinsonism associated deglycase
PASE	Physical Activity Scale
PD	Parkinson's disease
PDQ8	8-item Parkinson's disease questionnaire

PDSS	Parkinson's disease sleep scale
PET	Positron-emission tomography
PIGD	Postural instability gait disorder
PINK1	PTEN induced kinase 1
PPMI	Parkinson's Progression Markers Initiative
PRADO	Pradivel (brand name for bromocriptine) and L-dopa study
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analyses
PRKN	parkin RBR E3 ubiquitin protein ligase
PRoBaND	Parkinson's Repository of Biosamples and Network Datasets
PSP	Progressive supranuclear palsy
PSPS	Progressive supranuclear palsy syndrome
QUIP	Questionnaire for Impulsive-Compulsive Disorders in PD
RBD	REM sleep behaviour disorder
REM	Rapid eye movement
SCOPA-AUT	Scales for outcomes in PD – Autonomic Dysfunction
SD	Standard deviation
SDR	Short-duration response
SDR	Standard deviation
SLC6A3	Solute carrier family 6 member 3
SNCA	Alpha-synuclein
SNpc	Substantia nigra pars compacta
SPECT	Single-photon emission computed tomography
STAI	State-Trait Anxiety Inventory for Adults
SWEDDs	Scans without evidence of dopaminergic deficits

TD	Tremor-dominant
UDysRS	Unified Dyskinesia Rating Scale
UPDRS	Unified Parkinson's disease Rating Scale
UPSIT	University of Pennsylvania Smell Identification
VMAT-2	Vesicular monoamine transporters type 2
WOQ	Wearing-off Questionnaire
YOPD	Young-onset Parkinson's disease

Chapter 1: General Introduction

1.1 L-dopa in Parkinson's disease

L-dopa is commonly used as the short name for L-3,4-dihydroxyphenylalanine, an amino acid which has been the gold standard for the treatment of mainly motor function impairment in Parkinson's disease (PD) since its introduction in the 1960s.

PD is a progressive neurodegenerative disorder, and the cause of the disease is unknown. Substantial loss of dopaminergic neurons in an area of the brain called *substantia nigra pars compacta* (SNpc), induces impairment of motor function, which is often the first noticeable symptom of the disease.

L-dopa is the metabolic precursor of dopamine and can be taken orally. It helps to restore the low levels of dopamine that are caused by the dopaminergic cell loss, and this in turn improves the patient's motor symptoms.

The identification of L-dopa as the main treatment for PD is relatively recent. A brief history of its discovery now follows.

1.1.1 A brief history of L-dopa

The idea of L-dopa as the "miracle drug" manifested in the late 1960s but started much earlier with Torquato Torquati, a scientist who in 1913 reported a naturally occurring nitrogenous substance in the seedlings of the bean plant *Vicia faba*. Markus Guggenheim, a Swiss biochemist, followed up on his report, suspecting the substance to be adrenaline related, a research topic of great interest at the time. He investigated the substance and first established the chemical structure of L-dopa. Self-administration did not cause any adverse effects, which led him to conclude the substance to be harmless (Guggenheim, 1913).

Unaware of the importance of his discovery, research around the substance stagnated for more than two decades until the work of the German pharmacologist Peter Holtz in 1938. He discovered the key enzyme for the conversion of L-dopa to dopamine: dopa decarboxylase (DDC) (Holtz, 1939). Through decarboxylation, the removal of a carboxyl group (COOH), L-dopa is converted into the biologically active catecholamine dopamine. At the time, the link between L-dopa and dopamine was not of great research interest, whereas the confirmation that dopamine plays a key role in the synthesis of noradrenaline and eventually adrenaline, was an exciting new finding.

Over a decade later in 1950, Arvid Carlsson caused a paradigm shift, by proving the existence of chemical neurotransmission in the brain. His experiments showed that dopamine itself is not able to pass through the protective blood-brain barrier, whereas its precursor L-dopa has the properties to do so.

Seven years later, Wilhelm Raab provided the first evidence for the existence of dopamine in the human brain (Raab and Gigee, 1951). In the next years, several studies showed that dopamine is located in the striatum, suggesting an involvement in "extrapyramidal control" and "reserpine parkinsonism". Reserpine, an antipsychotic and antihypertensive drug, had been shown to deplete dopaminergic neurons in the rabbit brain, effectively inducing parkinsonism. In this study, the depletion could be replenished by the application of L-dopa and furthermore, it had an excitatory effect on the animal (Carlsson et al., 1958).

To continue the mapping of dopaminergic neurons in the human brain, Sano found that dopamine levels were high in the basal ganglia in 1959 (Sano et al., 1959). In 1961, another key figure in the development of L-dopa as a Parkinson's treatment, Oleh Hornykiewicz, investigated dopamine levels in post-mortem brains of Parkinson's patients. He realised that a substantial loss of dopaminergic neurons in the striatum is related to Parkinson's disease (Ehringer and Hornykiewicz, 1960). Immediately after this, he convinced the medical doctor Walter Birkmayer to conduct the first study in patients, by intravenous injection of L-dopa to 20 cases with severe Parkinson's. The results were astonishing: for a few hours, the patients could move their rigid limbs, showing the miraculous effect of the drug (Birkmayer and Hornykiewicz, 1961).

Only two years later, Hornykiewicz defined a dopaminergic deficit in the substantia nigra in post-mortem brains of Parkinson's patients, which indicated a nigrostriatal dopaminergic pathway in the human brain (Hornykiewicz, 1963). The short-acting benefits of intravenous L-dopa were enhanced when George Cotzias started trials of an oral form of L-dopa in 1968 (Cotzias et al., 1969), and the drug was approved by the Food and Drug Administration (FDA) in 1970 for use as a treatment for PD.

Despite the introduction of drugs such as dopamine agonists and enzyme inhibitors for the dopamine metabolic pathway, L-dopa remains the most effective and universally applied drug for Parkinson's, leaving these other drug classes in a supportive rather than central role.

1.1.2 Motor function in Parkinson's disease

PD is a common disorder of the central nervous system. It is twice as common in men than women (Taylor et al., 2007, Wooten et al., 2004). Around 2% of people over the age of 65, and 3.5% older than 85 (de Lau and Breteler, 2006, Twelves et al., 2003, de Rijk et al., 1997) are affected. Epidemiological studies show that, as a result of the ageing population in the developed world, the prevalence of Parkinson's is steadily increasing (Collaborators, 2018).

Genetics

PD was traditionally considered as non-genetic, but this understanding has changed substantially in the past 30 years. Today we still refer to idiopathic PD, but genetic types are recognised, either 'monogenic', or 'genetic risk'. 'Monogenic' describes forms of PD caused by a single gene mutation, while 'genetic risk' types relate to gene mutations that are presumed to work in combination with other factors to increase the risk of developing PD. Both monogenic and genetic risk types can also be of reduced penetrance.

Since 1990, it has been generally recognised that genetically caused forms of PD affect 5-10% of cases (Antony et al., 2013). The monogenic forms of PD involve mutations on the genes *SNCA* (*alpha-synuclein*), *LRRK2* (*Leucine-rich repeat kinase 2*), *PINK1*, *PARK7* (also known as *DJ-1*), *ATPase type 13A2* (*ATP13A2*), or *PARK2* (also known as *Parkin*). The *PINK1*, *Parkin*, and *DJ-1* types are associated with young-onset PD disease (YOPD), which is generally diagnosed before the age of 50 years (Singleton et al., 2013).

In addition to the disease-causing genetic mutations, there is a wide range of genetic risk variants. These contribute to the risk of developing PD, and often to variations in the clinical presentation and evolution of the disease (Iwaki et al., 2019). The most common genetic risk variant for the development of PD is the *GBA* (*Glucosylceramidase beta*) gene, affecting around 10% of cases (Malek et al., 2018). There are multiple further genetic risk variants which collectively are estimated to explain 22-27% of overall heritability of the condition (Iwaki et al., 2019, Blauwendraat et al., 2019).

Dopamine depletion and motor function

Severe loss of dopaminergic neurons and Lewy body formation with alpha-synuclein inclusions are the main neuropathological features of PD (Elbaz et al., 2016, Dickson et al., 2009). Lewy bodies are present in several neurodegenerative disorders and contain abnormal protein aggregates. In Parkinson's, those formations contain misfolded alpha-synuclein (Spillantini et al., 1997) which is encoded by the *SNCA* gene. Alpha-synuclein plays a crucial role in synaptic activity, and it is hypothesized that such Lewy body formation in a nerve cell leads to the failure of the cell's protective mechanisms like autophagy and proteasomal processes, which then results in cell death (Webb et al., 2003). Even though alpha-synuclein is present throughout the brain, the greatest impact of PD related alpha-synuclein is on dopaminergic neurons and accordingly dopamine levels in the striatum (Perez et al., 2002, Venda et al., 2010).

The degree of dopaminergic depletion in the SNpc correlates with the severity of motor impairment (Vingerhoets et al., 1997, Greffard et al., 2006). Around 4-5% of dopamine is lost per decade in PD, and it takes the loss of around 40-60% of dopaminergic neurons before motor symptoms appear (Fearnley and Lees, 1991, Burke and O'Malley, 2013). The duration of this prodromal phase of progressive cell loss before the onset of motor symptoms ranges from 5 to 20 years (Greffard et al., 2006, Kalia and Lang, 2015).

During this preclinical phase, other non-motor symptoms may manifest and are increasingly recognised as early indicators of disease. Such symptoms include disorders of mood like apathy, and psychosis; sleep disorders like insomnia, excessive daytime sleepiness, rapid eye movement (REM) sleep behaviour disorder (RBD); autonomic dysfunction like orthostatic hypotension, dysfunction of the urogenital tract, constipation; and sensory symptoms such as olfactory dysfunction (hyposmia), abnormal sensations, and pain. Hyposmia and RBD have been studied extensively as prodromal features of PD and may in future form a component of screening procedures (Poewe, 2008).

However, as motor dysfunction is more specific, it is often the first noticeable impairment, and the clinical diagnostic focus in PD remains largely with the motor symptoms. The involuntary tremor of the extremities in resting positions (resting tremor) is often the first symptom associated with PD. It is often accompanied by another cardinal symptom called bradykinesia, describing slowness of movement with a progressive reduction in speed and/or amplitude of movement. General muscle stiffness

(rigidity) completes the trio of the main symptoms of PD (Gelb et al., 1999, Hughes et al., 1992, Postuma et al., 2015). In most patients, the progression of the disease is often accompanied by a worsening of existing and the development of new symptoms. Many patients also experience disturbances in gait and the sudden involuntary freezing while walking. Additionally, impaired handwriting, deficits in grip force, distinctive speech abnormalities, or abnormal contraction of eyelid muscles (blepharospasm) can occur (Gelb et al., 1999, Postuma et al., 2015, Moustafa et al., 2016).

The annual NHS cost of treating Parkinson's in 2017 was £2,118 per patient, whereas the financial burden for the patient, their carers, and families added up to a total of £20,123 per year (Rogers et al., 2017). Management of such a variety of symptoms requires a close patient-clinician relationship, constant monitoring of the symptoms, and a well-fitted treatment schedule consisting of exercise, therapies, and medication.

Available antiparkinsonian medications address the broad range of symptoms (dopamine replacements, receptor activators, enzyme inhibitors), sometimes supplemented with other drug classes (anticholinergics, amantadine). Medication often comes with the risk of adverse effects, which then require additional medication to be taken.

1.1.3 Pharmacology and side effects

L-dopa treatment has been available for several decades and is effective but associated with emerging side effects over time. Depending on the clinical phenotype, it restores the motor deficit with a return to an almost normal level of function in Parkinson's patients. However, many patients have lesser degrees of improvement and most patients eventually develop a fluctuating motor response. These problems relate in part to the pharmacology of L-dopa, which will now be further explored.

Chemical structure and pharmacology

The chemical structure of L-dopa is summarised as $C_9H_{11}NO_4$. It is part of the catecholamine synthesis pathway. We naturally derive L-dopa directly from the amino acid L-Tyrosine. As a medication, L-dopa is mainly administered orally in the form of capsules or tablets.

L-dopa is rapidly metabolised in the gastrointestinal tract where DDC is highly enriched. Before even reaching the brain, DDC already converts L-dopa to dopamine. Only 10-30% of biological L-dopa can now be further metabolised, with less than 1% of unchanged L-dopa reaching the brain (Contin and Martinelli, 2010, Freitas et al., 2016, Di Stefano et al., 2011). This rapid 'presystemic' metabolism led to the development of the DDC inhibitors carbidopa and benserazide. In combination with L-dopa, those inhibitors delay its conversion to dopamine and increase the amount of available L-dopa (Seeberger and Hauser, 2015).

However, if DDC is inhibited in the periphery, L-dopa metabolism is diverted to another enzyme called Catechol-O-methyltransferase (COMT) (Nutt and Fellman, 1984). COMT methylates L-dopa to 3-O-methyldopa by adding a CH3 group (Nutt and Fellman, 1984). This process reduces the amount of biological L-dopa, and in addition produces a substance that competes with L-dopa for transport across the blood-brain barrier. Therefore, COMT inhibitors (entacapone, opicapone) are used to improve the transport of L-dopa through the blood-brain barrier and therefore improve treatment efficacy (Nissinen et al., 1992, Learmonth et al., 2004, Tohgi et al., 1991). Other commonly used enzyme blockers inhibit the metabolic activity of monoamine oxidase (MAO)-B. This process slows the breakdown of dopamine into its degradation products, which results in higher dopamine levels for a longer period of time (Finberg et al., 1998) (Figure 1-1).

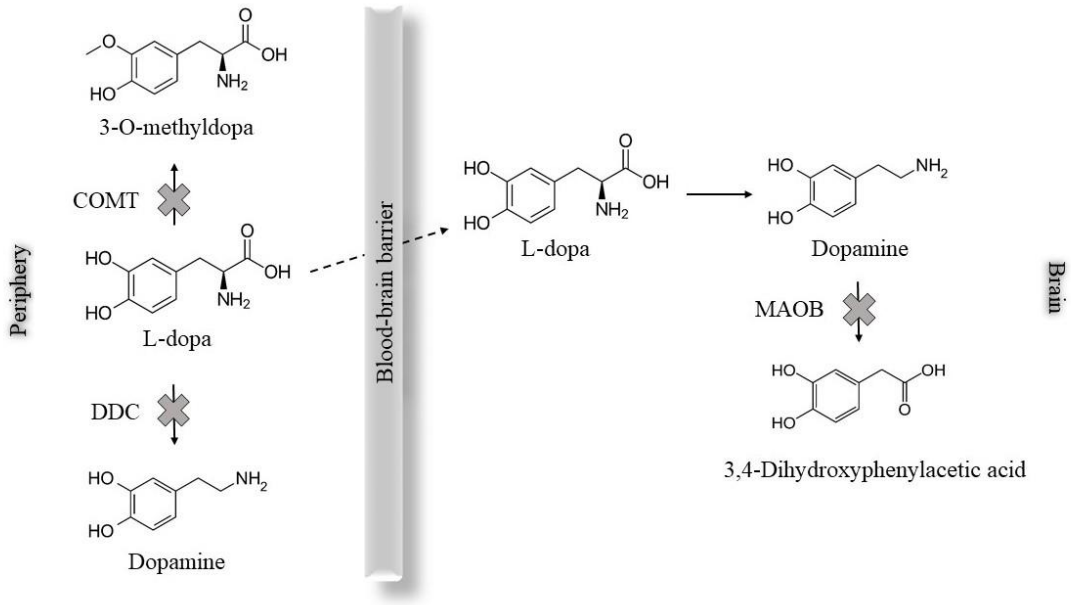

Figure 1-1: Schematic of the L-dopa metabolism.
Mechanism of action of L-dopa and routinely prescribed enzyme inhibitors to improve treatment efficacy (adapted from (Krauss and Bracher, 2018)).

Treatment complications

The fast metabolism of L-dopa results in an inconsistent plasma concentration. With increasing disease progression and treatment duration, for some patients those plasma fluctuations can induce changes in the treatment response: side effects such as motor fluctuations, dyskinesia, and dystonia.

The most commonly known phenomenon is motor fluctuations, which describes the inconsistent and shortened therapeutic response, depending on the time point of drug administration (van Laar, 2003). Motor fluctuations define if a patient is in an "off" or "on" period. During "off" periods, tremor, bradykinesia and/or rigidity are present because the medication has not been taken yet, the drug's effect has already worn off, or the medication was taken but it was ineffective. In contrast, "on" periods refer to the time where those "off" symptoms are alleviated due to effective drug treatment. Depending on the motor fluctuation episode that patients are experiencing, other complications can arise.

Dyskinesia, the involuntary choreic movement mainly affecting the extremities, occurs for 70-80% of dyskinetic patients during "on" periods when dopamine levels are at their highest ("peak-dose") (Zesiewicz et al., 2007). Yet, they can also appear when

dopaminergic stimulation is at their lowest ("off") or when patients are just transitioning from "on" to "off" periods, which often happens at night (nocturnal wearing-off) or before the first morning dose (early-morning off periods) (Hametner et al., 2010). Additionally, diphasic dyskinesia can occur at the beginning and the end of a dose cycle (Muenter et al., 1977), and often presents as rapid dystonic flexion/extension foot movements (Obeso et al., 1989).

Dystonia describes the sustained and repetitive muscle twisting, spasm or cramping of the lower extremities and mainly appears during "off" periods of chronic dopaminergic treatment (Hametner et al., 2010, Quinn, 1998). While dystonia can occasionally be seen in untreated PD, the majority of dystonia relates to medication (Rivest et al., 1990) and it improves when dopamine levels are restored after a further dose of medication.

Around 10% of patients treated with L-dopa develop motor complications per year (Hauser et al., 2007, Katzenschlager et al., 2008), but the extent and severity of motor complications varies between patients. This suggests different underlying pathophysiological mechanisms which are yet to be fully explored.

Pathophysiology

The striatum is a brain area in the basal ganglia, a structure in the forebrain. It is critically involved in motor and reward systems and communicates with the SNpc. It receives excitatory dopaminergic input and sends inhibiting signals using the neurotransmitter gamma-aminobutyric acid (GABA).

In recent years, extensive research has been conducted relating to the underlying mechanism of how motor complications manifest.

The most common hypothesis is that a continuous dopaminergic stimulation (CDS) of receptors in the striatum is the natural state and that the pulsatile stimulation of short-acting dopaminergic drugs, at the postsynaptic receptors, results in the development of motor complications. The use of longer-acting agents, such as by continuous infusion, therefore reduces those complications by preventing or reversing abnormal sensitisation of these postsynaptic receptors (Stocchi and Olanow, 2004, Chase, 1998).

Functional brain imaging studies support this hypothesis, showing higher amounts of dopamine being released over shorter periods of time after L-dopa administration (Stoessl, 2015). Contrary observations suggest that even in the healthy brain, striatal

dopamine release is not constant, but changes during the conduct of different physical tasks (Koepp et al., 1998, Goerendt et al., 2003, Ouchi et al., 2002).

There is additional evidence that L-dopa induces plastic synaptic changes in D1 and D2 striatal projection neurons (Suarez et al., 2014, Suarez et al., 2016, Nutt, 2007, Picconi et al., 2018). Those changes lead to a reorganisation of the neurotransmitters dopamine, serotonin, and glutamine (Picconi et al., 2018). Limited evidence suggests that this reorganisation results in a more sensitive response to the drug, which may result in a greater motor function improvement for longer periods and/or tolerance, which describes the more moderate response for shorter periods of time (Nutt et al., 1992, Mouradian et al., 1988, Castro et al., 1985, Post, 1980, Nutt, 2007). Dyskinesia partly resembles the concept of sensitisation, whereas motor fluctuations follow the concept of drug tolerance (Nutt, 2007). However, it is important to note that current studies suggest that an early initiation of L-dopa does not lead to accelerated dyskinesias or disease pathology (Cilia et al., 2014, Verschuur et al., 2019).

Research into the development of motor complications is mainly based on animal research, calling for more clinical approaches to give more conclusive evidence about how exactly the different complications manifest.

The overall goal of medication in PD is to achieve relatively constant drug levels during the waking day. Various formulations have been developed to assist with this, including longer-lasting release (controlled-release) and liquid forms of L-dopa (Thanvi and Lo, 2004). Additionally, a multitude of different delivery options like microspheres, pulmonary inhalation, nasal or transdermal delivery, or gastrointestinal infusion in the jejunum are available (Ngwuluka et al., 2010, Olanow and Stocchi, 2018). Generally, simpler treatment options progress to more complicated or invasive regimens with progressing disease severity (Fabbrini et al., 2010, Amjad et al., 2019, Luinstra et al., 2019).

1.2 Clinical features

Parkinsonism is an umbrella term for a set of clinical features involving motor function impairment. Especially in the early stages of the disease, it remains challenging to differentiate PD from other forms of parkinsonism, often described as 'atypical parkinsonism' or 'parkinson-plus syndrome'.

A major clinical difficulty is the differentiation between parkinsonism with or without a loss of dopamine in the SNpc, which in turn has an impact on prescribed treatment but also the treatment response for the patient. Depending on the expertise of the movement disorder specialist, around 5-20% of PD cases do not have their diagnosis confirmed at death (Hughes et al., 2002, Litvan et al., 1998). Clinical criteria and methods aim to reduce this error rate and will now be reviewed.

1.2.1 Clinical diagnostic criteria

Parkinsonism presents differently between affected individuals. Such heterogeneity can complicate early recognition of the disease and later, the ideal treatment approach. In 2015, the Movement Disorder Society (MDS) published a revised version of the UK Brain Bank Criteria (Hughes et al., 1992), the "Clinical Diagnostic Criteria for Parkinson's disease" (MDS-PD Criteria) (Postuma et al., 2015). This comprehensive set of criteria aims to standardise the process of PD diagnosis and to improve their application even for less experienced physicians (Postuma et al., 2015).

A clinical diagnosis of PD is made when at least two cardinal symptoms are present: bradykinesia, resting tremor, and/or rigidity.

In addition to motor features, a 'clear and dramatic beneficial response to dopaminergic therapy' and the presence of levodopa-induced dyskinesia (LID) are part of the supportive diagnostic criteria for PD (Hughes et al., 1992, Postuma et al., 2015). The lack of an observable response to high doses of L-dopa in cases with at least moderate severity is an absolute exclusion criterion for a PD diagnosis (Postuma et al., 2015). The reason for this strong distinction is the assumption that PD is L-dopa responsive, whereas atypical forms of parkinsonism do not show a sustained response to the drug. Yet, PD is largely clinically overdiagnosed, and pathological examination remains the diagnostic gold standard (Hughes et al., 1992, Marshall et al., 2009).

Atypical forms of parkinsonism with dopaminergic loss in the SNpc are multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration

(CBD). Diagnostic challenges and clinical features that are often confused with PD will now be described.

MSA is characterised by parkinsonian motor symptoms, impairment in coordinated motor skills due to damage to the cerebellum (cerebellar ataxia), autonomic failure including urogenital dysfunction, and corticospinal disorders. A *definite* diagnosis requires autopsy confirmation of cytoplasmatic inclusions of glial cells containing alpha-synuclein in the CNS (Gilman et al., 2008). A *probable* diagnosis of MSA only requires the presence of clinical features, including a poor response to L-dopa with the presence of the main parkinsonian symptoms bradykinesia with rigidity and/or tremor (Gilman et al., 2008).

A diagnosis of **PSP** is made with different levels of certainty in the presence of the four core features: eye motor dysfunction (ocular motor dysfunction), postural instability, akinesia, and cognitive dysfunction (Hoglinger et al., 2017). Dysfunction of eye movements especially in the early stages of the disease is difficult to identify even for specialised physicians (Phokaewvarangkul and Bhidayasiri, 2019).

The complexity of clinical and pathological features complicates the development of clinical diagnostic criteria for **CBD** (Alexander et al., 2014). Four phenotypes are clinically identified: Corticobasal syndrome (CBS), progressive supranuclear palsy syndromes (PSPS), frontotemporal dementia (FTD), and Alzheimer-like dementia (AD-like dementia). A combination of different phenotypes results in a diagnosis of probable sporadic CBS or possible CBS (Armstrong et al., 2013). Again, the motor symptoms of the different phenotypes are PD-like and the broad set of CBS criteria can lead to false-positives (Armstrong et al., 2013).

Even though these criteria aim to refine the differential diagnosis of parkinsonism, they remain imperfect (Marsili et al., 2018). In addition to clinical criteria, brain imaging methods have rapidly developed in the past decade with the aim of unravelling the underlying processes of parkinsonism but also differentiating degenerative forms from non-degenerative ones.

1.2.2 Brain imaging in parkinsonism

An accurate clinical diagnosis, especially with regards to a confirmed dopaminergic deficit, is of high importance in clinical practice. Patients with parkinsonism but without dopaminergic depletion will not respond to dopamine replacement therapy. This applies for essential tremor, drug-induced parkinsonism, psychogenic parkinsonism, and dystonic tremor (with the rare exception of dopa-responsive dystonia), and vascular parkinsonism except where the vascular insult causes only presynaptic dopamine loss.

Essential tremor is defined with "bilateral upper extremity action tremor" as its main symptom (Bhatia et al., 2018). A resting state is however present in between 2 and 46% of cases, which complicates the differentiation from PD (Shanker, 2019).

Vascular parkinsonism presents clinically with parkinsonian features, however, those symptoms must be preceded by, and directly linked to an ischemic stroke event (Zijlmans et al., 2004).

Mainly antipsychotic drugs but also dopamine depleting drugs, drugs improving gastrointestinal mobility (antiemetics), or calcium-channel blockers can lead to **drug-induced parkinsonism**, presenting with clinical symptoms mimicking PD (Shin and Chung, 2012). Where possible, stopping drug treatment is often sufficient for the symptoms to disappear after a few weeks to months (Rajput et al., 1982).

Very rarely, psychological disorders can lead to motor symptoms of PD (**psychogenic parkinsonism**). Clues like a non-progressive course of the disease, inconsistent motor symptoms, and the disappearance of the symptoms with distraction of the patient can help to identify this form of parkinsonism (Deuschl et al., 1998). Dystonia and tremor are strongly associated with features of parkinsonism. Tremor can occur as a result of dystonia in the same or different body parts. Dystonic tremor can occur at any possible state: rest, sustained postures, voluntary movements, or task specific. As dystonia and tremor are its only features, it is often mistaken for essential tremor or PD (Deuschl, 2003, Deuschl, 2016).

Single-photon emission computed tomography (SPECT) with DaTSCAN was developed as a tool for the differential diagnosis in patients with suspected parkinsonism. The main focus was on the differentiation between non-degenerative essential tremor and degenerative PD (Benamer et al., 2000). SPECT with DaTSCAN uses a radioactive tracer called ^{123}-I-ioflupane (DaTSCAN^{TM}), a substance that specifically binds to dopamine transporters of the presynapse in the striatum. This

binding process is only temporary and can be quantified. Lower tracer levels reflect fewer dopamine transporters are present in the striatum, indicating a greater degree of dopaminergic loss in the striatum. Besides the risks associated with the use of a radioactive tracer, a safety analysis of 10 clinical trials has shown that the scanning method is well tolerated (Grosset et al., 2014).

Scans with normal uptake rates in their SPECT with DaTSCAN are called 'scans without evidence of dopaminergic deficits' (SWEDDs) (Schneider et al., 2007). Only a small number of suspected parkinsonism SWEDD cases have been shown to slightly degenerate over a 5.4-year follow-up period (Batla et al., 2014).

DaTSCAN helps in the differentiation of parkinsonism with dopaminergic deficit versus non-dopaminergic deficit in essential tremor (Hauser and Grosset, 2012) but also in clinically diagnosed essential tremor cases (Benamer et al., 2000). Another study showed the successful differentiation of vascular parkinsonism from PD with 'a good degree of certainty' (Contrafatto et al., 2012). In the case of vascular vs drug-induced parkinsonism, a meta-analysis suggested the need for more studies before reaching a definite conclusion (Brigo et al., 2014).

While some DaTSCAN studies suggest that there are differences for MSA, PSP, CBD, and dementia with Lewy bodies (DLB) compared to PD, it is generally accepted that individual case diagnosis cannot be made accurately between PD and these disorders (Davidsson et al., 2014).

Unsurprisingly, the outcome of SPECT imaging impacts clinician's confidence in diagnosing parkinsonism (Graebner et al., 2017), the management of patients (Sadasivan and Friedman, 2015, Kupsch et al., 2013), and the patient's psyche (Graebner et al., 2017). However, given that neuropathological brain examination is the gold standard in diagnosing PD, only a few PD cases have undergone SPECT brain imaging and a post-mortem examination (de la Fuente-Fernandez and Lovblad, 2014), which challenges over-reliance on imaging results for clinical decisions (de la Fuente-Fernandez, 2012).

In addition to SPECT with DaTSCAN, the use of cardiac scintigraphy to image the function of the heart muscle further refines the differential diagnosis of parkinsonian syndromes (Langston et al., 2018, Shin et al., 2006). A 'cardiac sympathetic denervation' confirmed by heart scintigraphy using the substance ^{123}I cardiac meta-

iodiobenzylguanidine (MIBG) is part of the supportive diagnostic criteria of the Movement Disorder Society (Postuma et al., 2015).

Other imaging techniques like magnetic resonance imaging (MRI), and positron emission tomography (PET) help to unravel parts of the many manifestations of PD. Another application of brain imaging in PD is the selection of candidates for deep brain stimulation (DBS) (Asahi et al., 2016, Nakajima et al., 2018). However, despite all of these observations, SPECT brain imaging is not required in all cases in routine clinical practice (Pagano et al., 2016).

1.2.3 Assessing responsiveness with rating scales

In clinical practice, motor function is assessed by patient-clinician interactions. Rarely in clinical practice and more commonly in clinical studies, motor function in PD is assessed with standardised clinical rating scales. Assessing motor function alone or as a result of treatment responsiveness requires the translation of the patient's subjective impression of improvement or worsening of symptoms into more objective measures.

The Unified Parkinson's disease Rating Scale (UPDRS) was developed in 1987 and revised by the MDS in 2008. The new version of the MDS Unified Parkinson's disease Rating Scale (MDS UPDRS) is more detailed and assesses motor and non-motor elements of PD (Goetz et al., 2008). The scale consists of four parts: Part 1 - Non-Motor Aspects of Experiences of Daily Living (nM-EDL), Part 2 - Motor Aspects of Experiences of Daily Living (M-EDL), Part 3 - Motor Examination, and Part 4 - Motor Complications.

The nM-EDL covers aspects of cognition, hallucinations, and psychosis; mood disorders like depression, anxiety, apathy; features of dopamine dysregulation syndrome; sleep disorders; pain; bladder and gastrointestinal dysfunctions; and aspects of fatigue. M-EDL comprises speech abnormalities; saliva production and drooling; issues with chewing and swallowing; requiring assistance with everyday tasks like dressing or personal hygiene; changes in own handwriting; if disease symptoms prevent the patient from participating in hobbies or other activities; some motor aspects like tremor, problems with turning over in bed or getting up, walking and balance, and sudden freezing in motion while walking. Motor examination of the PD patient includes the assessment of facial expression; rigidity; fine motor tasks like finger and toe-tapping; hand movements; the agility of the legs; gait; freezing of gait; postural stability; bradykinesia; postural and kinetic tremor; presence and interference of

dyskinesia with the rating; and disease severity with the Hoehn & Yahr (H&Y) scale. Motor complications assess the presence and time spent with dyskinesia, motor fluctuations, and dystonia.

Most items on the scale are assessed on a range from 0 (normal), 1 (slight), 2 (mild), 3 (moderate) to 4 (severe), allowing a maximum total score of 199 for the worst possible disability from PD.

To assess the severity of motor impairment from PD, MDS UPDRS 3 is the most commonly used scale. It consists of 23 items and all items added together can result in a maximum total MDS UPDRS 3 score of 72. Together with the Hoehn & Yahr (H&Y) score, this gives a good indication of the disease progression and severity of motor function (Goetz et al., 2008).

This part of the rating scale is also useful to directly measure the patient's motor response to dopaminergic treatment, either on an out-patient basis or by challenge testing. Challenge testing is often conducted in the morning with the patient in a defined 'off' medication state, as the last overnight dose was omitted. After getting assessed in the 'off' state, the usual morning dose can be taken, and the patient is assessed again (usually within 1-3 hours after drug administration). Deriving the percentage change between the 'off' and 'on' MDS UPDRS 3 score is a good indication for how well the drug works for the patient. Even though challenge testing only captures a small time window of responsiveness, challenge testing on L-dopa treatment is a dependable measure in the early stages of the disease, when in some cases diagnosis is difficult (Schade et al., 2017). Another study showed that this challenge test had the potential to predict long-term responsiveness to L-dopa at 2-year follow-up (Merello et al., 2002). However, such challenge testing fell out of favour in the 1990s, except in the research setting, due to mismatches in some cases between the acute and chronic responses observed.

As previously elaborated, the responsiveness to L-dopa also covers the development of motor complications on long-term treatment (MDS UPDRS 4). The time spent with dyskinesia, defined as percentage of a waking day and their functional impact, the time spent in the 'off' motor fluctuation state, their functional impact, and complexity, and the time spent in painful 'off' dystonia thoroughly captures drug-induced side effects. They are also rated from 0-4, allowing a maximum total score of 24.

The MDS UPDRS is not the only available PD rating scale but it is the most recent and validated one (Postuma et al., 2018). Especially in terms of L-dopa responsiveness and motor complication development, the 'Wearing Off Questionnaire' (WOQ) assesses several motor and non-motor symptoms that can appear during 'off' periods or transition periods. The Unified Dyskinesia Rating Scale (UDysRS) is another MDS rating scale from 2008, assessing mainly aspects of the M-EDL in relation to the presence of dyskinesia. In addition to the MDS UPDRS, those rating scales can contribute to an extremely thorough picture of the occurrence of side effects, mainly for research purposes rather than clinical practice.

1.2.4 Factors affecting L-dopa responsiveness

Responsiveness to L-dopa depends on a multitude of pharmacological and molecular factors but also a variety of clinical variables. Research has focused far more on the investigation of motor complication, than on the degree of improvement of motor function from L-dopa. In clinical practice, a subjective interpretation of motor improvement is often considered sufficient for assessment. An overview of such research now follows.

Motor improvement is often categorised into short- (SDR) and long-duration response (LDR) (Muenter and Tyce, 1971, Nutt et al., 1992), which describe measurable responsiveness after a few hours of and a sustained response for up to two (and possibly four) weeks after drug administration.

Challenge testing assesses the SDR. A greater percentage change is seen in patients with a younger age at diagnosis, and better motor performance (lower MDS UPDRS 3) (Malek et al., 2019). A greater motor improvement on long-term L-dopa is associated with female gender (Hauser et al., 2009), postural instability (Hauser et al., 2009), and L-dopa dose (Fahn et al., 2004, Fahn and Parkinson Study, 2005). Greater improvement in motor function was often associated with motor fluctuations (Fahn et al., 2004, Hauser et al., 2009, Clissold et al., 2006).

Predictive factors for the development of dyskinesia, motor fluctuations, and dystonia have been studied extensively.

A young age at onset of PD symptoms contributes to the development of all of these adverse events (Dos Santos et al., 2018, Fraix et al., 2000, Gershanik and Leist, 1987, Ilson et al., 1984, Kadastik-Eerme et al., 2017, Kelly et al., 2019, Manson et al., 2012, Muenter et al., 1977, Olanow and Schapira, 2013, Ouma et al., 2017, Quinn, 1993, Thenganatt and Jankovic, 2014). Dyskinesia and motor fluctuations are impacted by female gender (Dos Santos et al., 2018, Olanow and Schapira, 2013, Ouma et al., 2017), longer disease duration (Ahlskog and Muenter, 2001, Aquino and Fox, 2015, Blanchet et al., 1996) and greater motor severity (Group, 1996, Dos Santos et al., 2018, Horstink et al., 1990, Quinn et al., 1987), motor phenotypes like tremor dominance (Nicoletti et al., 2016), akinetic-rigid dominant (Kadastik-Eerme et al., 2017), and right side onset (Bay et al., 2019), worse scores for motor experiences of daily living (MDS UPDRS 2) (Olanow and Schapira, 2013), and better/worse scores for non-motor experiences of daily living (MDS UPDRS 1) (Kelly et al., 2019).

Additionally, dyskinesia development is contributed by low body weight (Olanow and Schapira, 2013) and body mass index (BMI) (Kelly et al., 2019), whereas motor fluctuations are linked to a higher level of education (Kelly et al., 2019), and higher motor examination scores (MDS UPDRS 3) (Olanow and Schapira, 2013).

Clinically, it is known that the degree of striatal dopaminergic degeneration and the duration and dose of L-dopa treatment are the main drivers of motor complication development. A higher daily total L-dopa dose (Ahlskog and Muenter, 2001, Aquino and Fox, 2015, Dos Santos et al., 2018, Kadastik-Eerme et al., 2017, Kelly et al., 2019, Olanow and Schapira, 2013), and a shorter time to treatment start (Kadastik-Eerme et al., 2017) are associated with the development of dyskinesia and motor fluctuations. Development of dyskinesia alone is associated with a shorter duration of L-dopa therapy (Jenner, 2008, Manson et al., 2012). It was also shown that the combination of different antiparkinsonian medications is relevant (Olanow and Schapira, 2013), with L-dopa monotherapy or MAO-B inhibitors plus L-dopa (Giannakis et al., 2018), but also dopamine agonists (Dos Santos et al., 2018) putting the patient at a greater risk of developing the condition.

Drug-induced dystonia can be reduced by reduction or withdrawal of L-dopa treatment (Melamed, 1979) or the administration of amantadine (Uitti et al., 1996), apomorphine (Esteban Munoz et al., 1997), and other dopamine agonists (Poewe et al., 1988).

Fluctuations of non-motor symptoms as a response to L-dopa treatment can be directly tied into fluctuations of motor function. Especially neuropsychiatric symptoms like anxiety and depression; autonomic symptoms like sweating (Raudino, 2001), urinary problems (Christmas et al., 1988), constipation (Witjas et al., 2002), pulmonary function (Rice et al., 2002), and sensory symptoms like inner restlessness (akathisia) (Witjas et al., 2002); and pain (Chaudhuri and Schapira, 2009) are linked to 'on' and 'off' periods and can vary in the presence of motor complications.

Clinical strategies can be put into place to at least delay the onset of this wide range of treatment complications. Early prescription of dopamine agonists as monotherapy was proven to be effective in large randomised trials (Bracco et al., 2004, Hauser et al., 2010, Holloway et al., 2004, Lees and Stern, 1981, Olanow and Obeso, 2000, Watts et al., 2010, Goetz et al., 2005) and also delayed the introduction of L-dopa into the treatment schedule (Hubble, 2002, Chung et al., 2018). However, after the introduction of L-dopa, the risk of developing dyskinesia again, increases in a dose-dependent

manner (Chondrogiorgi et al., 2014). If the maximum tolerated dose of L-dopa treatment is introduced early on, it does not predispose those patients to major disabling dyskinesia (Turcano et al., 2018), as severe forms would have manifested right away (Holloway et al., 2004, Onofrj et al., 1998). Another strategy could be the prescription of lower doses ('underdosing'), as patients have been shown to be much less likely to develop L-dopa induced motor complications (Ahlskog and Muenter, 2001, Montastruc et al., 1994). Even though those strategies may only provide a temporary solution, they are not the most ideal treatment approach and highlight the extent to which motor complications affect therapeutic choices (Rascol et al., 2003).

Genetics of L-dopa responsiveness

Genetics play a key role in disease development, especially for YOPD cases, which are often associated with dyskinesia development. This suggests genetic involvement in the manifestation of motor complications (Dekker et al., 2003). Current evidence is often contradictory, probably in part due to fast evolving techniques in the genetic field but also the lack of standardised parameters in the different studies conducted.

Some studies found that *LRRK2* cases had a higher rate of dyskinesia in comparison to idiopathic PD (Lesage et al., 2008, Nishioka et al., 2010), whereas others could not confirm this finding (Healy et al., 2008, Yahalom et al., 2012). Parkin cases had a delayed dyskinesia onset in one study (Lohmann et al., 2009).

Beyond the typical monogenic PD genes, studies investigated other genetic variants that are associated with L-dopa conversion and dopamine receptor stimulation. Genes encoding for Homer scaffold protein 1 (HOMER1) (Schumacher-Schuh et al., 2014), adenosine A2a receptor (ADORA2A) (Rieck et al., 2015), DRD2 (Rieck et al., 2012, Oliveri et al., 1999, Strong et al., 2006, Zappia et al., 2005), ankyrin repeat and kinase domain containing 1 (ANKK1) (Rieck et al., 2012), brain-derived neurotrophic factor (BDNF) (Foltynie et al., 2009), CYP2D6 (Stefanovic et al., 2000), glutamate ionotropic receptor NMDA type subunit 2A (GRIN2A) (Ivanova et al., 2012), COMT (de Lau et al., 2012), and solute carrier family 6 member 3 (SLC6A3) (Kaplan et al., 2014), and GBA (Iwaki et al., 2019) were associated with dyskinesia development.

The manifestation of motor fluctuation was linked to variants in the genes encoding for CYP2D6 (Stefanovic et al., 2000), COMT (Wu et al., 2014), GBA (Iwaki et al., 2019), and DRD2 (Lee et al., 2011).

Limited research has been conducted to investigate genetic aspects of dystonia, but it appears to be linked to the dopamine receptor D3 (DRD3) (Paus et al., 2009), DJ-1 (Bonifati et al., 2003, van Duijn et al., 2001), PINK-1 (Bonifati et al., 2005), and PRKN (Abbas et al., 1999, Lucking et al., 2000) genes.

Genotypes of specific polymorphisms of the *DDC* (Devos et al., 2014) and *SLC6A3* (Moreau et al., 2015) genes were associated with the degree of motor improvement at challenge testing, whereas carriers of the G2019S mutation on the *LRRK2* gene presented with a slower decline in MDS UPDRS 3 throughout disease progression (Saunders-Pullman et al., 2018).

1.3 Longitudinal observational studies

To increase our understanding of PD, several long-term studies monitoring from disease onset to death with the collection of information about disease severity and progress, medical history, and biosamples have been commenced in recent years. Earlier observational studies largely captured snapshots of the disease in smaller numbers of cases and over shorter time frames, although with some notable exceptions (Rascol et al., 1998, Hely et al., 1994, Shoulson, 1989). However, those studies predated modern genetic methods and had other limitations like high drop-out rates, so in order to extend our understanding of the different shapes of manifestations of the disease and generate more useful outcomes, longitudinal studies over several years with many more cases are required. Two ongoing studies with a comprehensive longitudinal clinical, genetic, and brain imaging data set who have been followed for around 8 years are of particular interest, and several aspects from these studies will be assessed in this thesis.

1.3.1 The Parkinson's Progression Markers Initiative (PPMI)

The Parkinson's Progression Markers Initiative (PPMI) is an ongoing American-led study that started in 2011. Participants were recruited worldwide as a multi-centre project in the US, Europe, Israel, and Australia. PPMI is mainly funded by The Michael J. Fox Foundation for Parkinson's Research (MJFF) and enrolled a total of 748 subjects, grouped into a *de novo* PD cohort consisting of untreated PD patients at enrolment (n=423), a control cohort (n=196), SWEDDs (n=64), and prodromal subjects (n=64). The initiative is still recruiting for genetic cohort subjects for *LRRK2*, *GBA*, or *SNCA* mutations, and genetic registry subjects including siblings of participants of the genetic cohort.

PD subjects had to be diagnosed with resting tremor, bradykinesia and/or rigidity, or either asymmetric resting tremor or asymmetric bradykinesia. Additionally, patients were 30 years of age or older, male or female, diagnosed with PD for 2 years or less at screening, H&Y stage 1 or 2 at baseline, SPECT with DaTSCAN™ or vesicular monoamine transporters type 2 (VMAT-2) PET scan confirmed dopamine transporter deficit, and patients were not expected to require antiparkinsonian medication for the next 6 months from baseline. Subjects were excluded if they were taking antiparkinsonian medication prior to, or at baseline, received neuroleptics or other drugs that might interfere with dopamine transporter SPECT imaging, or were treated with anticoagulants or other investigational drugs within 60 days before baseline.

The study (www.ppmi-info.org) collects demographics, vital signs, socio-economics, disease progression and severity related scores on motor (MDS UPDRS, H&Y) and non-motor function (MDS UPDRS; Modified S&E Activities of daily living; Physical Activity Scale, PASE; Hopkins Verbal Learning Test; Beton Judgment of line Orientation; Semantic Fluency; Letter Number Sequencing; Symbol Digit Modalities Test; Montreal Cognitive Assessment, MoCA; Epstein Sleepiness Scale, ESS; REM; Geriatric Depression Scale, GDS-15; State-Trait Anxiety Inventory for Adults, STAI; Questionnaire for Impulsive-Compulsive Disorders in PD, QUIP; Scales for outcomes in PD, SCOPA-AUT; Cognitive Categorization; Olfactory testing by the University of Pennsylvania Smell Identification, UPSIT); medical history; family history; brain imaging data; and biologic samples to establish markers of disease progression in PD.

1.3.2 The Tracking Parkinson's (PRoBaND) study

The Parkinson's Repository of Biosamples and Network Datasets (Tracking Parkinson's) (PRoBaND) study is the largest longitudinal follow-up study into PD (Study ID: GN11NE062 and NCT02881099, www.trackingparkinsons.org.uk). The study is multicentre across 70 UK, coordinated from Glasgow, and funded by the charity Parkinson's UK. From 2012 to 2015, the study enrolled 2,614 participants, grouped into 3 cohorts: Patients diagnosed within the last three years (recent onset, n=2008), patients diagnosed before the age of 50 years (young-onset, n=263) and a relatives cohort with siblings of existing participants (relatives, n = 344). In addition to those specific cohort criteria, eligible suspects were aged 18 to 90 years, of any gender, and the PD diagnosis was based on UK Brain Bank criteria, including the presence of the cardinal motor features of parkinsonism, i.e. bradykinesia and one or both of resting tremor and rigidity. Participants were excluded if they had severe comorbid illness preventing study participation, degenerative parkinsonism (e.g. PSP), drug-induced parkinsonism, symmetrical lower body parkinsonism with subcortical cerebrovascular disease, negative or normal functional imaging of presynaptic dopamine system, and the presence of UK Brain Bank diagnostic exclusion criteria at baseline. The study is scheduled to continue until at least November 2021.

Data were collected on patient demographics, medication, vital signs, motor function (MDS UPDRS), generic health status (EuroQuol- 5 Dimension, EQ5D; 8-item Parkinson's disease questionnaire, PDQ8; social history), environmental exposures with the Mini Environmental Risk Questionnaire for PD patients (MERQ-PD), non-motor function (MDS UPDRS; SCOPA-AUT; Non-Motor Symptoms Scale, NMSS;

Parkinson's Disease Sleep Scale, PDSS; ESS; REM sleep behaviour disorder screening questionnaire, RBD; smell testing; Hospital Anxiety and Depression Scale, HADS; QUIP; MoCA; big five index (BFI) of personality traits), and brain computerised tomography (CT) and/or MRI and dopamine transporter scans, and biologic samples.

1.4 Aims and structure of the thesis

This thesis aims to review the manifestation, development, and associations of responsiveness to dopaminergic therapy (L-dopa and dopamine agonists) in Parkinson's. Different perspectives including pathology, clinical variables, pharmacology, and brain imaging aspects are considered. The primary concept is that an increased understanding of the reasons for variation in the dopaminergic response will help to inform our understanding of PD, as well as the approaches taken in clinical practice.

A total of four new analyses was conducted to reach this goal. The objectives of these analyses were:

- To establish the extent of variation in L-dopa responsiveness by systematically reviewing pathologically confirmed Parkinson's and analysing clinical studies;
- To update current literature on the extent of long-term motor complication prevalence under current treatment schedules;
- To clarify the association of challenge testing and motor complication development for the assessment of L-dopa responsiveness;
- To determine the true dopaminergic loss at motor symptom onset

A variety of literature of different areas in Parkinson's research was reviewed to place the findings of these analyses in context with and draw conclusions. The remainder of the thesis is divided into six chapters, as follows:

- **Chapter 2** is a systematic review on the analysis of L-dopa responsiveness in pathologically confirmed PD. A standardised literature search for articles of autopsy-confirmed PD cases and information on their use of and response to L-dopa treatment was performed. The aim was to quantify the extent of variation in the L-dopa response in definite PD, to avoid the problems of interpretation of clinical studies which are likely to include cases with diagnostic error. Motor responsiveness on L-dopa, the development of motor complications, and the effects of comorbid pathologies were all assessed. A sensitivity analysis was conducted to remove potential atypical cases and test if the conclusion could be biased (Pitz et al., 2020).

- **Chapter 3** is a combined approach, using the two large longitudinal cohort studies Tracking Parkinson's and PPMI, to assess the relationship between motor complication development and drug treatment over time. Prior studies on motor complications in PD are not well defined since they used small sample sizes, are short-term, and treatment schedules are outdated. Therefore, this chapter aims to update and extend prior observations, reflecting more recent changes in therapy approaches, and quantifying the evolution of motor complications over time, analysing data in 2-year intervals from 0-6 years of disease duration.
- **Chapter 4** combines the analysis of the two main clinical measures to assess L-dopa responsiveness: motor complication development and the response to challenge tests. Both, Tracking Parkinson's and PPMI had challenge testing performed at a follow-up visit of around 3 years of disease duration (Malek et al., 2019). This chapter examines associations of L-dopa response variables with general cohort demographics and disease variables like other MDS UPDRS scores.
- **Chapter 5** establishes the degree of dopaminergic depletion in the striatum of an early disease Parkinson's cohort, using functional imaging SPECT with ^{123}I-Ioflupane (DaTSCAN™). Data from the PPMI study allowed the analysis of data available for up to 6 years disease duration, to estimate the true extent of dopaminergic depletion at symptom onset in context with pathological and imaging studies, but also assess the duration of the preclinical phase, and the annual rate of degeneration.
- **Chapter 6** discusses the findings of all chapters presented to draw conclusions about the impact of this work in clinical practice, clinical trials, and Parkinson's diagnostics. The relevance of individual findings but also the potential relevance of L-dopa responsiveness in neuroprotective trials is discussed to make recommendations for future trial cohorts.

Chapter 2: L-dopa responsiveness in definite PD

2.1 Introduction

When considering the factors that may influence responsiveness to dopaminergic medication in PD, a key factor in clinical studies is the accuracy of the diagnosis. It is known that other conditions may mimic PD and that these alternative diagnoses have a lesser or absent response to PD medication. Accordingly, improving the accuracy of diagnosis is the subject of a set of criteria, as follows.

A clinical diagnosis for idiopathic PD requires the presence of bradykinesia in combination with resting tremor and/or rigidity (Hughes et al., 1992, Postuma et al., 2015). Additionally, responsiveness to L-dopa is a supportive diagnostic criterion for the condition, defined as 'an excellent response' in the UK Brain Bank criteria (Hughes et al., 1992) and a 'clear and dramatic beneficial response to dopaminergic therapy' with return to a 'normal or near-normal level of function' during initial treatment in the Movement Disorder Society (MDS) clinical diagnostic criteria (Postuma et al., 2015). If no response to adequate doses of L-dopa in patients with at least moderate parkinsonism is observed, this counts as an exclusion criterion for PD, and points towards an alternative diagnosis (Hughes et al., 1992, Postuma et al., 2015).

Those clinical criteria help to reduce diagnostic error rates, which currently apply to between 5 and 25% of cases which are diagnosed in life as idiopathic PD but are not confirmed at autopsy. Those cases are likely to have another neurodegenerative disorder, or secondary parkinsonism (Hughes et al., 2002, Hughes et al., 1992, Rajput et al., 1991, Tolosa et al., 2006, Litvan et al., 1998). These observations might lead to the conclusion that a less-than-excellent response to L-dopa is incompatible with a diagnosis of PD. The range of L-dopa responses seen in clinical trials and studies of patients with a clinical diagnosis of PD would then be explained by diagnostic error, rather than biological variability.

In the only placebo-controlled dose-ranging study of L-dopa in *de novo* PD, there was an overall dose-related improvement in motor scores from baseline to 9 and 24 weeks, but a significant proportion of cases 'did not experience a robust response to levodopa' (Hauser et al., 2009). Variation in L-dopa motor responsiveness is also seen in other clinical trials (Holloway et al., 2004, Parkinson Study Group, 2009, Rascol et al., 1998, Rascol et al., 2006). Clinically, diagnostic specificity is refined by assessing only cases with confirmed presynaptic dopamine deficiency using functional neuroimaging (Rajput

et al., 2015, Hauser et al., 2009), but even within this subgroup, the motor response may be limited: after 24 weeks 27.1% of L-dopa treated patients experienced a 10% or less improvement (Hauser et al., 2009). While this excludes patients with more benign disorders (e.g. essential and dystonic tremor) it does not exclude other neurodegenerative parkinsonian conditions like PSP or MSA. Diagnostic clues for PSP include 'Levodopa-resistance' and further define it as 'improvement of the MDS-UPDRS motor scale by ≤30%' (Hoglinger et al., 2017). MSA patients are considerably less responsive to dopamine replacement therapy (Gilman et al., 2008); the 30% of cases that have an initial motor improvement do not sustain this as the disease progresses (Hughes et al., 1992).

While clinical criteria undoubtedly increase diagnostic accuracy, they remain imperfect, and a pathological assessment remains the gold standard for diagnosing PD. Therefore, assessing only pathologically confirmed PD cases should give a clearer indication of the degree of variation in L-dopa response, and was the objective of the present study.

A clinical diagnosis can be further supported by the development of motor complications like motor fluctuations, including wearing-off and dyskinesia, which manifest on long-term L-dopa treatment (Hughes et al., 1992, Postuma et al., 2015). The development of these features varies both the time to onset and the severity of such motor complications. Several factors contribute to this variation, including disease duration (Aquino and Fox, 2015), L-dopa treatment duration (Jenner, 2008) and dose (Warren Olanow et al., 2013), the presence of autosomal recessive PD gene mutations (Manson et al., 2012, Thenganatt and Jankovic, 2014), and genetic variation in enzymes involved in dopamine metabolism (Sampaio et al., 2018). Clinical diagnostic accuracy also affects the interpretation of these phenomena, as patients with benign tremor disorders do not develop motor complications with L-dopa (Rajput et al., 2015) and those affected by other degenerative parkinsonian disorders may have an earlier onset or different pattern of motor complications compared to PD (Gilman et al., 2008). We therefore also examined for variation in the prevalence of motor complications ('wearing off', 'on-off' fluctuations, and dyskinesia) in the pathologically confirmed PD cases identified in this systematic review. The main outcome measure of this review was the responsiveness to L-dopa with grading reported as binary or in four categories.

2.2 Materials and Methods

2.2.1 Main analysis

Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009), a literature search of the databases PubMed, Embase and Latin American and Caribbean Health Sciences Literature (LILACS) was conducted for full-text articles between 1971 and March 2018, using the combined medical subheading terms 'levodopa', 'L-dopa', 'Parkinson's disease', and 'post-mortem'. The search was limited to humans, research articles, and the English language. Duplicates, book chapters, and reviews were excluded. We also searched the reference list in papers that met search criteria. Studies had to include the following: 1) pathologically confirmed idiopathic PD, 2) patient demographics, and 3) motor improvement with L-dopa. One researcher (VP) screened the abstracts and identified potentially eligible studies, which were then assessed by a second researcher (DG), and disagreements were resolved by consensus.

2.2.2 Sensitivity analysis

A sensitivity analysis was conducted to replicate the findings of the main analysis. Single case reports potentially contain cases with clinically atypical parkinsonism which were therefore excluded in this analysis. Study inclusion criteria from the main analysis were therefore refined such that only articles including five or more pathologically confirmed cases fulfilling the same criteria as in the main analysis were eligible.

2.3 Results

2.3.1 Main analysis

The literature search identified 893 studies, from which 757 full-text articles were assessed. 26 of those studies reporting a total of 469 pathologically confirmed PD cases met eligibility criteria for the main analysis. The pathological PD diagnosis was made (in all 26 papers) when there was severe depletion of pigmented neurons and Lewy body formation in the substantia nigra pars compacta. In addition, detailed immunohistochemistry was reported in 22/26 papers, including staining for alpha-synuclein inclusions in 14 of those 22 studies. Pathological rating scales were reported in 6 papers, including 1 or more of: a 4-point neuronal loss scale (Hughes et al., 1993, Hughes et al., 1992), Lewy body counts (McKeith criteria) (De Pablo-Fernandez et al., 2017, McKeith et al., 2005), Braak staging (Kiely et al., 2015), Queen Square Brain Bank semi-quantitative grading (De Pablo-Fernandez et al., 2017, Kiely et al., 2015), and Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria (Joyce et al., 2002). Two papers recorded prospective clinical data; the remainder extracted data retrospectively from patient files.

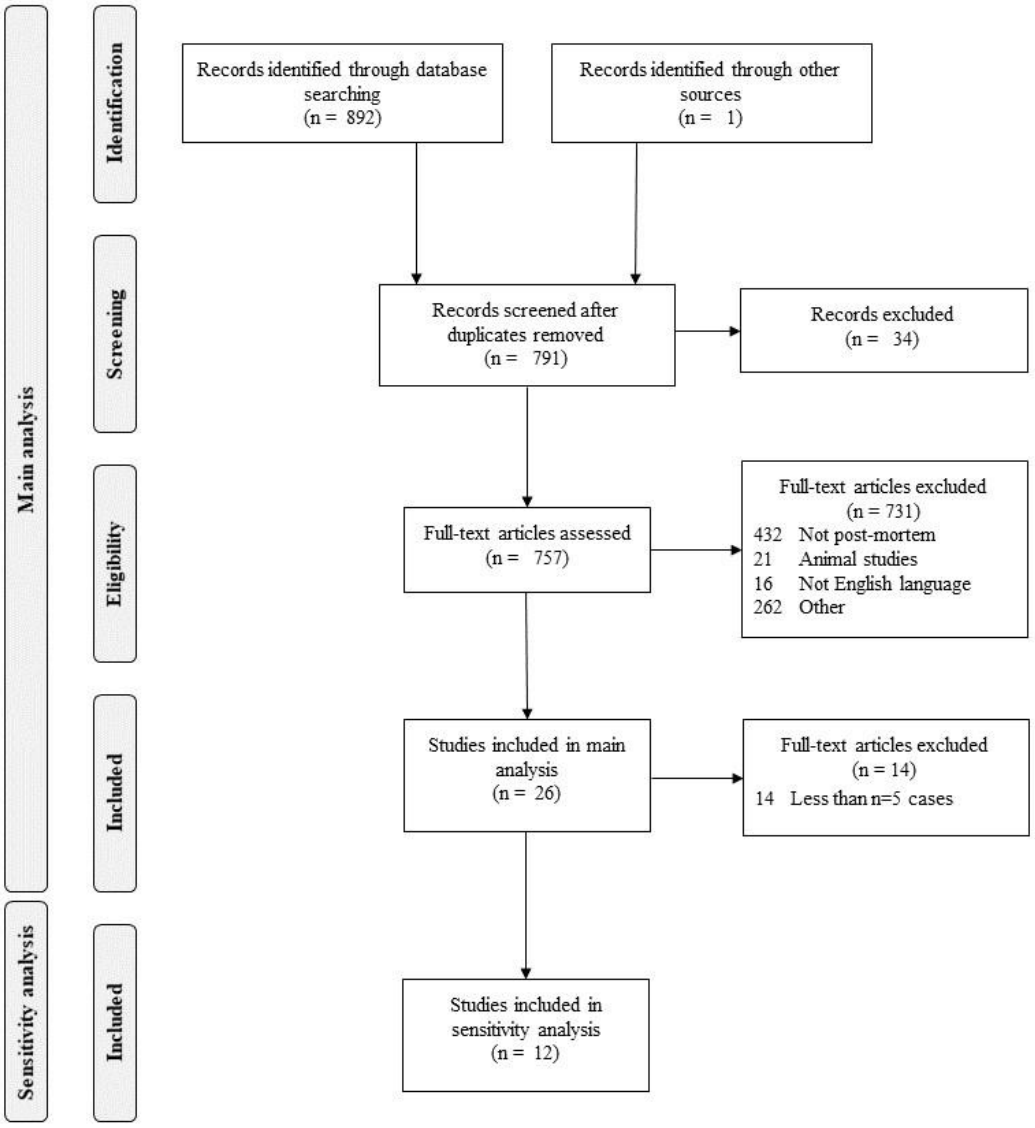

Figure 2-1: Flow diagram showing the study selection process.
The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) process was used to identify papers for both the main analysis (26 studies) and the sensitivity analysis (12 studies) (adapted from (Moher et al., 2009)).

2.3.1.1 Clinical assessments

The assessment of disease severity was based on the Hoehn and Yahr (H&Y) scale in 11/26 papers, and/or Unified PD Rating Scale (UPDRS) in 4/26 papers. 26 papers assessed the motor improvement on L-dopa, 16 had information on the occurrence of motor complications, 13 investigated comorbid pathologies, and 8 looked into genetic factors. The degree of motor improvement with L-dopa was defined according to 4 categories: >70% (excellent), 50-70% (good), 30-50% (moderate), and <30% (none-to-

poor) in 17/26 papers following UK Brain Bank descriptors (Hughes et al., 1993, Hughes et al., 1992). However, treatment responses were not calculated numerically in any paper. In the remaining 9 papers, the L-dopa motor response was only differentiated into responsive or non-responsive (Benarroch et al., 2001, de Vos et al., 1995, Halliday et al., 1996, Joyce et al., 2002, Louis et al., 1997, Pramstaller et al., 2005, Rajput et al., 1993).

2.3.1.2 Features of the L-dopa response

Demographics

A total of 469 cases had pathologically confirmed PD, of whom 61.3% were male. Patients had a mean age at onset of 63.4 (SD 10.3) years, a mean disease duration at death of 13.1 (SD 6.6) years, and a mean age at death of 76.6 (SD 7.7) years. Treatment with L-dopa was introduced after a mean disease duration of 3.3 (SD 4.3) years (Table 2-1, Table 2-2).

Degree of motor response

A motor response to chronic L-dopa therapy was reported in 423 of 469 PD cases (90.2%) (De Pablo-Fernandez et al., 2017, Halliday et al., 1996, Hughes et al., 1993, Hughes et al., 1992, Louis et al., 1997, Rajput and Rajput, 2017, Rajput et al., 1993). It was graded in 298 cases, and was excellent in 113 (37.9%), good in 135 (45.3%), moderate in 35 (11.7%), and none-to-poor in 15 (5.0%) of cases. In the remaining 125 cases, a binary response to L-dopa was reported: 93 (74.4%) of these were L-dopa responsive, and 32 (25.6%) were unresponsive (Figure 2-2, Table 2-1). Information on L-dopa doses was given in 13/26 papers, ranging from single case and dose reports to the mention of an 'adequate trial' of 1,000mg/day.

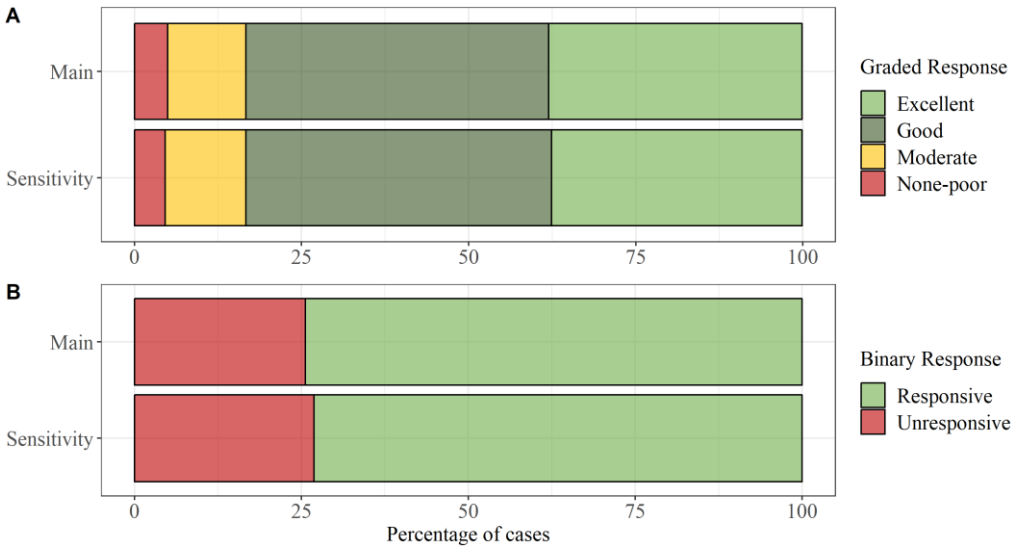

Figure 2-2: Motor improvement on L-dopa in 423 pathologically confirmed PD.
The motor response was graded (A) or binary (B) and was variable by both methods. A substantial proportion of cases had a response to L-dopa that was graded as less than excellent. Findings were very similar comparing the main analysis (all cases) and the sensitivity analysis (excluding 24 cases from individual reports containing less than 5 cases).

Motor complications (motor fluctuations and dyskinesia)

Motor complications were reported in 160 patients in 16 papers (Gaig et al., 2008, Gouider-Khouja et al., 2003, Henderson et al., 2001, Hughes et al., 1993, Kiely et al., 2013, Kiely et al., 2015, Koh et al., 2006, Lesage et al., 2013, Liang et al., 2005, Litvan et al., 1998, Pramstaller et al., 2005, Puschmann et al., 2012, Rajput and Rajput, 2017, Rajput et al., 1993, Sage et al., 1990, Uitti et al., 1995). After 5.1 (SD 1.7) years of L-dopa treatment, 71 (44.4%) reported motor fluctuations, whereas dyskinesia was present in 89 (55.6%), with mean onset after 6.2 (SD 2.4) years of L-dopa treatment.
Unbundling summary reports of motor responsiveness proved to be difficult, so that the degree of motor responsiveness could only be directly compared with the occurrence of motor complications in 43 single case reports. 35 of these 43 (81.4%) responders were reported as good/excellent (graded response), or responsive (binary response). Of these, 19/35 (54.3%) developed motor complications (26.3% developed motor fluctuations, 47.4% dyskinesia, and 26.3% both). 16/35 (45.7%) did not have any motor complications. Considering the 8 remaining cases (18.6% of the 43) the response was moderate/none to poor (when graded) or unresponsive (binary response). 3 of these 8

(37.5%) developed motor complications, and 5 of these 8 (62.5%) did not have any motor complications (Table 2-1). The daily L-dopa dose was only reported in 9 of these 43 cases, with a range from 200 to 5,000mg, so further analysis of the relationship between dose and motor complications was not possible.

Comorbid brain pathology

247 patients were assessed for the L-dopa motor response and comorbid brain pathology. 144 of these 247 (58.3%) had additional brain pathology at autopsy. 63 of the 144 (43.8%) had cerebrovascular pathology, 56 (38.9%) had Alzheimer's disease (AD) or Alzheimer-type pathology, 18 (12.5%) had amyloid angiopathy, 6 (4.2%) had diffuse Lewy body disease (DLBD), and one case (0.7%) showed additional progressive supranuclear palsy (PSP) pathology (Figure 2-3).

There was information about both the L-dopa response (motor improvement and development of motor complications) and comorbid pathology, which was sometimes multiple, in 43 cases. 35 of these (81.4%) were responsive to L-dopa, of whom 9 cases (25.7%) had one or more coexistent pathology (AD n=4, DLBD n=4, cerebrovascular disease n=2). There were 8 cases (18.6%) that were not responsive to L-dopa, of whom 5 (62.5%) had one or more coexistent pathology (AD n=2, DLBD n=2, cerebrovascular n=2, PSP n=1, Figure 2-3). In the L-dopa responsive patients, 19 (54.3%) developed motor complications, while in the 8 that were unresponsive to L-dopa, 3 (37.5%) developed motor complications.

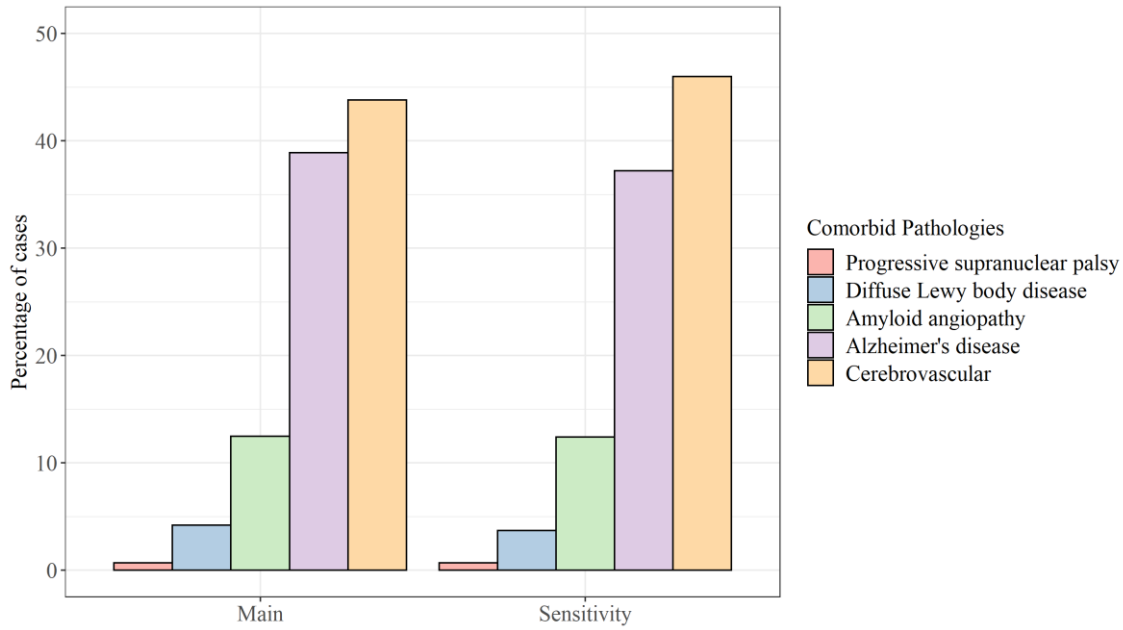

Figure 2-3: Comorbid pathologies in 423 cases with pathologically confirmed PD. Most PD cases showed cerebrovascular changes (n=63), followed by AD (n=56), amyloid angiopathy (n=18), DLBD (n=6), and PSP (n=1). Findings were very similar comparing the main to the sensitivity analysis (excluding 24 cases from publications reporting less than 5 cases).

Sensitivity analysis

445 pathologically confirmed PD cases in 12 studies met inclusion criteria for the sensitivity analysis (Table 2-1, Table 2-2). The pathological diagnosis was made (in all 12 papers) by microscopic confirmation of severe depletion of pigmented neurons and Lewy body formation in the substantia nigra pars compacta. In addition, immunohistochemistry was reported in 7 of 12 papers, including alpha-synuclein staining in 5 of 12. Pathological rating scales were reported in 3 papers. Two papers (Hughes et al., 1993, Hughes et al., 1992) recorded prospective clinical data; the remainder extracted data retrospectively from patient files. 3 studies also used standardised forms (Hughes et al., 1993, Louis et al., 1997, Litvan et al., 1998). All studies reported the chronic out-patient L-dopa response.

2.3.2 Sensitivity analysis

2.3.2.1 Clinical assessments

Disease severity grading was based on Hoehn and Yahr (H&Y) in 6 of 12 papers, and/or Unified PD Rating Scale (UPDRS) in 1 of 12 papers. 12 papers assessed the motor improvement on L-dopa (De Pablo-Fernandez et al., 2017, de Vos et al., 1995,

Halliday et al., 2008, Halliday et al., 1996, Hughes et al., 1993, Hughes et al., 1992, Joyce et al., 2002, Litvan et al., 1998, Louis et al., 1997, Matsumoto et al., 2014, Rajput and Rajput, 2017, Rajput et al., 1993), 4 the occurrence of motor complications, and 6 investigated comorbid pathologies. The degree of motor improvement with L-dopa was defined in 5 of 12 papers following UK Brain Bank descriptors (Hughes et al., 1993, Hughes et al., 1992). In the remaining 7 papers, the L-dopa motor response was categorised as either responsive or non-responsive (de Vos et al., 1995, Halliday et al., 1996, Joyce et al., 2002, Louis et al., 1997, Rajput et al., 1993, Rajput and Rajput, 2017, Matsumoto et al., 2014) Table 2-2).

2.3.2.2 Features of the L-dopa response

Demographics

Of the 445 pathologically confirmed PD patients (61.7% male), age at onset was 64.0 (SD 9.6) years, L-dopa treatment was started 3.1 (SD 3.6) years after diagnosis, and disease duration at death was 13.0 (SD 6.5) years. Age at death was 77.1 (SD 7.2) years (Table 2-1, Table 2-2).

Degree of motor response

The L-dopa response was reported in 399 of 445 PD cases (89.7%) (De Pablo-Fernandez et al., 2017, de Vos et al., 1995, Halliday et al., 2008, Halliday et al., 1996, Hughes et al., 1993, Hughes et al., 1992, Joyce et al., 2002, Litvan et al., 1998, Louis et al., 1997, Matsumoto et al., 2014, Rajput and Rajput, 2017, Rajput et al., 1993). It was graded in 280 cases: excellent in 105 (37.5%), good in 128 (45.7%), moderate in 34 (12.1%), and none-to-poor in 13 (4.6%). In the remaining 119 cases, a binary response to L-dopa was reported: 87 (73.1%) of these were L-dopa responsive, and 32 (26.9%) were unresponsive (Figure 2-2). L-dopa doses were reported in 5 of 12 papers but were largely declared as 'adequate' (often defined as 1000mg per day), rather than quantified. Where quantified, the mean daily L-dopa dose was 917mg (SD 446) in 23 cases (De Pablo-Fernandez et al., 2017).

Motor complications (motor fluctuations and dyskinesia)

Motor complications were reported in 148 patients in 4 papers (Hughes et al., 1993, Litvan et al., 1998, Rajput and Rajput, 2017, Rajput et al., 1993), being motor fluctuations in 63 cases (42.6%), and dyskinesia in 79 cases (53.4%).

Comorbid brain pathology

235 patients were assessed for the L-dopa motor response and comorbid brain pathology. 137 of these (58.3%) had additional brain pathology, most commonly cerebrovascular disease (46.0%) and AD (37.2%), followed by Amyloid angiopathy (12.4%), DLBD (3.6%), and PSP (0.7%) (Figure 2-3, Table 2-1). Data about the L-dopa response, motor complications, and comorbid pathology were only available in 25 cases but did not readily explain the degree of L-dopa responsiveness.

Table 2-1: Clinical and pathological features in pathologically confirmed PD.

	Main analysis		**Sensitivity analysis**	
	PD cases (n=469)	**Publications (n=26)**	**PD cases (n=445)**	**Publications (n=12)**
Age at onset (years)	63.4 (10.3)	26	64.0 (9.6)	12
Disease duration at death (years)	13.1 (6.6)	26	13.0 (6.5)	12
Age at death (years)	76.6 (7.7)	25	77.1 (7.2)	12
Symptom onset to starting L-dopa treatment (years)	3.3 (4.3)	9	3.1 (3.6)	2
L-dopa motor response reported	423/469 (90.2%)	26	399/445 (89.7%)	12
Graded	298/423 (70.4%)	17	280/399 (70.2%)	5
Excellent (>70%)	113 (37.9%)		105 (37.5%)	
Good (50-70%)	135 (45.3%)		128 (45.7%)	
Moderate (30-50%)	35 (11.7%)		34 (12.1%)	
None-to-poor <30%)	15 (5.0%)		13 (4.6%)	
Binary	125/423 (29.6%)	9	119/399 (29.8%)	7
Responsive	93 (74.4%)		87 (73.1%)	
Unresponsive	32(25.6%)		32 (26.9%)	
Treatment duration (years)	10.9 (0.7)		10.9 (0.7)	4
Assessed for comorbid pathology	247/423 (58.4%)	12	235/399 (58.9%)	6
Comorbid pathology present	144/247 (58.3%)		137/235 (58.3%)	
Cerebrovascular	63 (43.8%)		63 (46.0%)	
Alzheimer-type	56 (38.9%)		51 (37.2%)	
Amyloid angiopathy	18 (12.5%)		17 (12.4%)	
Diffuse Lewy body disease	6 (4.2%)		5 (3.7%)	
Progressive supranuclear palsy	1 (0.7%)		1 (0.7%)	

Table 2-2: Demographics in pathologically confirmed PD patients with a reported motor response to L-dopa.

Study	PD patients total, n	Male, n (%)	PD patients with reported L-dopa response, n (%)	Type of L-dopa response grading	Clinical rating scales used	Mean age at PD onset, years (SD)	Mean age at death, years (SD)	Disease duration years (SD)	Onset to starting L-dopa, years (SD)
TOTAL (main analysis)	469 (100%)	234 (61.3%)	423 (90.2%)			63.4 (10.3)	76.6 (7.7)	13.1 (6.6)	3.3 (4.3)
Sage et al. 1990 (Sage et al., 1990)	5	4	5	Graded	None	49.6 (18.1)	67.4 (10.6)	17.8 (14.3)	Not stated
Hughes et al. 1992 (Hughes et al., 1992)*	76	Not stated	69	Graded	H&Y	63.6 (13.3)	76.4 (10.25)	12.8 (7.0)	Not stated
Hughes et al. 1993 (Hughes et al., 1993)*	100	65	95	Graded	H&Y, MMSE, DSM 3	62.5 (9.2)	75.6 (6.7)	13.1 (6.3)	3.2 (3.7)
Rajput et al. 1993 (Rajput et al., 1993)*	26	18	20	Binary	H&Y, Webster	58.8 (8.8)	70.8 (8.5)	11.7 (9.3)	Not stated
Uitti et al. 1995 (Uitti et al., 1995)	2	2	2	Graded		56.5 (14.8)	74.5 (3.5)	18.0 (11.3)	4.5 (2.1)
De Vos et al. 1995 (de Vos et al., 1995)*	18	9	18	Binary	H&Y, MMSE, DSM 3, HAM-D	66.2 (NS)	76.3 (NS)	10.1 (NS)	Not stated
Halliday et al. 1996 (Halliday et al., 1996)*	11	8	6	Binary	H&Y, CDR	67.4 (8.7)	77.6 (5.4)	10.3 (5.7)	Not stated
Louis et al. 1997 (Louis et al., 1997)*	34	22	14	Binary	None	62.0 (NS)	76.0 (NS)	14.5 (NS)	Not stated
Litvan et al. 1998 (Litvan et al., 1998)*	11	Not stated	11	Graded	None	54.4 (4.0)	Not stated	15.6 (1.6)	Not stated

Study	PD patients total, n	Male, n (%)	PD patients with reported L-dopa response, n (%)	Type of L-dopa response grading	Clinical rating scales used	Mean age at PD onset, years (SD)	Mean age at death, years (SD)	Disease duration years (SD)	Onset to starting L-dopa, years (SD)
Henderson et al. 2001 (Henderson et al., 2001)	1	0	1	Graded	H&Y, ADL, Columbia, CDR	56.0	71.0	15.0	4.0
Benarroch et al. 2001 (Benarroch et al., 2001)	5	2	5	Binary	None	64.6 (9.1)	75.6 (8.4)	11.0 (2.6)	Not stated
Joyce et al. 2002 (Joyce et al., 2002)*	23	15	23	Binary	None	65.0 (10.9)	78.1 (6.1)	13.2 (7.9)	Not stated
Gouider-Khouja et al. 2003 (Gouider-Khouja et al., 2003)	1	1	1	Graded	H&Y, UPDRS	34.0 (0.0)	47.0	13.0	Not stated
Kotzbauer et al. 2004 (Kotzbauer et al., 2004)	1	1	1	Graded	None	37.0	57.0	20.0	Not stated
Pramstaller et al. 2005 (Pramstaller et al., 2005)	1	1	1	Binary	H&Y, UPDRS	49.0	74.0	25.0	Not stated
Liang et al. 2005 (Liang et al., 2005)	1	0	1	Graded	None	59.0	67.0	8.0	2.0
Koh et al. 2006 (Koh et al., 2006)	1	1	1	Graded	None	53.0	63.0	10.0	Not stated
Halliday et al. 2008 (Halliday et al., 2008)*	7	2	7	Graded	H&Y, CDR	59.4 (8.6)	73.4 (9.3)	14.0 (3.4)	1.7 (0.6)
Gaig et al. 2008 (Gaig et al., 2008)	1	0	1	Graded	None	51.0	78.0	27.0	1.0

Study	PD patients total, n	Male, n (%)	PD patients with reported L-dopa response, n (%)	Type of L-dopa response grading	Clinical rating scales used	Mean age at PD onset, years (SD)	Mean age at death, years (SD)	Disease duration years (SD)	Onset to starting L-dopa, years (SD)
Puschmann et al. 2012 (Puschmann et al., 2012)	1	0	1	Graded	H&Y, UPDRS	50.0	69.5	19.5	Not stated
Kiely et al. 2013 (Kiely et al., 2013)	1	1	1	Graded	MMSE	19.0	49.0	29.0	6.0
Lesage et al. 2013 (Lesage et al., 2013)	1	0	1	Graded	None	60.0	67.0	7.0	Not stated
Matsumoto et al. 2014 (Matsumoto et al., 2014)*	16	12	16	Binary	None	63.6 (10.9)	72.8 (8.4)	10.2 (6.1)	Not stated
Kiely et al. 2015 (Kiely et al., 2015)	2	0	2	Graded	MMSE	54.5 (23.3)	66.0 (24.0)	12.0 (0.0)	Not stated
De Pablo-Fernandez et al. 2017 (De Pablo-Fernandez et al., 2017)*	100	60	98	Graded	None	63.9 (10.3)	78.5 (6.9)	14.6 (7.7)	Not stated
Rajput et al. 2017 (Rajput and Rajput, 2017)*	23	10	22	Binary	H&Y, Webster/UPDRS, MMSE	82.7 (2.2)	91.2 (3.0)	8.5 (2.7)	Not stated

ADL, Activity of Daily Living Scale; CDR, Clinical Dementia Rating; DSM, Diagnostic and Statistical Manual of Mental Disorders; HAM-D, Hamilton Depression Rating Scales; H&Y, Hoehn and Yahr; MMSE, Mini Mental State Examination; PD, Parkinson's disease; UPDRS, Unified Parkinson's Disease Rating Scale. *Papers included in the sensitivity analysis.

2.4 Discussion

There is a wide variation in the motor response to L-dopa treatment in PD. The inclusion of only pathologically confirmed cases and replication of the results in both of our analyses allow us to reliably conclude that errors in the clinical diagnosis of PD do not fully explain variability in the L-dopa motor response. It is also clear that a substantial proportion of cases of definite PD have a response to L-dopa that is less than excellent. An exploration of potential reasons for this striking finding now follows.

The definitions of what is 'excellent' regarding the motor response to L-dopa clearly influence this categorisation of patients, and it is evident that such definitions have evolved. Before the publication and use of the MDS criteria for PD (Postuma et al., 2015), the most widely applied criteria were the UK Queen's Square Brain Bank criteria. The L-dopa response was defined in those criteria as a clear and persisting motor improvement and was based on case record review (Hughes et al., 1992). Although the percentage improvement for an excellent response was stated as being '70-100%' this interpretation of case records was *subjective* and not based on a numerical calculation of a motor score change on a rating scale (Hughes et al., 1992). The definition of responsiveness based on the UK Brain Bank criteria was predominantly applied, being used in 70.4% of the 423 cases in the papers of the current review. It is therefore not possible to accurately define the motor response to L-dopa in these pathologically confirmed cases in accordance with the more *objective* and latest MDS criteria definition, where an excellent response is defined as a >30% improvement in UPDRS Part 3 (Postuma et al., 2015) or ≥24.5% improvement in the MDS UPDRS 3 (Merello et al., 2011). The recent validation of the MDS clinical diagnostic criteria for PD resulted in 73.4% of 434 patients with an excellent L-dopa response (assessed in routine clinical practice) and is therefore a close match to those earlier pathological findings (Postuma et al., 2018). Considering the open-label methodology of assessing L-dopa responsiveness, that is known to over-emphasise the benefits of drug treatment (Witek et al., 2018), those more objective findings are crucial.

Issues about the definition of the grade of motor response and the largely retrospective clinical methodology in the pathological studies complicate the interpretation of the new results. It is therefore appropriate to compare the findings with those from clinical trials, to identify evidence of variation in L-dopa motor responsiveness on a clinical level. The standardised scoring with clinical rating scales (e.g. UPDRS 3), before and after starting L-dopa treatment, is an appropriate testing method against current definitions of an

excellent L-dopa response. After 9 weeks on L-dopa treatment, the mean UPDRS improvement was 27.4% in 260 patients, and after 24 weeks it was 26.2% in 247 patients (Hauser et al., 2009). Limiting the analysis to cases with a proven dopaminergic deficit on SPECT imaging showed similar results: mean percentage improvement was 23.4% at 9 weeks in 72 patients, and 26.8% at 24 weeks in 70 patients (Hauser et al., 2009). Therefore, at each time point and regardless of case refinement, less than half of PD cases had an excellent L-dopa response (i.e. exceeding 30% motor improvement). In another study, 42% of 89 L-dopa treated patients had a less-than-excellent motor improvement at 6 months (Rascol et al., 1998). Clinical trials have also shown clear evidence for a variation in the L-dopa responsiveness, noting that some cases with benign tremor disorders (between 4 and 14%, (Erro et al., 2016)) are likely to have been included and that clinical studies slightly underestimate the proportion of true PD cases with an excellent L-dopa response. However, the two lines of evidence - the pathological studies, which benefit from a confirmed diagnosis, and the clinical studies, which benefit from objective motor scoring – inform us that at least one quarter, and more likely one half of PD cases do not have an excellent response to L-dopa.

There are several potential explanations for these findings. A worse motor score in men than women despite higher L-dopa doses (Lyons et al., 1998) may indicate gender differences, an observation that is supported by the lesser improvement in motor score seen in men in one study (Hauser et al., 2009). Also, the postural instability gait difficulty phenotype is less therapy responsive than tremor-dominant Parkinson's (Hauser et al., 2009). However, an exception is benign tremulous PD: in pathologically confirmed cases, the L-dopa response during the first 8 years of treatment was definite in only 6 of 16 cases (37.5%), and 3 of 16 (18.8%) had no L-dopa response (Selikhova et al., 2013). Slower progression in younger patients (Wickremaratchi et al., 2009) may be partly due to better L-dopa responsiveness. These factors are contributed by genetic variations, such as in the dopamine metabolizing enzymes (Guin et al., 2017). However, the pathological studies did not include demographic or genetic data to allow these factors to be examined in detail.

Further important variables in assessing the L-dopa response are the dose (Hauser et al., 2009) and duration (Hughes et al., 1992, Postuma et al., 2015) of treatment. A few cases in the pathological studies had low tolerability of L-dopa which was dose-limiting (Kempster et al., 2007), and overall the detail regarding the L-dopa doses used was

limited but the average follow-up of more than 13 years before death was clearly adequate to assess treatment responses.

The development of motor complications (motor fluctuations or dyskinesia) is a further key feature of evolving PD. Dyskinesia was present in around half of the post-mortem confirmed PD cases in this review in both analyses conducted. This number is similar to the approximate 50% dyskinesia rate after 10 years of treatment in patients that were initially treated with a dopamine agonist in a clinical trial setting while noting a higher dyskinesia rate of 78% when L-dopa was used as the initial treatment (Hauser et al., 2007). Although after 20 years of PD diagnosis, nearly all of the 90% of survivors had mild to moderate dyskinesia in a long-term observational study, and 74% of the original cohort had died by this time (Hely et al., 2008). This leaves open the possibility that patients who died earlier had either more severe dyskinesia, or did not develop dyskinesia, and that one or both of those situations is associated with shorter survival times. The relationship between longer disease duration and a higher rate of dyskinesia which is observed clinically (Ku and Glass, 2010) was also evident in the pathological studies (Kempster et al., 2007). However, the pathological studies generally did not report motor complication data by gender, so we could not assess whether the clinical observations of more dyskinesia in women (Lyons et al., 1998) held true in pathologically confirmed cases. The clinical pattern of PD is also contributory to the development of motor complications: in benign tremulous PD, despite the limited motor response to L-dopa mentioned earlier, most cases (81.3% of 16) developed dyskinesia (Selikhova et al., 2013). A postural instability gait disorder (PIGD) PD phenotype is associated with more motor complications, and a faster disease progression, compared to tremor-dominant cases (van der Heeden et al., 2016).

The assessment of comorbid brain pathology in relation to the motor response to L-dopa in PD cases suggested a relation of cerebrovascular changes to an impaired L-dopa response. This has support from clinical and imaging findings in a large observational study (Malek et al., 2016, Malek et al., 2017). There was insufficient data to assess the effect of comorbid Alzheimer pathology on the L-dopa response; indicative information about this may emerge from clinical studies in PD that assess L-dopa responsiveness and include testing for Alzheimer genetic factors (Barrett et al., 2016).

The studies in the current review largely predate developments in genetic testing in PD, so that data regarding the L-dopa response in relation to genetic mutations were limited.

Reported pathological cases with mutations in *SNCA*, *LRRK2*, and *PRKN* were all associated with L-dopa responsiveness, but the small number of cases do not allow meaningful comparisons of the response against sporadic PD cases and were therefore omitted from sensitivity analysis. Studies that reported the L-dopa response in cases with PD-related mutations, but *without* pathological confirmation of PD, were not included in the current review. Specific pathogenic mutations in the PD related genes in the presence of parkinsonism assist in clinically confirming PD, while accepting that there is age-dependence in disease presentation and that the penetrance of many variants is not complete.

No studies fulfilled our selection criteria and reported *GBA* mutations (Malek et al., 2018), but one report noted that out of 17 pathologically confirmed *GBA* positive cases, 2 (11.8%) 'did not respond to L-dopa', mentioning that a positive response was at least a 30% improvement after the first introduction of L-dopa (Neumann et al., 2009). In *GBA* cases there was more dyskinesia than in cases of sporadic PD, but higher L-dopa doses were possibly contributory (Schneider and Alcalay, 2017). An initial L-dopa response that waned over the course of 5 years was seen in one of 31 *GBA* cases, but there was no pathological confirmation (Neumann et al., 2009) .

In 356 clinical *LRRK2* cases, the L-dopa response was good or excellent in 88%, modest in 9%, and poor in 3%, and similar in a comparative group of sporadic cases (Healy et al., 2008). However, the time to onset of dyskinesia was significantly longer, and the proportion affected by dyskinesia was lower. Especially at 5 years, 11% of cases had dyskinesia after 5 years of treatment, which in comparison was much higher in sporadic cases with 25%. After 10 years of treatment, 32% of LRRK2 cases reported dyskinesia, whereas 41% of sporadic cases were affected.

In *PRKN* cases, an excellent (Gouider-Khouja et al., 2003, Pramstaller et al., 2005) or striking (Khan et al., 2002) L-dopa response is frequently described. The response often sustains but *PRKN* cases often show severe motor fluctuations (Doherty et al., 2013). *PINK1* cases had an 'excellent' and 'sustained' response to L-dopa (Healy et al., 2004, Ibanez et al., 2006). A comparison of *PINK1* and *PRKN* PD cases indicated no phenotypic differences, but *PINK1* cases had an even better L-dopa response than *PRKN* cases (Ibanez et al., 2006). Compared to idiopathic PD, both *PRKN* and *PINK1* cases developed dyskinesia earlier (Ibanez et al., 2006).

Taking the small number of genetic PD in the pathological series together with the clinical cases with likely pathogenic mutations, there is a variation in both the L-dopa motor response and motor complications across and between genetic PD types. However, one outlying *PRKN* observation was the most L-dopa responsive and typified by severe dyskinesia.

Although pathology is the gold standard pathological definition of PD, just over half of the studies that we included relied on dopaminergic cell loss and Lewy body formation in the substantia nigra, as they predated the landmark observations about alpha-synuclein (Polymeropoulos et al., 1997). As many studies derived clinical information retrospectively, the interpretation of the L-dopa response may be over-optimistic, and there might be a lack of recording of milder motor complications. Two studies with a total of 176 cases had a partial overlap (estimated to be 69 cases) which could not be unbundled accurately, so our results are affected by this duplication (Hughes et al., 1993, Hughes et al., 1992). One study in 23 patients had a high age at onset of 82.7 (2.2) years, and a disease duration of 8.5 (2.7) years at time of death, which is therefore an outlier. That study had a relatively high rate of 22% for cerebrovascular changes, but commented that they were possibly age-related rather than pathological (Rajput and Rajput, 2017).

In conclusion, there is substantial variation in the L-dopa response in pathologically confirmed PD, so diagnostic error does not explain this observation. Around 10% of pathologically confirmed PD cases are unresponsive to L-dopa treatment, and an additional 12% have a modest response. These findings broadly match clinical trial data. In clinical practice, limited L-dopa responsiveness may therefore be diagnostically misleading. This is particularly the case in a patient with parkinsonism and a confirmed dopaminergic deficit on brain imaging. Comorbid pathology did not readily explain these findings, and reports about the L-dopa response in the presence of genetic mutations linked to PD was not sufficient to reach a conclusion. Variation is also seen in motor complications, both time to onset and severity, but their development is not inevitable in definite PD.

To further understand the causes of variation in the L-dopa motor response, and to better link the findings in early disease (relating to motor improvement) with the later development of motor complications, analysis of other modifying clinical and genetic factors is required.

Chapter 3: Relationship of motor complications and drug treatment over time

3.1 Introduction

Motor complications are debilitating drug-related side effects in PD patients and are clinically difficult to address (section 1.1.3). Many studies have investigated motor complications in relation to drug treatment in PD, but those studies have shown variability. This chapter briefly reviews the history of antiparkinsonian treatments, and the initial recognition of motor complications, along with the results of comparative drug trials. A novel analysis of the findings from two large observational clinical studies of PD offers an excellent opportunity to examine this, in large patient numbers, and across different healthcare settings. In addition, they give an opportunity to explore the relationship of motor complications with other disease factors, including the degree of motor control from lower dose treatment.

Motor complications: a general perspective

Motor complications like motor fluctuations, dyskinesia, and off dystonia are well-recognised in clinical practice and among PD patients on dopamine replacement therapy. However, as L-dopa is still the most effective antiparkinsonian treatment, most patients tolerate those complications in exchange for a certain period with improved motor function, which would not be achieved with other drug treatments.

The discovery of L-dopa is relatively recent, and it remains the foundation of the range of antiparkinsonian medications that are also approved and still used for the symptomatic treatment of PD. Those other treatment options can be categorised into anticholinergics, dopamine agonists (including apomorphine), amantadine, and enzyme inhibitors like MAO-B, COMT, and DDC. Their clinical use and benefit are now briefly reviewed.

History of antiparkinsonian medication

From 1945, **anticholinergics** were used to treat PD symptoms. Anticholinergics work with a different mechanism than dopamine replacement, as they block the relative overactivity of the neurotransmitter acetylcholine (Brocks, 1999). Acetylcholine is primarily involved in muscle contraction. Blocking this neurotransmitter would cause healthy people to experience muscle weakness, whereas in PD patients it can reduce involuntary muscular movements like tremor (Schrag et al., 1999).

Shortly after, the first **dopamine agonist** apomorphine was tested in a clinical study that reported a short but marked improvement in PD patients (Schwab et al., 1951). Similarly to L-dopa, dopamine agonists also affect dopamine transmission. They mimic the action of dopamine and bind to postsynaptic dopaminergic receptors, increasing the synthesis of dopamine. Other dopamine agonists like bromocriptine, cabergoline, pergolide, lisuride, pramipexole, and ropinirole followed.

The discovery of L-dopa and its promising first intravenous administration in humans (Birkmayer and Hornykiewicz, 1961) resulted in first attempts to administer the drug orally. However, oral L-dopa administration was not as effective (McGeer and Zeldowicz, 1964) and had to be improved. This resulted in the introduction of DDC inhibitors. In combination with L-dopa they led to a "prolonged efficacy and better tolerability" compared to L-dopa monotherapy (Bartholini et al., 1967, Pletscher and DaPrada, 1993). This effect was achieved by preventing peripheral dopa decarboxylation, thereby increasing the bioavailability of dopamine that is derived from L-dopa in the CNS compartment. Other enzyme inhibitors like **MAO-B** were tested alone or in combination with L-dopa around the same time and provided additive benefit (Bernheimer et al., 1961, Birkmayer and Hornykiewicz, 1961, Birkmayer and Hornykiewicz, 1962, Birkmayer and Hornykiewicz, 1964).

Amantadine is an antiviral drug that has been used since 1969 (Schwab et al., 1969). It was initially used to treat influenza and was coincidentally found to improve PD symptoms. Its exact mechanism of action is unclear, but it increases dopamine synthesis, blocks the reuptake of dopamine and noradrenaline, and blocks N-methyl D-aspartate (NMDA) (Bailey and Stone, 1975). It can restore the ability for voluntary movement in people with severe akinesia, reduce rigidity, and sometimes also improve rest tremor (Kornhuber et al., 1995).

In 1971, the first studies of a combination therapy of L-dopa and **COMT** inhibitors were undertaken but results were not satisfactory (Reches and Fahn, 1984). Later, new inhibitors were developed and notably improved and prolonged the L-dopa effect (Kaakkola et al., 1994, Mannisto and Kaakkola, 1990).

First occurrence of motor complications

The first trial of intravenous L-dopa administration in 1961 showed 'miraculous' results (Birkmayer and Hornykiewicz, 1961). In hope for a similar outcome but more easily accessible administration, oral forms of L-dopa were administered shortly thereafter. However, oral L-dopa did not show the same efficacy and it was even questioned if it was useful at all (McGeer and Zeldowicz, 1964). In addition to those observations, adverse events like nausea, light-headedness, and weakness were discovered (Cotzias et al., 1967, McGeer and Zeldowicz, 1964).

In 1967, Birkmayer and colleagues showed a "prolonged efficacy and better tolerability" of L-dopa when it was combined with DDC inhibitors (Pletscher and DaPrada, 1993). Another study also saw positive results in the combination of L-dopa with DDC inhibitors, and instead of administering a high dose right away, it described a slow increase of the L-dopa dose. This was a successful strategy to limit adverse effects and is still used today (Cotzias et al., 1969).

Despite the success in minimising systemic side effects, the same treatment approach led to the manifestation of other adverse events affecting the motor function: motor complications. First reports described "involuntary movements ranging from fleeting to severe" (Cotzias et al., 1969). Today we know that this was the first description of **dyskinesia**. Early on, it was already clear that dyskinesia was dose-dependent and occurred after 3 weeks of L-dopa monotherapy (Calne et al., 1971). The same study reported the dystonic presentation as part of peak-dose dyskinesia and was therefore the first description of **dystonia** in relation to PD treatment (Calne et al., 1971). Only in 1974, clinicians became increasingly aware of the terms "on-off" (Fahn, 1974, Sweet and McDowell, 1974, Yahr, 1974), "wearing-off" (Fahn, 1976), and "end-of-dose" (Marsden and Parkes, 1976) to describe the different stages of plasma level **fluctuation**.

Early studies investigated motor complications using small numbers of patients over short periods of time, and outdated treatment schedules. With more studies conducted, such treatment schedules and strategies have changed.

Early administration of **dopamine agonists** instead of L-dopa helped to delay the onset of motor fluctuations (especially "wearing-off") and dyskinesia. After 4 years follow-up of a de novo PD cohort (n=526), the Pradivel and L-dopa (PRADO) study concluded that a partial substitution of L-dopa with bromocriptine prevented the development of

L-dopa induced side effects, compared to L-dopa monotherapy (Przuntek et al., 1996). Other studies reported less dyskinesia and dystonia, and a lower daily dose of L-dopa with the same bromocriptine and L-dopa combination therapy (Rinne, 1987, Moreau et al., 2015). Another study followed up 69 patients for 10 years, and a comparison of ropinirole vs L-dopa showed a lower incidence of dyskinesia and moderate "wearing-off". The study however, stressed that both treatment options are useful in early PD (Hauser et al., 2007).

The prescription of **MAO-B inhibitors** like selegiline in combination with the standard treatment of L-dopa and DDC inhibitors was successful in the reduction of motor fluctuations, especially "wearing-off" (Golbe et al., 1988, Parkinson Study, 2005) and a shortening of "off" time (Rascol et al., 2005). A 5-year follow-up of 520 PD cases compared the effect of L-dopa with DDC inhibitor vs L-dopa with DDC inhibitor and selegiline. The study reported no clinical benefit of the combination therapy with selegiline over L-dopa only (Lees, 1995).

The **COMT** inhibitors tolcapone and entacapone were also shown to significantly increase "on" periods and therefore the L-dopa response (Davis et al., 1995, Kurth et al., 1997, Limousin et al., 1993, Merello et al., 1994, Ruottinen and Rinne, 1996, Ruottinen and Rinne, 1996). Some studies even saw a reduction of the L-dopa dose needed (Kurth et al., 1997, Ruottinen and Rinne, 1996, Ruottinen and Rinne, 1996), but some patients in turn reported more dyskinetic movements after treatment with entacapone (Kaakkola et al., 1994).

As problems with **L-dopa** treatment are so common, different ways of drug delivery have also been studied (section 1.1.3). A randomised 5-year study compared immediate-release vs controlled-release L-dopa in 380 early PD cases. However, the study concluded that there was no significant difference in the prevalence of motor complications between immediate- and controlled-release formulations (Koller et al., 1999).

Earlier reports of motor complications from L-dopa generally suggested that half of the patients develop problems at 5 years and around 90% at 10 years of disease duration. This has had an effect on the reputation of what began as a so-called miracle drug (Fahn, 2006). However, clinical practice has evolved with the use of lower doses of L-dopa in more recent years, such that dose-related motor complications would be expected to be less common. Although some longitudinal approaches in the

investigation of motor complications have been undertaken (Kim et al., 2020, Kelly et al., 2019, Clissold et al., 2006), there has been limited study of this so far, largely including small numbers of patients and short-term clinical trials in what is a long-term disease, so the 'true' rates of motor complications and their progression are not well defined.

Accordingly, an analysis of two such large cohort studies, the Tracking Parkinson's (PRoBaND) and PPMI was performed and is now reported.

3.2 Materials and Methods

Data

Data were analysed from the two long-term and observational studies, the Tracking Parkinson's (PRoBaND) study and the Parkinson's Progression Markers Initiative (PPMI). Both studies were introduced in section 1.3. Data from the PPMI study were obtained from https://www.ppmi-info.org/ (download: 03/2018) (Parkinson Progression Marker, 2011). Data from the Tracking Parkinson's study were release 2.0 (April 2020).

Data preparation

The recently diagnosed cohort from Tracking Parkinson's was assessed for MDS UPDRS at 0 (baseline), 18, 36, 54, and 72 months follow-up. The drug-naïve and recently diagnosed cohort in PPMI was assessed for MDS UPDRS at least every 6 months.

Data were categorised in two-year intervals from 0-2, 2-4, 4-6 years of disease duration from diagnosis. To avoid double-counting of patients in the same 2-year interval, only their last entry was used for the analysis. As both studies are ongoing and have dropouts, visits with a follow-up period greater than 6 years were disregarded because of small numbers.

Motor complications presence, severity, and impact

The presence, severity, and impact of motor complications were assessed using the MDS UPDRS 4 items.

Each motor complication was defined as present when the score was greater than 0 from the relevant item: Dyskinesia was assessed from items 4.1 (time spent with dyskinesia), motor fluctuations were assessed from items 4.3 (time spent in the off state), and dystonia was assessed from item 4.6 ("off" dystonia). All those items were rated on a severity scale from 0-4, with 0 being normal or absent, 1 slight, 2 mild, 3 moderate, and 4 severe.

Additionally, the impact of motor fluctuations was assessed with item 4.4 (functional impact of fluctuations), and dyskinesia impact with item 4.2 (functional impact of dyskinesia). Those items as well were rated on a scale from 0-4.

Medication

Antiparkinsonian medication was analysed by drug class: L-dopa (with DDI), dopamine agonists, COMT inhibitors, MAO-B inhibitors, amantadine, and anticholinergics. Medication start and stop dates were used to define which drug was taken at the date of clinical scoring. When drug doses had been changed (which was indicated by multiple records of the same drug with different doses in the same time interval), the last record was used for analysis. When different medication types were started and stopped during the same visit month, only the newly started drugs were included. Drug-naïve cases were excluded from the analysis. The presence or absence of each drug class was assessed using a binary variable (yes/no).

Standard methods were used to define LEDD, which is a combined measure for daily drug dose usage, using L-dopa as a baseline. Other classes of antiparkinsonian medication had a dose adjustment using established conversion factors (see Table 3-1). The LEDD was calculated according to the defined protocol, which was the same for PPMI and Tracking Parkinson's, and excluded anticholinergic medication. The effect of COMT-I was calculated by a correction factor to concomitant L-dopa. In the case of the MAOB-I safinamide, the dose was set to 100mg, regardless of the actual dose, following recent recommendations (Schade et al., 2020).

Table 3-1: List of antiparkinsonian medication and their LEDD conversion factors

Drug Class	Active substance	Release form	Administration	Brand name (examples)	Conversion factor
Amantadine	Amantadine	Immediate	Oral	Symmetrel®	1
COMT Inhibitors	Entacapone	Immediate	Oral	Comtan®	L-dopa dose x1.33
	Opicapone	Immediate	Oral	Ongentys®	L-dopa dose x1.5
Dopamine agonists	Apomorphine	Immediate	Oral	Apokyn®	10
	Bromocriptine	Immediate	Oral	Parlodel®	10
	Piribedil	Immediate or controlled	Oral	Trivastal®	1
	Pramipexole	Immediate	Oral	Mirapex®	100
	Ropinirole	Immediate	Oral	Requip™	20
	Rotigotine	Controlled	Transdermal	Neupro®	30.3
L-dopa	L-dopa + benserazide	Immediate	Oral	Madopar®	1
	L-dopa + benserazide	Controlled	Oral	Madopar® CR	0.7
	L-dopa + carbidopa	Immediate	Oral	Sinemet®	1
	L-dopa + carbidopa	Controlled	Oral	Sinemet® CR, Rytary™	0.7
	L-dopa + carbidopa	Immediate	Intestinal	Duodopa®	1.11
	L-dopa + carbidopa + entacapone	Controlled	Oral	Stalevo®	L-dopa dose x1.33
MAO-B Inhibitors	Rasagiline	Immediate	Oral	Azilect®	100
	Safinamide	Immediate	Oral	Xadago®	Total Dose set to 100mg
	Selegiline	Immediate	Oral	Jumex®	10
	Selegiline	Immediate	Sublingual	Deprenyl™	80

The LEDD was grouped into dose quartiles (Q1-Q4) by using 25th, 50th, and 75th percentile cut-offs for each 2-year interval. The intervals were grouped as follows: Q1 if LEDD was <25th percentile cut-off dose, Q2 if LEDD was ≥25th and <50th, Q3 if LEDD was ≥50th and <75th, and Q4 if LEDD was ≥75th percentile dose.

Summary statistics for the different motor complications were derived for the same 2-year intervals, according to the LEDD quartiles.

Statistical analysis

All data were processed, and summary data were derived using RStudio (version 1.2.1335, RStudio, Inc). Normally distributed data were presented as mean and SD, and skewed data variables were shown as median and interquartile range.

Longitudinal analyses were performed to test if time affects the number of patients with motor complications or on a specific drug within each study. Generalised Estimating Equations (GEE) for longitudinal and clustered data were fitted to compare the patient counts over time, using the R packages 'gee' and 'geepack'. In this logistic GEE approach, the categorical time variable (0-2, 2-4, 4-6) was tested as the predictor variable for the binary outcome variable (yes/no) for the presence of each motor complication, and drug class usage. Model estimates were reported as p-value, odds ratio and 95% confidence intervals. A similar but linear approach was used for LEDD changes over time, reporting beta coefficient, standard error, and multiple R-squared.

Counts of cases with motor complications or on a specific drug were compared with the chi-square test for large data sets at each time point. As a simple follow-up measure, Bonferroni correction was performed for multiple comparisons.

Normality of residuals was tested to detect any outliers or influential points. All p-values were 2-tailed, and hypothesis testing was conducted at 5% statistical significance.

3.3 Results

The Tracking Parkinson's study enrolled 2000 patients in their recent-onset cohort, with 64.9% being male. The mean age at diagnosis was 66.2 (SD 9.3) years, and the mean disease duration was 1.3 (SD 0.9) years at study enrolment. Study drop-outs by 7 years after enrolment were 1194 (60%), consisting of 142 (12% of 1194) who died, and 1052 (88% of 1194) did not continue due for a range of reasons (e.g. intercurrent illness, travel problems, increased disease severity).

PPMI enrolled 423 patients (65.5% male) in the recent onset cohort with a mean age at diagnosis of 61.1 (SD 9.7) and disease duration of 0.5 (SD 0.5) years at study enrolment. At baseline, in Tracking Parkinson's 196 (9.8%) patients were drug naïve, whereas in PPMI per study entry criteria no patient has received any antiparkinsonian medication before enrolment, so that all cases were drug naïve. Study dropouts were reported as 17% of patients who did not continue after year 3, there were no reports on deaths (Hogue et al., 2018).

Only patients with a completed MDS UPDRS 4 visit and prescribed drug treatment were then used for further analysis. In Tracking, 449 (10%) records at any time interval were excluded because of incomplete or missing MDS UPDRS 4 assessments, and 202 (5%) were excluded because patients were not on prescribed medication. In PPMI, 163 (17%) records at any time point were excluded because of incomplete or missing MDS UPDRS 4 assessments, and 21 (2%) records were excluded because the patients were not on prescribed medication.

1174 (58.7%) patients from Tracking vs 233 (55.1%) patients from PPMI were included in the 0-2 year disease duration group, 1550 (77.5%) vs 356 (84.2%) with a disease duration of 2-4 years, and 1059 (53.0%) vs 350 (82.7%) had a disease duration of 4-6 years (Table 3-2).

Table 3-2: Demographics and summary data on motor complications in two large cohort studies.

	Tracking Parkinson's	**PPMI**
Demographics		
Number of cases enrolled, n	2000	423
Gender (male), n (%)	1298 (64.9)	277 (65.5)
Age at diagnosis, mean (SD)	66.2 (9.3)	61.1 (9.7)

	Tracking Parkinson's	PPMI
Disease duration at enrolment, mean (SD)	1.3 (0.9)	0.5 (0.5)
0-2 years after diagnosis		
Number of cases, n (%)	1174 (58.7)	233 (55.1)
Motor fluctuations, n (%)	222 (18.9)	23 (9.9)
Slight	200 (90.1)	20 (87.1)
Mild	16 (7.2)	1 (4.3)
Moderate	3 (1.4)	1 (4.3)
Severe	3 (1.4)	1 (4.3)
Dyskinesia, n (%)	49 (4.2)	5 (2.2)
Slight	40 (81.6)	2 (40.0)
Mild	6 (12.2)	2 (40.0)
Moderate	2 (4.1)	0 (0.0)
Severe	1 (2.0)	1 (20.0)
Dystonia, n (%)	73 (6.2)	13 (5.6)
Slight	53 (72.6)	12 (92.3)
Mild	12 (16.4)	1 (7.7)
Moderate	3 (4.1)	0 (0.0)
Severe	5 (6.9)	0 (0.0)
LEDD, median (IQR)	300.0 (160.0, 400.0)	260.0 (150.0, 400.0)
L-dopa, n (%)	842 (71.7)	91 (39.1)
L-dopa naïve, n (%)	332 (28.3)	142 (60.9)
Dopamine agonist, n (%)	355 (30.2)	106 (45.5)
COMT-I, n (%)	33 (2.8)	0 (0.0)
MAOB-I, n (%)	321 (27.3)	109 (46.8)
2-4 years after diagnosis		
Number of cases, n (%)	1550 (77.5)	356 (84.2)
Motor fluctuations, n (%)	396 (25.5)	76 (21.3)
Slight	350 (88.4)	52 (68.4)
Mild	37 (9.3)	14 (18.4)
Moderate	5 (1.3)	6 (7.9)
Severe	4 (1.0)	4 (5.3)
Dyskinesia, n (%)	136 (8.8)	33 (9.3)
Slight	103 (75.7)	26 (78.8)
Mild	18 (13.2)	6 (18.2)
Moderate	8 (5.9)	1 (3.0)
Severe	7 (5.2)	0 (0.0)
Dystonia, n (%)	151 (9.7)	38 (10.7)
Slight	116 (76.8)	30 (78.9)
	9 (6.0)	6 (15.8)

	Tracking Parkinson's	PPMI
Mild	13 (8.6)	1 (2.6)
Moderate	13 (8.6)	1 (2.6)
Severe		
LEDD, median (IQR)	400.0 (300.0, 574.0)	400.0 (300.0, 600.0)
L-dopa, n (%)	1298 (83.7)	242 (68.0)
L-dopa naïve, n (%)	252 (16.3)	114 (32.0)
Dopamine agonist, n (%)	583 (37.6)	160 (44.9)
COMT-I, n (%)	126 (8.1)	5 (1.4)
MAOB-I, n (%)	495 (31.9)	169 (47.5)
4-6 years after diagnosis		
Number of cases, n (%)	1059 (53.0)	350 (82.7)
Motor fluctuations, n (%)	389 (36.7)	139 (39.7)
Slight	326 (83.8)	109 (78.4)
Mild	49 (12.6)	27 (19.4)
Moderate	9 (2.3)	2 (1.4)
Severe	5 (1.3)	1 (0.7)
Dyskinesia, n (%)	198 (18.7)	70 (20.0)
Slight	131 (66.2)	49 (70.0)
Mild	25 (12.6)	13 (18.6)
Moderate	14 (7.1)	5 (7.1)
Severe	28 (14.1)	3 (4.3)
Dystonia, n (%)	162 (15.3)	57 (16.3)
Slight	118 (72.8)	40 (70.2)
Mild	25 (15.4)	11 (19.3)
Moderate	7 (4.3)	1 (1.8)
Severe	12 (7.4)	5 (8.8)
LEDD, median (IQR)	550.0 (400.0, 750.0)	523.0 (385.0, 800.0)
L-dopa, n (%)	990 (93.5)	292 (83.4)
L-dopa naïve, n (%)	69 (6.5)	58 (16.6)
Dopamine agonist, n (%)	485 (45.8)	150 (42.9)
COMT-I, n (%)	148 (14.0)	18 (5.1)
MAOB-I, n (%)	373 (35.2)	149 (42.6)

3.3.1 Prevalence of motor complications

The number of patients affected by **motor fluctuations** increased in a dose-dependent manner with longer disease duration in both studies. Fluctuations were significantly more common in Tracking Parkinson's compared to PPMI ($p<0.001$). Changes in prevalence (Figure 3-1, Table 3-2) and severity (Figure 3-4) over time were investigated.

At 0-2 years disease duration, 222 (18.9%) of Tracking Parkinson's patients vs 23 (9.9%) of PPMI patients reported motor fluctuations. The highest proportion of motor fluctuations was in the highest (4^{th}) quartile of antiparkinsonian medication doses: Tracking 27.0% and PPMI 15.4%. The LEDD for this quartile was median 470mg (IQR 400-600) in Tracking, and 510mg (IQR 500-588) in PPMI. Most motor fluctuations were slight at this 0-2-year time interval: 200 (90.1%) for Tracking vs 20 (87.0%) for PPMI. The more severe categories were: mild in 16 (7.2%) Tracking vs 1 (4.4%) PPMI, moderate in 3 (1.4%) vs 1 (4.4%), and severe in 3 (1.4%) vs 1 (4.4%).

At 2-4 years disease duration, motor fluctuations were more common: 396 (25.5%, $p<0.001$) in Tracking Parkinson's vs 76 (21.3%, $p<0.001$) in PPMI. Again, the highest dose quartile had the most fluctuations: 41.5% in Tracking vs 35.1% in PPMI; the LEDD for this quartile was 757mg (IQR 640-940) vs 800mg (IQR 633-1209). 350 (88.4%) vs 52 (68.4%) showed slight, 37 (9.3%) vs 14 (18.4%) mild, 5 (1.3%) vs 6 (7.9%) moderate, and 4 (1.0%) vs 4 (5.3%) had severe fluctuations.

At 4-6 years study duration, motor fluctuations were even more common: 389 (36.7%, $p<0.001$) vs 139 (39.7%, $p<0.001$). Once again, the highest dose quartile had the most fluctuations: 53.0% vs 51.1%, at an LEDD of 932 (IQR 800-1197) vs 964 (IQR 860-1200). Most cases had slight to mild fluctuations: slight 326 (83.8%) vs 109 (78.4%) and mild 49 (12.6%) vs 27 (19.4%). Under 5% had moderate or severe fluctuations: moderate 9 (2.3%) vs 2 (1.4%), severe 5 (1.3%) vs 1 (0.7%) (Figure 3-1 to Figure 3-4).

Dyskinesia generally affected fewer cases compared to motor fluctuations, but the increase over time, and with dose, was still largely observed. Dyskinesia was significantly more common in Tracking Parkinson's ($p=0.02$).

At 0-2 years, dyskinesia affected 49 cases (4.2%) in Tracking vs 5 (2.2%) in PPMI. The highest prevalence was in the highest (4^{th}) quartile of LEDD for Tracking Parkinson's

(7.0%) at a dose of 520mg (IQR 400-695), while in PPMI, dyskinesia was most likely in the second highest (3rd) quartile of LEDD (5.1% of cases) at a dose of 300mg (IQR 300-300). Overall, there was slight dyskinesia in 40 (81.6%) vs 2 (40.0%), mild dyskinesia in 6 (12.2%) vs 2 (40.0%), moderate dyskinesia in 2 (4.1%) vs 1 (20.0%), and severe dyskinesia in 1 (2.0%) vs 0 (0.0%).

At 2-4 years, 136 (8.8%, p<0.001) vs 33 (9.3%, p=0.003) patients had dyskinesia. Severity was slight in 103 (75.7%) vs 26 (78.8%%), mild in 18 (13.2%) vs 6 (18.2%), moderate in 8 (5.9%) vs 1 (3.0%), and severe in 7 (5.2%) vs 0 (0.0%). Dyskinesia was most common in the highest dose quartile: 18.0% vs 14.4% at an LEDD of 750mg (670-938) vs 775mg (633-1180).

At 4-6 years, the prevalence of dyskinesia was at its highest compared to the previous time interval: 198 (18.7%, p<0.001) vs 70 (20.0%, p<0.001) cases. Most cases remained slight to mild: 131 (66.2%) vs 49 (70.0%) were slight, and 25 (12.6%) vs 13 (18.6%) mild. However, dyskinesia was more likely to be graded moderate or severe than the comparable figures for motor fluctuations: 14 (7.1%) vs 5 (7.1%) moderate; 28 (14.1%) vs 3 (4.3%) severe. The highest dose quartile was associated with the largest proportion of cases in Tracking Parkinson's (32.7%) whereas PPMI dyskinesia prevalence was highest in the 3rd dose quartile (26.5%); the LEDD for this group was 932 (825-1210) vs 684 (600-740) (Figure 3-1 to Figure 3-4).

Off dystonia slowly increased with disease duration, but there was no clear dose relationship. There was no statistically significant difference in the prevalence of dystonia between the 2 studies (p=0.09).

At 0-2 years, 73 (6.2%) reported dystonia in the Tracking Parkinson's study vs 13 (5.6%) in PPMI. The severity slight in 53 (72.6%) vs 12 (92.3%), mild in 12 (16.4%) vs 1 (7.7%), moderate 3 (4.1%) vs 0 (0.0%), and severe in 5 (6.9%) vs 0 (0.0%). The highest number of cases was in the highest dose quartile in Tracking Parkinson's (9.9%), whereas PPMI reported a peak of 8.5% in Q3 (LEDD 478, IQR 400-600 vs 300, IQR 300-300).

At 2-4 years, dystonia was slightly more common: 151 (9.7%, p<0.001) vs 38 (10.7%, p=0.03) of cases. Of those, 116 (76.8%) vs 30 (78.9%) had slight, 9 (6.0%) vs 6 (15.8%) mild, 13 (8.6%) vs 1 (2.6%) moderate, and 13 (8.6%) vs 1 (2.6%) had severe

dystonia. The highest drug dose quartile had the greatest proportion of cases with dystonia (14.4% vs 20.6%) at an LEDD of 899mg (IQR 745-1101) vs 780 (IQR 630-1053).

At 4-6 years, dystonia was present in 162 (15.3%, p<0.0001) vs 57 (16.3%, p=0.01). 118 (72.8%) vs 40 (70.2%) were slight, 25 (15.4%) vs 11 (19.3%) mild, 7 (4.3%) vs 1 (1.8%) moderate, and 12 (7.4%) vs 5 (8.8%) severe. Most cases were associated with the highest drug dose (20.3% vs 22.8%) at a LEDD of 1040 (860 – 1304) vs 940 (875-1200) (Figure 3-1 to Figure 3-4).

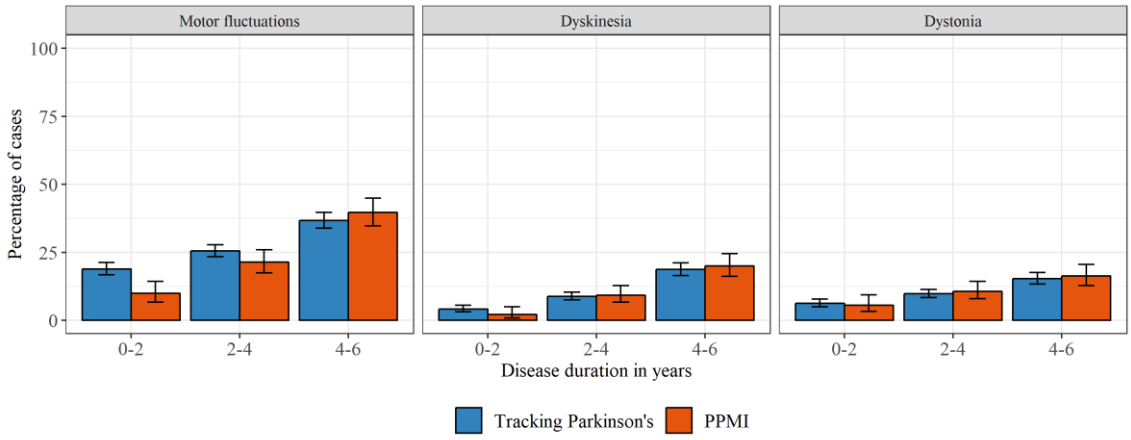

Figure 3-1: Prevalence of motor complications over time in two large cohort studies. Motor fluctuations were the most common, and dystonia the least common. There was an increase in motor complications over time, and findings were largely similar across the two studies. 95% confidence intervals of proportions are based on sample sizes.

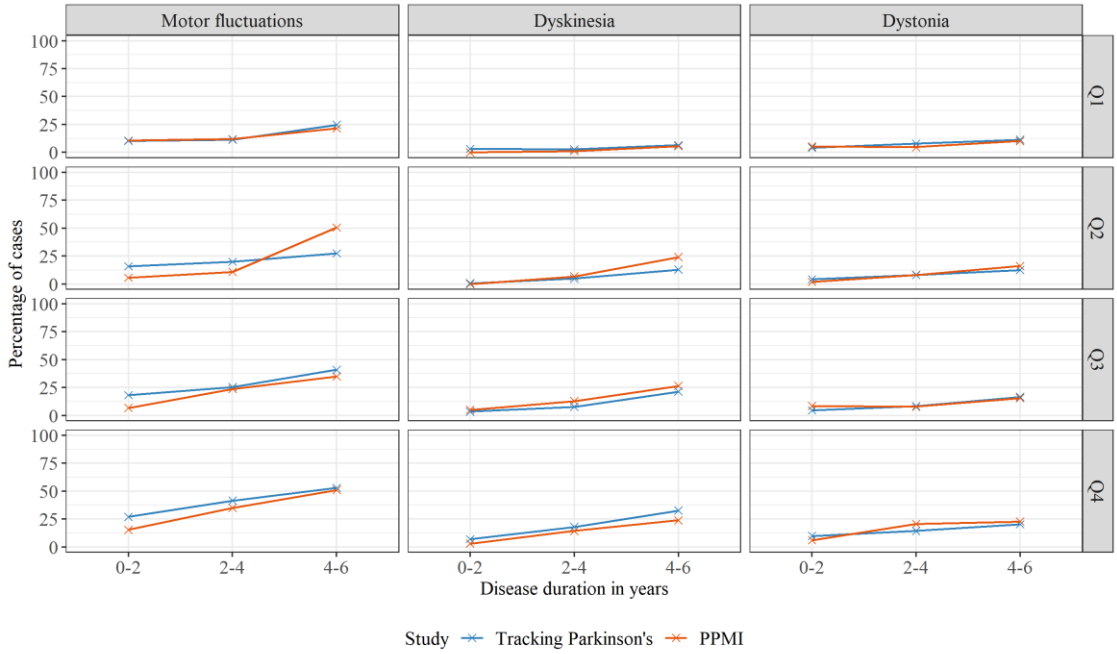

Figure 3-2: Motor complications according to antiparkinsonian drug doses and time, in two large cohort studies.

The daily dose of antiparkinsonian medication, expressed as LEDD, is grouped into quartiles from lowest (Q1) to highest (Q4) dose. The prevalence of motor fluctuations had a striking dose relationship, but a similar effect was observed for both dyskinesia and dystonia. Findings were similar across the two studies.

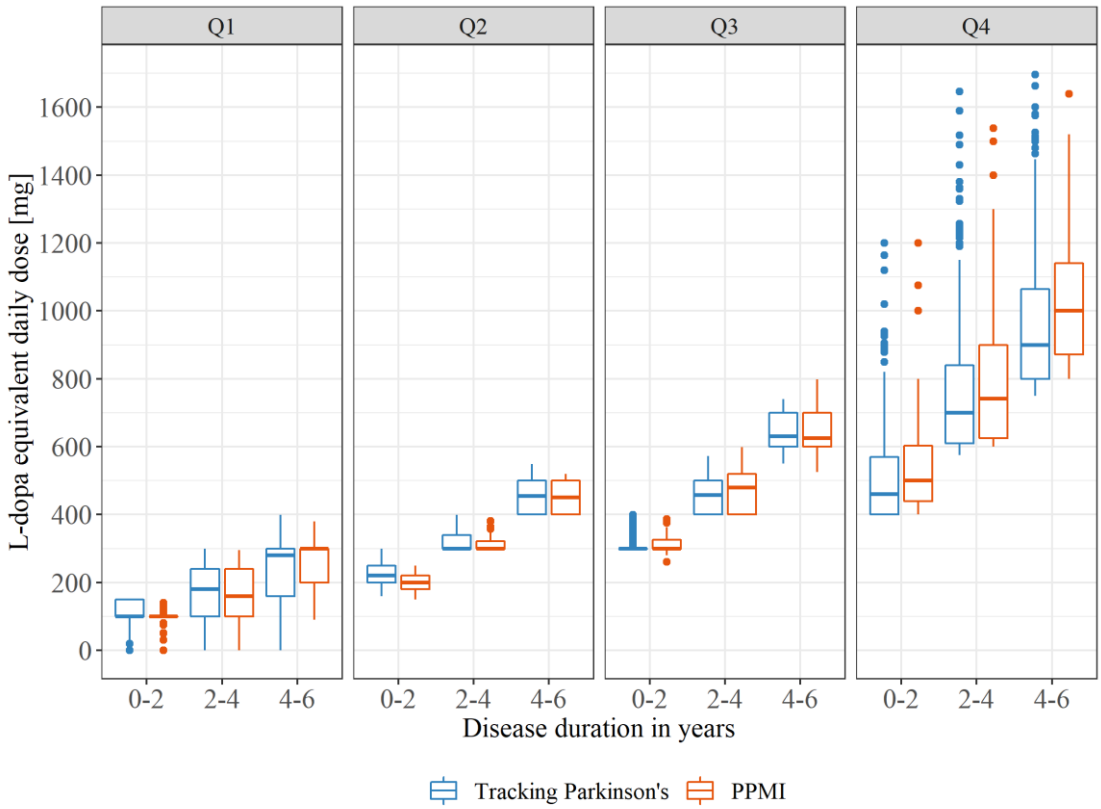

Figure 3-3: Antiparkinsonian medication doses (expressed as L-dopa equivalent units) in two cohort studies.

Dose levels are shown from lowest to highest quartiles (Q1 through Q4). Doses increased with longer disease duration. A subset of cases in the highest dose quartile had particularly high dose levels, represented by dots above the boxes. Data are median and interquartile range (box), minimum and maximum value (whiskers), and outliers (dots).

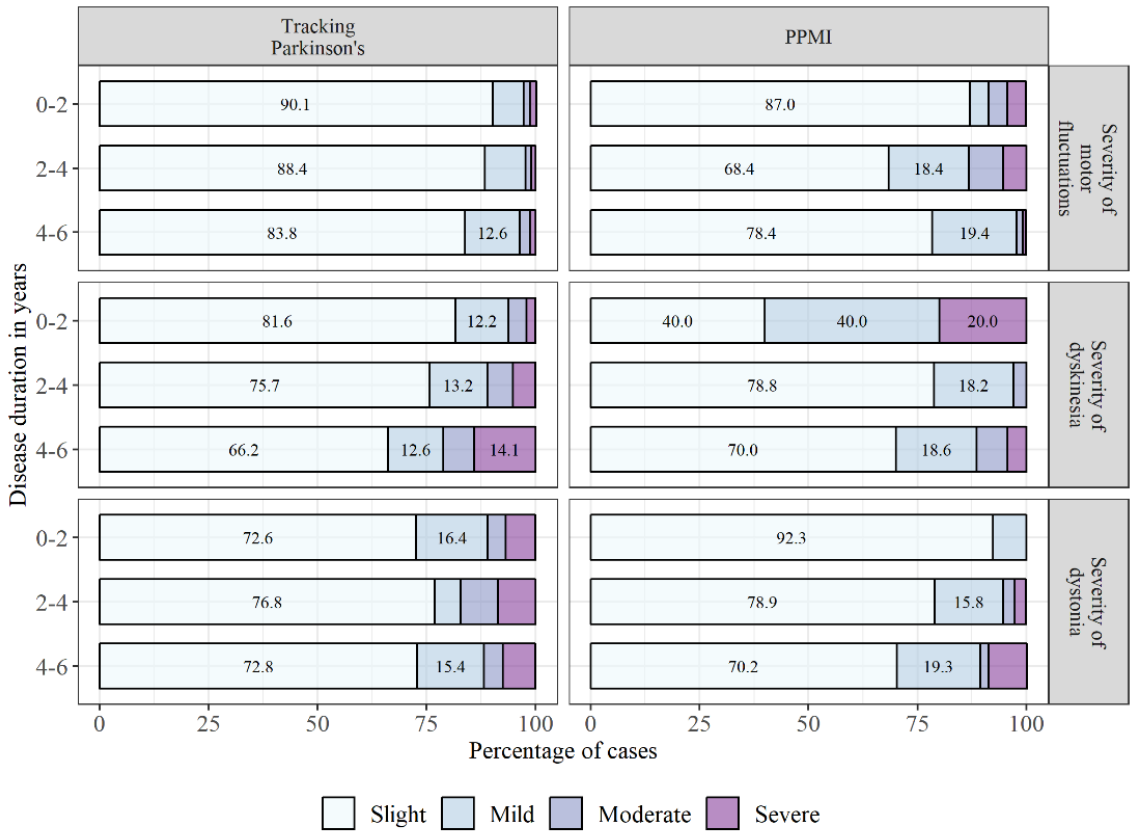

Figure 3-4: Severity of motor complications over time in two large cohort studies. The severity of each of the three main motor complications, when these were present, is shown. Motor complications were mostly slight initially, but greater grades of severity were observed with increasing disease duration in both studies. Values of less than 10% are not labelled.

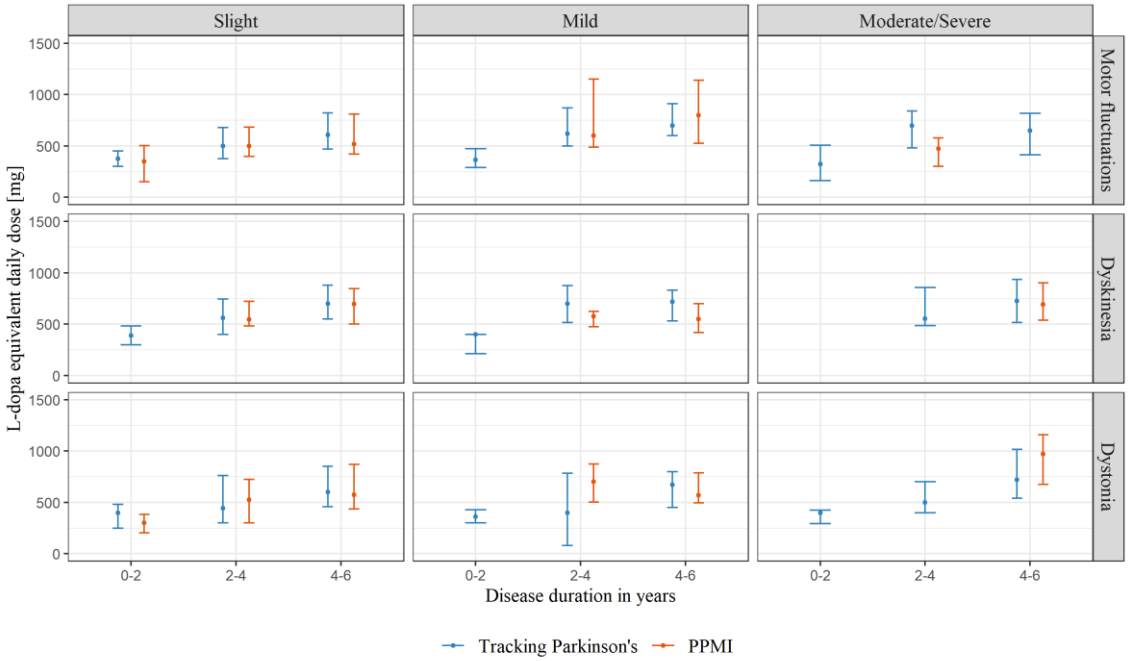

Figure 3-5: Severity of motor complications in comparison to antiparkinsonian medication doses in two large cohort studies.

Dose increases were observed over time within the slight and mild severity categories, indicating that dose increases are not necessarily associated with increasingly severe motor complications, at least in some patients. However, data were limited for moderate and severe motor complications, due to small numbers, preventing more substantial conclusions (groups of less than 5 cases omitted for clarity).

3.3.2 Impact of motor complications

Besides the presence and severity of motor complications, it is important to assess the impact those events have on everyday life in terms of activities and social interaction. The MDS UPDRS 4 assesses the impact for each of motor fluctuations and dyskinesia in a very similar four-level grading system, from low to high (no impact, slight, mild, moderate, and severe impact). All patients assessed for complication severity were also assessed for impact.

Motor fluctuations had a greater impact on the patients' daily life, than did dyskinesia, and this increased over time (Figure 3-6).

At 0-2 years, data were from 222 patients in Tracking Parkinson's vs 23 in PPMI. 141 (63.5 %) vs 9 (39.1%) categorised it as slight, 28 (12.6%) vs 2 (8.7%) as mild, 12 (5.4%) vs 1 (4.3%) was moderate, and 1 (0.5%) vs 0 (0.0%) were severe. Despite experiencing motor fluctuations, 40 (18.0%) vs 11 (47.8%) did not report an impact of fluctuations on their daily activities**.**

At 2-4 years, out of 396 vs 76 people, 220 (55.6%) vs 39 (51.3%) experienced a slight, 49 (12.4%) vs 9 (11.8%) mild, 48 (12.1%) vs 5 (6.6%) moderate, and 9 (2.3%) vs 0 (0.0%) severe impact on their daily lives. 70 (17.7%) vs 23 (30.3%) were not further impacted by motor fluctuations.

At 4-6 years, 389 vs 139 people graded the impact of motor fluctuations, with 192 (49.4%) vs 70 (50.4%) slight, 69 (17.7%) vs 14 (10.1%) mild, 63 (16.2%) vs 20 (14.4%) moderate, and 15 (3.9%) vs 1 (0.7%) severe. 50 (12.9%) vs 34 (24.5%) did not experience any inability with motor fluctuations.

The impact **dyskinesia** had on daily functions was overall lower than motor fluctuations, but it also increased over time (Figure 3-6).

At 0-2 years, 49 Tracking Parkinson's patients vs 5 from PPMI were assessed for the impact of dyskinesia on their lives, which was reported as slight in 25 (51.0%) vs 3 (60.0%), and mild in 5 (10.2%) vs 0 (0.0%). 19 (38.8%) vs 2 (40.0%) reported no impact at all.

At 2-4, the impact of dyskinesia was assessed in 136 vs 33 patients, and was slight in 56 (41.2%) vs 9 (27.3%), mild in 9 (6.6%) vs 2 (6.1%), moderate in 6 (4.4%) vs 0 (0.0%), and severe in 1 (0.7%) vs 0 (0.0%) cases. 64 (47.1%) vs 22 (66.7%) were not impacted by dyskinesia.

At 4-6, dyskinesia impact was graded in 198 vs 70, with 78 (39.4%) vs 25 (35.7%) reporting it as slight, 20 (10.1%) vs 2 (2.9%) mild, 12 (6.1%) vs 2 (2.9%) moderate, and 2 (1.0%) vs 0 (0.0%) severe. 86 (43.4%) vs 41 (58.6%) did not report any inability associated with dyskinesia.

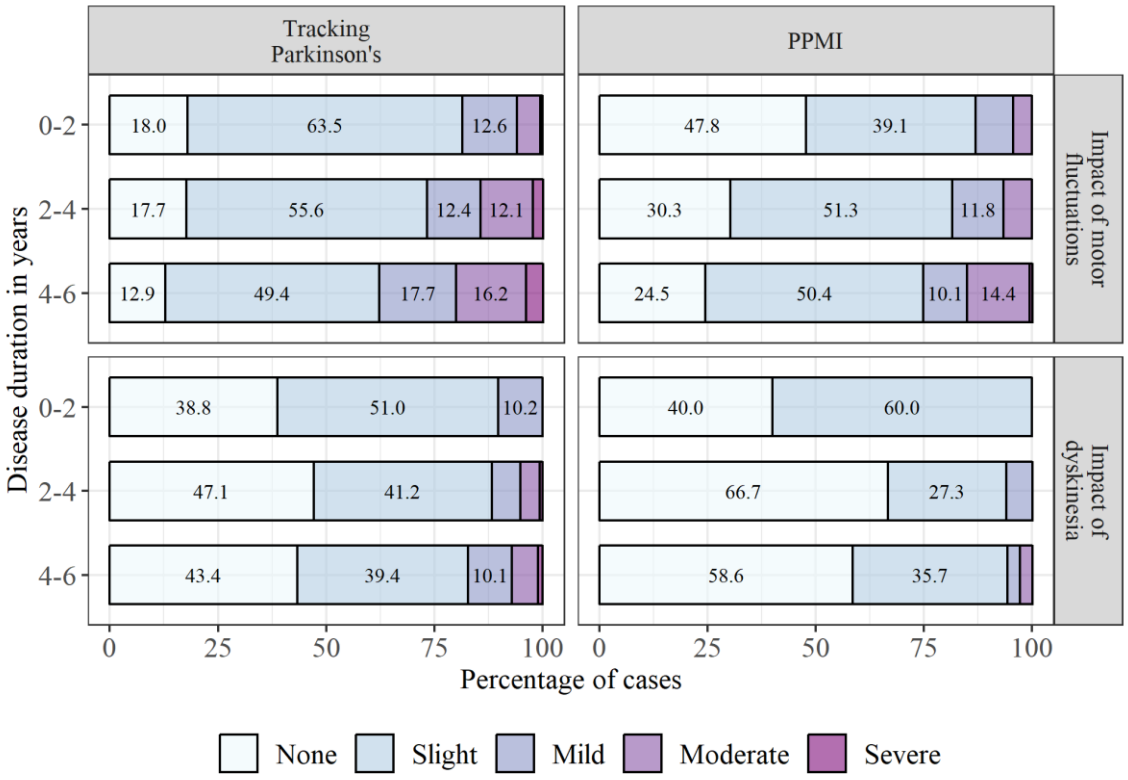

Figure 3-6: Graded impact of motor fluctuations and dyskinesia in both studies over time.

Overall, motor fluctuations caused a greater inability than dyskinesia in both studies, with the majority reporting no impact at all. Impact worsened over time for both motor fluctuations and dyskinesia similarly in both studies.

3.3.3 Drug treatments

Only patients on drug treatment were included in this analysis, and numbers of patients on each antiparkinsonian medication were reported. Use of each drug class was analysed, without consideration of combination treatments (Table 3-2 and Table 3-3, Figure 3-7).

Amantadine were taken by 8 (0.7%) in Tracking Parkinson's vs 27 (11.6%) in PPMI at 0-2 years. Numbers increased over time to 33 (2.1%, OR: 2.92, 95% CI: 1.50-5.69, p=0.002) vs 49 (13.8%) at 2-4, and 36 (3.4%, 1.66, 1.17-2.37, p= 0.005) vs 58 (16.6%) at 4-6 years. The number of patients on amantadine was not significantly different between the two studies (p=0.2) (Table 3-3).

Numbers of patients prescribed **anticholinergics** were low in both studies. At 0-2 years, 17 (1.5%) vs 4 (1.7%) were on anticholinergics, compared to 32 (2.1%,) vs 11 (3.1%), and 14 (1.3%) vs 12 (3.4%) at 4-6 years. Numbers of prescribed anticholinergics were not significantly different between the two studies (p=0.09).

COMT inhibitors were used as follows: At 0-2 years, 33 (2.8%) in Tracking Parkinson's vs 0 (0.0%) in PPMI. At 2-4 years, 126 (8.1%, 3.25, 2.33-4.54p<0.001) vs 5 (1.4%), and 148 (14.0%, 1.84, 1.53-2.20p<0.001) vs 18 (5.1%, p<0.001) at 4-6 years. COMT inhibitors were prescribed significantly more often in the Tracking Parkinson's study compared to PPMI (p=0.02).

Dopamine agonists were commonly prescribed in both studies. At 0-2 years numbers of cases on dopamine agonists were 355 (30.2%) vs 106 (45.5%), 583 (37.6%, 1.26, 1.14-1.38, p<0.001) vs 160 (44.9%) at 2-4 years, and 485 (45.8%, 1.21, 1.11-1.32, p<0.001) vs 150 (42.9%) at 4-6 years. There was no significant difference in the prescription of dopamine agonists between the two studies (p=0.6).

Proportions of patients on **L-dopa** increased significantly in both studies over time, with Tracking Parkinson's having the highest proportion of patients on L-dopa therapy. At 0-2 years, 842 (71.7%) patients in Tracking vs 91 (39.1%) in PPMI were on L-dopa. At 2-4 years, numbers increased to 1298 (83.7%, 2.32, 2.03-2.65, p<0.001) vs 242 (68.0%, 3.42, 2.64-4.43, p<0.001), and 990 (93.5%, 3.26, 2.52-4.21p<0.001) vs 292 (83.4%, 2.49, 1.96-3.17, p<0.001) at 4-6 years of disease duration. Patients on L-dopa treatment were significantly more common in Tracking Parkinson's compared to PPMI (p<0.001).

MAO-B inhibitors were quite common in both studies. At 0-2 years, 321 (27.3%) vs 109 (46.8%) were on MAO-B inhibitors. At 2-4 years, numbers increased to 495 (31.9%, 1.23, 1.11-1.35, p<0.001) vs 169 (47.5%), and 373 (35.2%, 1.16, 1.06-1.26, p<0.001) vs 149 (42.6%, 0.84, 0.73-0.97, p=0.02) at 4-6 years. There was no statistically significant difference in the prescription of MAO-B inhibitors between the two studies (p=0.4).

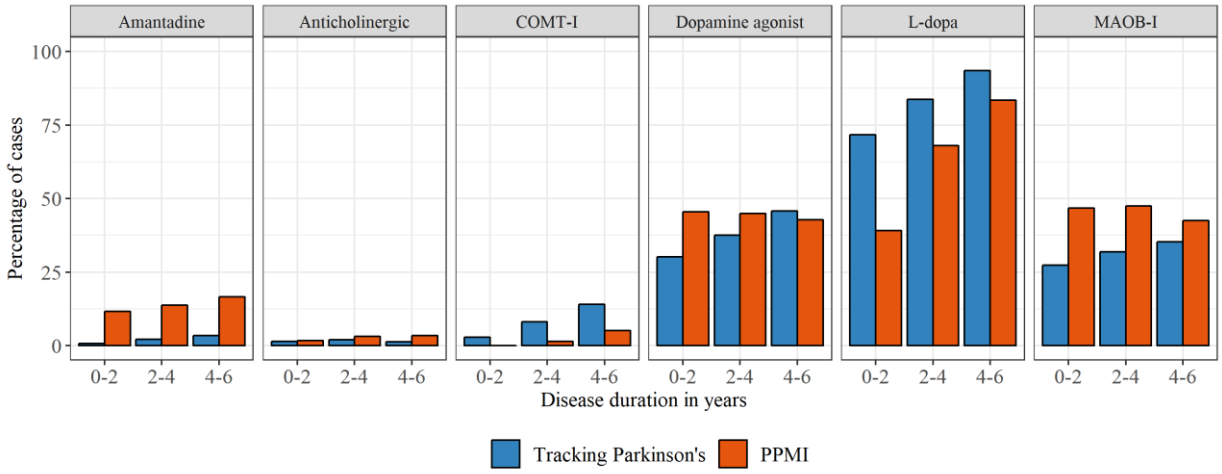

Figure 3-7: Distribution of prescribed antiparkinsonian medication over time.
L-dopa was the mainstay of treatment in both studies, but usage was lower in PPMI, particularly initially, indicating more of an L-dopa delaying strategy through dopamine agonist and MAOB-I usage, in PPMI than in Tracking Parkinson's.

Table 3-3: Output from longitudinal statistical analyses on the prevalence of motor complications and drug dose.

	Tracking Parkinson's[1]		**PPMI[1]**		**Between studies[2]**			
	0-2 to 2-4	2-4 to 4-6	0-2 to 2-4	2-4 to 4-6	χ^2	0-2	2-4	4-6
Motor fluctuations								
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.2	**<0.001**
OR (95% CI)	1.53 (1.29-1.81)	1.74 (1.51-2.01)	2.77 (1.69-4.55)	2.48 (1.87-3.30)				
Dyskinesia								
p-value	**<0.001**	**<0.001**	**0.003**	**<0.001**	**0.02**	0.1	1.0	0.09
OR (95% CI)	2.27 (1.64-3.14)	2.42 (1.98-2.96)	6.82 (1.91-24.33)	2.58 (1.76-3.79)				
Dystonia								
p-value	**<0.001**	**<0.001**	**0.03**	**0.01**	0.09			
OR (95% CI)	1.65 (1.26-2.17)	1.70 (1.39-2.09)	2.02 (1.08-3.79)	1.63 (1.10-2.41)				
LEDD								
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	NA	**0.001**	0.6	1.0
Beta-coefficient (SE, multiple R-squared)	1.48 (0.07, 0.15)	1.39 (0.08, 0.15)	1.81 (0.16, 0.11)	1.66 (0.16, 0.11)				

LEDD adjusted for 100mg/day unit changes.

[1] Logistic General Estimating Equation (GEE) fitted for binary outcome variables; linear GEE fitted for continuous LEDD

[2] Chi-square for testing relationship between studies (χ^2) and post hoc with Bonferroni correction when χ^2 <0.05. Kruskal-Wallis rank sum test applied for comparison of continuous LEDD between studies.

3.4 Discussion

The findings indicate the rates of motor complications that may be expected in patients with Parkinson's, during the first 6 years after diagnosis. The large size of the two longitudinal and observational studies, and their largely comparable findings from entirely different healthcare settings, suggests that these observations are representative of current treatment disease management approaches. Given the evolution of drug treatments and the use of different dose schedules and varied combinations of drug classes used historically, the current findings update the 'benchmark' figures that exist from earlier literature.

General numbers

The number of Parkinson's patients affected by motor complications varies greatly in the literature, ranging from 10-85%, and covering a treatment duration of around 4-13 years (Bjornestad et al., 2016, Chung et al., 2018, Kaiser et al., 2003, Koller et al., 1999, Purcaro et al., 2019). Motor fluctuations and dyskinesia have been the most studied complications, in comparison to dystonia for which data are more limited. In studies with a sample size greater than n=100, motor fluctuations were reported for 5% at up to 2.5 years of treatment (Kadastik-Eerme et al., 2017), 50% of patients at 5 years, around 60% at up to 9 years, and over 80% at 13 years of treatment duration. (Chung et al., 2018, Kaiser et al., 2003, Koller et al., 1999, Purcaro et al., 2019). Dyskinesia was prevalent in 3% with less than 2.5 years treatment duration, 40% at 5 years (Kaiser et al., 2003, Koller et al., 1999), and over 50% at up to 9 years (Purcaro et al., 2019). Dystonia was reported for 30% of patients after 8 years (Kidron and Melamed, 1987), and 48% at 9 years of treatment duration (Luquin et al., 1992).

Impact of time

The duration of treatment is a crucial factor in the development of motor complications, which are reported as early as 6 months into treatment (Fahn and Parkinson Study, 2005), and which persist for decades after starting antiparkinsonian medication (Hauser et al., 2007, Hely et al., 2008).

The Tracking Parkinson's and PPMI studies both reproduced prior observations of an increasing proportion of patients developing motor complications over time. The highest prevalence in both cohorts was for motor fluctuations (compared to dyskinesia and off dystonia) which affected over a third of cases by 4-6 years of disease duration.

This is a lower rate than in other reports: over a follow-up duration of 5 years, a recent observational study reported that 54% had fluctuations (Kim et al., 2020), and another study investigating L-dopa treatment only reported 60% (Purcaro et al., 2019), while other studies reported a prevalence of 43-50% at 5 years disease duration (Bjornestad et al., 2016, Chung et al., 2018, Kaiser et al., 2003). The factors that may explain this difference are discussed below.

Dyskinesia was less common, but had a similarly steep increase over time, ultimately affecting just under 1 in 5 cases at the 4-6-year time point. Compared with other observational studies reporting the LEDD, this finding is similar to one cohort reporting 24.3% at 5 years (Bjornestad et al., 2016), although another study from 2019 reported only 14.5% of cases with dyskinesia at 5 years (Kim et al., 2020). A retrospective cohort analysis showed a much higher prevalence of dyskinesia with 43.2% cases at 4.5 years of treatment in a cohort reporting the L-dopa dose only (Kaiser et al., 2003).

The longer-term prevalence of off dystonia, which showed a very slow increase over time in both the Tracking and PPMI studies, and reached 15-16% by 4-6 years of disease duration, is within the published range of between 10-30% at 4-5 years of treatment duration (Lees, 1995, Rinne, 1987), with the 10% rate coming from an observational study from 2016 (Bjornestad et al., 2016).

The present findings allow for a re-evaluation of the relationship between drug doses, disease duration, and the development and progression of each of the motor complications. This needs to be considered against the evolving background of the general understanding of the relationship between disease or treatment duration, drug doses, and the development of motor complications. These considerations have been influential on treatment approaches for some decades. Many earlier studies concluded that strategies to delay L-dopa treatment, either by leaving the patient drug-naïve for as long as possible or by using alternative drug classes as initial treatment and thereby delaying the start of L-dopa, were beneficial in lowering motor complication rates.

Impact of medication

The strategy to always delay L-dopa treatment was adopted by around a fifth of neurologists treating patients with late-onset PD (>50 years of age at onset) in a 1999 report (Fahn, 1999). Later guidelines suggest starting treatment at the point of functional impairment (Ferreira et al., 2013, NICE, 2017), but the subject has remained

controversial. Partly, this is related to a perception that dyskinesia was the most troublesome of the motor complications, which led to 'L-dopa phobia' in some patients (Kurlan, 2005). The PPMI study shows evidence of an L-dopa delaying strategy: the proportion of patients on L-dopa treatment was significantly lower in the baseline time period, around 4 in 10 cases in PPMI on L-dopa within 2 years of diagnosis, compared to 7 in 10 cases on L-dopa in Tracking Parkinson's for the same time period. Even at 4-6 years after diagnosis, around 10% fewer cases were prescribed L-dopa in PPMI (around 8 in 10 cases) compared to Tracking Parkinson's (around 9 in 10 cases). One possible explanation for different strategies is that the Tracking Parkinson's study (based in the United Kingdom) was much more influenced by the results of the British PD MED study which concluded that overall quality of life during the first 7 years of Parkinson's was greater when L-dopa was used as initial treatment, compared to alternative drug strategies (Group et al., 2014). However, the study design has been criticised as the study was randomised but was open-label and unblinded, which may have limited its impact in other countries, including the PPMI study which was primarily performed in the United States of America. Another interpretation is that even the provisional results of the PD MED (which were published in full in 2014) came too late to influence treatment choices for PPMI, which enrolled from 2010, compared to Tracking Parkinson's, which enrolled from 2012.

While the set-up of the Tracking Parkinson's and PPMI studies are overall similar, being observational studies of recent onset PD with follow-up for at least 8 years, there are some differences. In PPMI, participants could be included with either resting tremor or bradykinesia in combination with rigidity, whereas in Tracking Parkinson's, patients had to exhibit bradykinesia as a component of fulfilling standard clinical diagnostic criteria. Additionally, PPMI did not anticipate their participants to require any antiparkinsonian medication within at least 6 months from baseline. PPMI therefore defined entry parameters for milder, earlier-disease in comparison to Tracking Parkinson's.

The examination of the functional impact of motor fluctuations and dyskinesia in Tracking and PPMI lends support to the concept of motor fluctuations being more troublesome. In both studies, and at all time points, motor fluctuations had greater functional impact than dyskinesia. Therefore, for the first 6 years after diagnosis, 'off' periods have a greater adverse effect on activities of daily living than has dyskinesia. Whether this situation reverses in later years cannot be answered yet from these cohort

studies, as they are ongoing and have insufficient data for later time points. However, more recently available strategies to lower dyskinesia moderate the adverse effects of dyskinesia in eligible patients. Dopamine agonists, COMT-I, and MAOB-I were shown to reduce off time (Pahwa et al., 2007), apomorphine results in less on/off fluctuations, amantadine reduces dyskinesia, and COMT-I in combination with L-dopa improves motor fluctuations (Olanow et al., 2004). More invasive therapies, such as enteral administration of L-dopa and deep brain stimulation (DBS) are both effective for the reduction of dyskinesia, (Liu et al., 2019), so that, at least for some patients, the earlier concerns about dyskinesia being irreversible have been reduced.

Accordingly, the present analysis indicates that motor fluctuations ('off' periods) are more negative in their effect on the patient's condition than dyskinesia, during the first 6 years after diagnosis. At the same time, the results reinforce the far lower prevalence of dyskinesia than the observations made in the earlier era when drug doses were higher than today's. This suggests that there is a largely unrealised but clinically significant problem of the lower dosage approach, which affects patients adversely through having more 'off' time. Other disease factors and patient age should be considered against these findings when constructing the optimum long-term treatment plan for antiparkinsonian drugs. The presence of early markers of cognitive decline, or of comorbidities that affect patient survival, both of which interact with patient age, may point to the need for targeting 'off' time reduction as the priority, and largely ignoring later risks of dyskinesia. Dyskinesia may never emerge in some patients and even if it does, its impact on functional performance is less marked.

Drug Dose

The development of motor complications is strongly influenced by the drug dose administered. The LEDD is a combined measure to account for the dose of different antiparkinsonian medications used. Data from the Tracking Parkinson's and PPMI studies confirmed that a higher dose of combined antiparkinsonian medication shows the increased proportion of patients affected by all the complications: motor fluctuations, dyskinesia, and dystonia (Aquino and Fox, 2015, Kadastik-Eerme et al., 2017, Kelly et al., 2019).

Around a fifth of patients in the highest quartile of drug doses in Tracking Parkinson's and PPMI had motor fluctuations in the 0-2 years disease duration group, with a median LEDD of between 470mg and 510mg. In the ELLDOPA study, patients were

randomised into 3 different doses of L-dopa, of which the highest 600mg L-dopa dose also had the highest number of motor fluctuators with 33% after 0.8 years of treatment (Fahn et al., 2004). The comparability of Tracking and PPMI with the ELLDOPA study is limited to this short period of disease duration. The current work therefore helpfully extends our understanding beyond this constrained time period which was necessitated by the placebo-controlled design of the ELLDOPA study.

Dyskinesia can occur in any fluctuation state, but peak-dose dyskinesia is the most common and is expected to be the main type captured in the current cohort studies, being defined under the relevant questions of the MDS UPDRS scoring system (Goetz et al., 2008, Thanvi et al., 2007). They occur when L-dopa plasma levels are at their highest and depend on the dose administered (Zesiewicz et al., 2007). PPMI and Tracking Parkinson's both had 30% of cases affected by dyskinesia when given the highest dose of a median LEDD of 700-900mg. A 10 year-follow up comparative drug trial reported 78% of patients with dyskinesia on a mean daily dose of 862mg (Hauser et al., 2007). Another study reported 53% on 725-972mg L-dopa only at 5 years (Purcaro et al., 2019). Those studies were in rather younger patients (by around 6 years compared to Tracking and 1 year compared to PPMI), being biased towards participants who could take part in clinical trial research, and it is known that younger patients have a greater risk of motor complications. However, the analysis of the Tracking Parkinson's and PPMI cohorts suggests that the 'true' rate of motor complications is lower than the clinical trial studies suggest. It is important to mention that the current analysis focused on patients on antiparkinsonian medication only. Although there was a proportion of drug-naïve cases in the 0-2 years disease duration groups, we would not expect them to develop treatment-related motor complications, which is why they were not included in the analysis. The results should be viewed with this in mind, as the natural history of Parkinson's obviously includes untreated subjects and doing so would further reduce the overall motor complication rate.

Dystonia is generally an 'off' phenomenon, such as early morning dystonia related to falling dopamine levels due to the overnight drop caused by dose schedules that favour the waking day. However, dystonia can sometimes occur as part of a peak-dose dyskinetic effect. This potentially complicates analyses of the relationship between drug doses and the development of dystonia. In the Tracking Parkinson's and PPMI studies, 15% of patients had off dystonia at 4-6 years and the associations with drug doses were much less clear than for the other motor complications. In another study, 10% of

patients on L-dopa treatment had dystonia on a mean dose of 423mg at 5 years of treatment duration (Schrag and Quinn, 2000). After 8 years of treatment, studies report 30% of affected patients on a range of 500-2250mg of L-dopa (Luquin et al., 1992); and at 9 years, numbers increase to 43% with a mean L-dopa dose of 766.6mg (Kidron and Melamed, 1987). The rate of off dystonia in Tracking and PPMI is much lower than other reports, but those suggest that we will see higher rates later in the disease course, although the dystonia prevalence showed little evolution across the first 6 years.

There is very limited longer-term data to guide further on what will be observed in future years in the currently analysed but ongoing studies. However, the Sydney multicenter study did report for up to 20 years, but by this time the mortality rate was 74%. This study described dyskinesia as being only mild to moderate, which raises questions about the negative effect of motor complications on survival (Healy et al., 2008).

In conclusion, the present analysis of 2 major cohort studies identifies motor fluctuations as having an important adverse effect on patient's abilities, even within the first 6 years after diagnosis. These findings update and extend the results from clinical drug studies, which are necessary on a smaller scale and almost always over shorter time periods. The use of higher antiparkinsonian drug doses may be most beneficial in the older patient, or those with other health problems that are likely to limit remaining lifespan, so that they can have an optimised quality of life. There are 2 possible explanations for the important role of drug treatment in the manifestation of motor complication: Firstly, patients with fluctuations are undertreated, while dyskinetic cases are over-treated, and secondly, people with more severe disease are on higher medication and therefore develop more complications. Therefore, a more in-depth analysis of the association of motor complications with disease and clinical variables follows in the next chapter.

Chapter 4: Motor complications and challenge test responses

4.1 Introduction

A thorough assessment of L-dopa responsiveness does not only require the presence or absence of motor complications, it also must consider other clinical and disease variables, like treatment efficacy (section 1.2.3). Other factors than time and medication dose as shown in chapter 3 can affect motor complication development and therefore L-dopa responsiveness (section 1.2.4). This chapter further investigates predictors of motor fluctuations, dyskinesia, and off dystonia like demographics, disease severity but also non-motor factors like anxiety and depression. Furthermore, it assesses the use of medication challenge tests as a tool in clinical practice for early recognition of complication development in two large cohort studies.

Frequently observed motor complications are motor fluctuations (wearing-off, on-off), dyskinesia, and off dystonia which are recognised as such in the Movement Disorders Society (MDS) Unified Parkinson's disease rating scales (UPDRS) assessment for motor complications (MDS UPDRS 4) (Goetz et al., 2008). These complications usually manifest after years of treatment, although they can also occur after a couple of weeks (Ahlskog and Muenter, 2001, Manson et al., 2012), and numbers of patients affected can vary from 10-85% (Bjornestad et al., 2016, Chung et al., 2018, Kaiser et al., 2003, Koller et al., 1999, Purcaro et al., 2019).

The phenotypic heterogeneity of Parkinson's disease (PD) is well recognised (Szewczyk-Krolikowski et al., 2014), and includes variability in L-dopa responsiveness (Fahn et al., 2004, Hauser et al., 2009). While an excellent (Hughes et al., 1992) or clear and dramatic (Postuma et al., 2015) response to L-dopa is a supportive feature in the diagnostic criteria for idiopathic PD, a less marked response does not rule out the diagnosis of PD (Hughes et al., 1992, Postuma et al., 2015).

Both, the presence of L-dopa induced dyskinesia (LID) and L-dopa responsiveness are part of the supportive diagnostic criteria for a Parkinson's disease diagnosis (Postuma et al., 2015), and therefore crucial for a thorough assessment for responsiveness.

Many studies have shown variation in the response to either an acute L-dopa challenge dose (Hughes et al., 1991, Merello et al., 2011, Merello et al., 2002), or chronic L-dopa therapy (Hauser et al., 2009, Hughes et al., 1992, Hughes et al., 1991, Merello et al., 2002) both in

clinically diagnosed PD (Hauser et al., 2009, Hughes et al., 1992, Hughes et al., 1991, Merello et al., 2002) and in pathologically confirmed cases (Pitz et al., 2020). Clinically, factors like demographics (Hassin-Baer et al., 2011, Ku and Glass, 2010), treatment variables like dose and duration (Ahlskog and Muenter, 2001, Fahn et al., 2004, Luquin et al., 1992), but also genetics (Guin et al., 2017, Sampaio et al., 2018) have been associated with the development of motor complications. Additionally, other motor and non-motor clinical rating scores, like high UPDRS 1 or 2 scores, and even levels of education have been shown to be predictive for motor complication development (Olanow and Schapira, 2013, Warren Olanow et al., 2013), which also suggests the investigation of other rating (sub-)scores but also the existence of combinations of motor complications.

However, the definition of these clinical correlates and the significance of this variation in responsiveness remain unclear. A better and more comprehensive understanding of the manifestation of motor complications could be a useful tool to identify patients at a greater risk of developing such and putting treatment strategies into place early-on.

The aim of the study was to quantify the emergence of motor complications in an early cohort of PD, examine factors known to be associated with motor complications and assess the association of motor complications with the degree of responsiveness to dopamine replacement therapy.

4.2 Materials and Methods

Data

Data were again analysed from the two long-term and observational studies, the Tracking Parkinson's (PRoBaND) study and the Parkinson's Progression Markers Initiative (PPMI). Both studies were introduced in section 1.3. Data from the PPMI study were obtained from https://www.ppmi-info.org/ (download: 03/2018) (Parkinson Progression Marker, 2011). Data from the Tracking Parkinson's study were release 2.0 (April 2020). Patients with a reported change in diagnosis such as other forms of parkinsonism were excluded from the analysis.

Data preparation

Patients from the Tracking Parkinson's study were assessed for dopaminergic replacement challenge testing at the 24 months follow-up visit, whereas PPMI had annual challenge tests. However, to analyse data based on the same disease duration of both cohorts, the 36 months visit was chosen for PPMI. Tracking did not conduct challenge testing and MDS UPDRS assessments at the same visits, so that data on motor complications and other clinical scales were taken from the preceding 18 months visit to match the time points as good as possible.

Challenge testing and motor complications

Challenge testing for dopamine replacement therapy is a measure to test the efficacy of medication by comparing 'off' medication scores to 'on' medication scores. In more detail, patients withheld their medication for 6 hours (12 hours for long-acting drugs) and were assessed for their MDS UPDRS 3 score. Patients then take their usual morning dose and after 1-3 hours undergo another MDS UPDRS 3 assessment. The percentage change from the 'off' score to the 'on' score was derived as follows:

$$\left(\frac{OFF - ON}{OFF}\right) * 100$$

The resulting percentage changes were then grouped into definite and limited responders, according to the established method for the MDS UPDRS 3 scale.

Differences between the two studies were that in PPMI, patients who were on dopamine agonists and/or L-dopa conducted the challenge, whereas in patients enrolled with Tracking the challenge test was performed only if the patient was prescribed L-dopa. . The challenge test dose in PPMI was derived from medication frequency (less or more often than 3 times

daily) and depending on the combination of frequency with immediate or controlled-release drugs. This method was undertaken following clinical practice approaches. Levodopa equivalent daily dose was derived using conversion factors from study documentation (Table 3-1).

The change in MDS UDPRS 3 was dichotomised around a 24.5% improvement, which is equivalent to the 30% change in the UPDRS 3 score (Merello et al., 2011) and defined as 'definite' when improvement was ≥24.5%, and 'limited' when <24.5%.

Prevalence of motor complications was assessed using the same methods as described in 3.2. The same applies for medication usage and the calculation for LEDD.

Treatment duration and drug dose

Treatment duration was derived from the first start date of antiparkinsonian medication until the date of the follow-up visit. Treatment duration until the onset of each motor complication was derived using again the first start date of antiparkinsonian medication until the date of the first report of motor complications on the MDS UPDRS 4 scale which due to the longitudinal set up of both studies, might have been at an earlier time point than the visit chosen for this analysis.

Statistical analysis

Logistic regression models were fitted for the binary outcome variables of presence and absence of each motor complication (motor fluctuations, dyskinesia, and off dystonia), and for definite and limited responders to challenge testing. All models were adjusted for gender, age at diagnosis and disease duration. Statistically significant variables were then used to further adjust the model in a multivariate approach. Multicollinearity was considered and models were adjusted accordingly. Predictor variables were demographics, and scores from MDS UPDRS scales, assessing motor and non-motor function. Model estimates were reported as p-value, odds ratio and 95% confidence intervals. For a better interpretation of the results, odds ratios and confidence intervals were adjusted for a 5-unit increase for MDS UPDRS scores and a 100mg increase for LEDD. All data were processed using RStudio version 1.3.959.

4.3 Results

4.3.1 Cohort demographics

Tracking Parkinson's recruited 2000 patients, of whom 118 cases were excluded because of a change in diagnosis (n=34), they were drug-naïve (n=66), and did not have an MDS UDPRS 4 assessment (n=18), leaving 1501 cases for the assessment of motor complications at the 2-year time point of study duration. A further 567 did not have a challenge test, leaving 934 cases with complete data for the L-dopa challenge (Figure 4-1). The total 1501 cases had a mean age of 65.9 years (SD 9.0) at diagnosis and 68.8 (SD 9.0) years at study entry.

PPMI recruited 423 patients, of whom 95 cases were excluded because of missing data at the chosen time point (n=60), they were drug-naïve (n=19), and did not have an MDS UDPRS 4 assessment (n=16), leaving 328 cases for the assessment of motor complications at the 3-year time point of study duration. A further 149 did not have a challenge test, leaving 179 cases with complete data for the L-dopa challenge (Figure 4-1). These total 328 cases had a mean age of 61.0 years (SD 9.8) at diagnosis and 61.5 (SD 9.8) years at study entry.

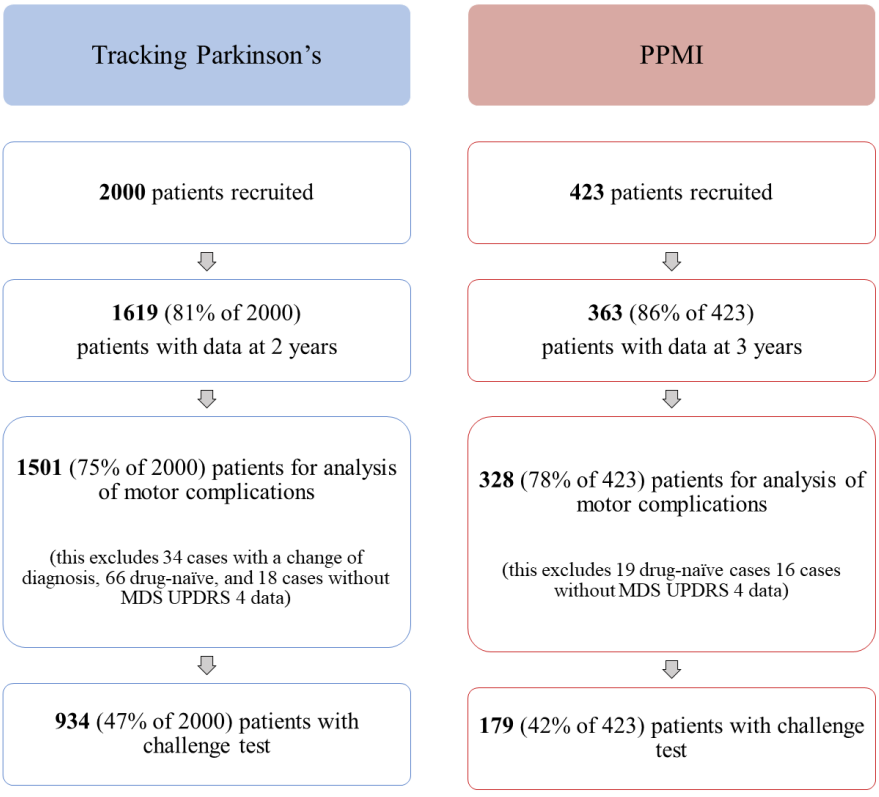

Figure 4-1: Cohort selection of two large clinical studies.
The main analysis was based on patients on antiparkinsonian treatment with data on MDS UPDRS 4 scoring. A subgroup had a reported response to challenge testing.

4.3.2 Motor complications

In the **Tracking Parkinson's** study, motor fluctuations were present in 381 (25.4%) patients after a median time to onset of 3.1 (1.9-4.6) years (Table 4-1 to Table 4-2).

Motor fluctuations were strongly associated with female gender (OR: 0.49, 95% CI: 0.32-0.75, p<0.001), a longer disease duration at study entry (1.39, 1.08-1.81, p=0.01) and assessment (1.42, 1.11-1.83, p=0.006), as well as higher LEDD (1.21, 1.11-1.32, p<0.001), a longer overall treatment duration (1.76, 1.36-2.30, p<0.001), and a shorter treatment duration until the first occurrence of motor fluctuations (0.54, 0.47-0.62, p<0.001). Other clinical variables like higher HADS anxiety score (1.40, 1.03-1.90, p=0.03), and a higher HADS depression score (1.69, 1.15-2.52, p=0.009) were also contributory.

Dyskinesia was present in 121 (8.1%) cases after a median time to onset of 4.2 (2.8-5.6) years. Dyskinesia was associated with a longer disease duration at study entry (2.67, 1.79-4.09, p<0.001) and assessment (2.73, 1.86-4.15, p<0.001), lower MDS UPDRS 1 (0.64, 0.41-0.98, p=0.04) and MDS UPDRS 3 (0.81, 0.68-0.95, p=0.01) scores at study entry, higher LEDD (1.20, 1.12-1.29, p<0.001), a longer treatment duration (3.14, 2.10-4.88, p<0.001) but also a shorter duration to the first onset of dyskinesia (0.35, 0.26-0.44, p<0.001).

Off dystonia first occurred after a median duration of 3.4 (2.1-5.0) years and manifested in 141 (9.4%) patients. Dystonia was predominantly associated with female gender (0.54, 0.30-0.97, p=0.04), longer disease duration at assessment (1.62, 1.13-2.36, p=0.01), no vascular risk at study entry (0.48, 0.26-0.88, p=0.02), higher MDS UPDRS 1 score (1.63, 1.16-2.33, p=0.006), higher LEDD (1.13, 1.03-1.25, p=0.01), a longer treatment duration (2.17, 1.48-3.26, p<0.001) but again a shorter duration to the first onset of dystonia (0.53, 0.43-0.64, p<0.001) (Table 4-1 to Table 4-2).

In the **PPMI** study, motor fluctuations were present in 68 (20.7%) patients after a median time to onset of 1.0 (0.4-1.9) years (Table 4-3 to Table 4-4).

They were strongly associated with a higher MDS UPDRS 1 and 2 scores at study entry (2.48, 1.09-5.81, p=0.03; 1.47, 1.05-2.07, p=0.03), and a lower STAI at study entry (0.87, 0.78-0.96, p=0.008). Fluctuators showed greater percentage changes at challenge testing (1.02, 1.00-1.04, p=0.02), had a higher L-dopa challenge dose (1.01, 1.01-1.01, p<0.001), and higher prescribed LEDD at challenge (1.19, 1.11-1.30, p<0.001).

Dyskinesia was present in 32 (9.8%) cases after a median time to onset of 1.6 (0.8-2.2) years. Dyskinesia was associated with female gender (0.38, 0.17-0.84, p=0.02), a greater percentage change at challenge testing (1.03, 1.01-1.06, p=0.02), and a higher LEDD (1.12, 1.04-1.22, p=0.003). Additionally, dyskinetic patients were strongly associated with a lower MDS UPDRS 3 score at challenge (0.83, 0.70-0.98, p=0.04).

Off dystonia first occurred after a median duration of 1.3 (0.5-1.8) years and manifested in 31 (9.5%) patients. Dystonia was predominantly associated with female gender (0.23, 0.09-0.58, p=0.02), a younger age at onset (0.92, 0.88-0.96, p<0.001), a younger age at diagnosis (0.92, 0.87-0.96, p<0.001), and a younger age at study entry (0.92, 0.88-0.96, p<0.001). Additionally, at study entry, a lower BMI score (1.09, 1.01-1.19, p=0.03), a higher UPDRS 1 score (3.38, 1.07-9.62, p=0.03), and a lower STAI (0.86, 0.74-0.99, p=0.04) contributed to dystonia. At challenge testing, both a higher total challenge test dose (1.00, 1.00-1.01, p=0.02), and a higher L-dopa challenge dose (1.01, 1.00-1.01, p=0.002), a higher LEDD (1.16, 1.07-1.28, p<0.001), and higher BMI (1.10, 1.02-1.19, p=0.01) were associated with dystonia (Table 4-3 to Table 4-4).

Table 4-1: Clinical variables including demographics and baseline characteristics in 1501 patients with Parkinson's in the **Tracking Parkinson's** study.

Tracking Parkinson's		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 1501	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
Male	978 (65.2%)	0.76 (0.60, 0.97)	**0.03**	0.49 (0.32, 0.75)	**0.001**	0.70 (0.48, 1.03)	0.06	0.89 (0.45, 1.75)	0.73	0.55 (0.39, 0.79)	**0.001**	0.54 (0.30, 0.97)	**0.04**
Female	523 (34.8%)	(reference)		(reference)		(reference)		(reference)		(reference)		(reference)	
Age at onset, years	64.1 (9.5)	0.97 (0.96, 0.99)	**<0.001**	0.99 (0.97, 1.01)	0.20	0.97 (0.95, 0.98)	**<0.001**	1.01 (0.97, 1.04)	0.76	0.95 (0.93, 0.97)	**<0.001**	0.98 (0.96, 1.01)	0.28
Age at diagnosis, years	65.9 (9.0)	0.97 (0.96, 0.99)	**<0.001**	0.98 (0.96, 1.01)	0.17	0.97 (0.95, 0.99)	**0.002**	1.01 (0.97, 1.04)	0.70	0.95 (0.94, 0.97)	**<0.001**	0.98 (0.95, 1.01)	0.26
Age at study entry, years	68.8 (9.0)	0.97 (0.96, 0.99)	**<0.001**	0.98 (0.96, 1.01)	0.17	0.97 (0.95, 0.99)	**0.002**	1.01 (0.97, 1.04)	0.70	0.95 (0.94, 0.97)	**<0.001**	0.98 (0.95, 1.01)	0.26
Disease duration at study entry, years	1.4 (0.9)	1.06 (0.93, 1.20)	0.40	1.39 (1.08, 1.81)	**0.01**	1.47 (1.20, 1.81)	**<0.001**	2.67 (1.79, 4.09)	**<0.001**	1.17 (0.97, 1.42)	0.10	1.41 (0.98, 2.06)	0.07
Disease duration at assessment, years	2.9 (0.9)	1.09 (0.96, 1.23)	0.20	1.42 (1.11, 1.83)	**0.006**	1.52 (1.25, 1.85)	**<0.001**	2.73 (1.86, 4.15)	**<0.001**	1.19 (0.99, 1.44)	0.06	1.62 (1.13, 2.36)	**0.01**
BMI at study entry	27.0 (4.7)	1.02 (0.99, 1.04)	0.22	1.00 (0.96,1.04)	0.86	1.04 (1.00, 1.07)	0.05	0.98 (0.92, 1.05)	0.64	0.99 (0.95, 1.02)	0.53	0.97 (0.92, 1.03)	0.30

Tracking Parkinson's		Motor fluctuations (Yes/No)		Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 1501	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
MDS UPDRS 1 at study entry	9.0 (5.0, 12.0)	1.37 (1.23, 1.54)	**<0.001**	0.79 (0.59, 1.06)	0.12	1.30 (1.10, 1.53)	**0.002**	0.64 (0.41, 0.98)	**0.04**	1.48 (1.27, 1.73)	**<0.001**	1.35 (0.93, 1.98)	0.11
MDS UPDRS 1 without 1.6 at study entry	8.0 (5.0, 12.0)	1.36 (1.21, 1.52)	**<0.001**	0.81 (0.60, 1.10)	0.18	1.32 (1.12, 1.55)	**0.001**	0.63 (0.40, 0.97)	**0.04**	1.46 (1.25, 1.70)	**<0.001**	1.36 (0.93, 2.02)	0.11
MDS UPDRS 2 at study entry	9.0 (5.0, 13.0)	1.43 (1.30, 1.58)	**<0.001**	1.05 (0.85, 1.30)	0.64	1.27 (1.10, 1.46)	**0.0009**	0.96 (0.69, 1.32)	0.80	1.42 (1.24, 1.62)	**<0.001**	1.00 (0.94, 1.06)	0.98
MDS UPDRS 3 at study entry	20.0 (14.0, 29.0)	1.22 (1.16, 1.29)	**<0.001**	1.04 (0.95, 1.15)	0.36	1.07 (0.98, 1.16)	0.12	0.81 (0.68, 0.95)	**0.01**	1.17 (1.09, 1.26)	**<0.001**	0.95 (0.84, 1.07)	0.41
HADS anxiety at study entry	4.0 (2.0, 8.0)	1.36 (1.18, 1.56)	**<0.001**	0.99 (0.70, 1.39)	0.94	1.56 (1.27, 1.92)	**<0.001**	1.51 (0.88, 2.62)	0.14	1.52 (1.25, 1.86)	**<0.001**	0.91 (0.56, 1.47)	0.70
HADS depression at study entry	4.0 (2.0, 7.0)	1.53 (1.29, 1.82)	**<0.001**	1.52 (0.92, 2.53)	0.11	1.68 (1.30, 2.19)	**<0.001**	0.91 (0.41, 2.00)	0.82	1.48 (1.16, 1.90)	**0.002**	0.73 (0.36, 1.44)	0.37
NMSS 7-12 at study entry	2.0 (0.0, 5.0)	1.11 (1.04, 1.19)	**0.003**	1.02 (0.85, 1.23)	0.86	1.13 (1.02, 1.24)	**0.01**	1.11 (0.83, 1.46)	0.45	1.09 (0.99, 1.19)	0.06	1.10 (0.85, 1.42)	0.44
Stroke history at study entry													
Yes	67 (4.5%)	1.02 (0.55, 1.78)	0.95	0.83 (0.28, 2.42)	0.73	3.03 (1.48, 5.80)	**0.001**	3.47 (0.92, 14.38)	0.07	1.93 (0.86, 3.88)	0.08	1.31 (0.35, 4.62)	0.67

Tracking Parkinson's		Motor fluctuations (Yes/No)		Dyskinesia (Yes/No)		Dystonia (Yes/No)					
Variable	Total N = 1501	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
No	1424 (95.5%)	(reference)		(reference)		(reference)		(reference)		(reference)		(reference)	
History of diabetes at study entry													
Yes	129 (8.7%)	1.49 (0.99, 2.22)	0.05	1.02 (0.50, 2.12)	0.96	1.00 (0.46, 1.95)	0.99	0.57 (0.17, 1.85)	0.36	1.32 (0.67, 2.41)	0.39	0.46 (0.14, 1.29)	0.16
No	1.61 (91.3%)	(reference)		(reference)		(reference)		(reference)		(reference)		(reference)	
Vascular risk at study entry													
Yes	731 (49.4%)	1.27 (0.99, 1.63)	0.06	0.99 (0.65, 1.52)	0.97	1.28 (0.86, 1.90)	0.22	0.98 (0.49, 1.95)	0.96	0.99 (0.68, 1.44)	0.97	0.48 (0.26, 0.88)	**0.02**
No	750 (50.6%)	(reference)		(reference)		(reference)		(reference)		(reference)		(reference)	

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. UPDRS1, 2, 3, HADS anxiety, HADS depression, and NMSS 7-12 per 5-unit change.

Abbreviations: HADS: Hospital Anxiety and Depression Scale, LEDD: Levodopa equivalent daily dose, NMSS: Non-Motor Symptoms Scale

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to motor fluctuations onset, MDS UPDRS 1, 2, 3, HADS anxiety, HADS depression, and NMS 7-12 at baseline.

[3] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to dyskinesia onset, MDS UPDRS 1, 2, and history of stroke at baseline, HADS anxiety, HADS depression, and NMSS 7-12 at baseline.

[4] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to dystonia onset, MDS UPDRS 1, 2, 3 at baseline, HADS anxiety and HADS depression at baseline.

Table 4-2: Clinical variables 2.9 years after diagnosis in patients with Parkinson's in **Tracking Parkinson's**.

Tracking Parkinson's		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 1501	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
Challenge test response, percentage change	30.5 (15.0, 45.4)	1.00 (1.00, 1.01)	0.17	1.00 (0.99, 1.01)	0.50	1.01 (1.00, 1.02)	0.13	1.01 (0.99, 1.02)	0.53	0.99 (0.98, 1.00)	0.19	0.98 (0.97, 1.00)	0.12
Challenge test dose, LD mg	100.0 (100.0, 112.5)	1.00 (1.00, 1.01)	**0.02**	1.00 (0.99, 1.00)	0.17	1.01 (1.00, 1.01)	**0.006**	1.00 (0.99, 1.01)	0.61	1.00 (1.00, 1.01)	0.15	1.00 (0.99, 1.00)	0.50
LEDD at follow-up**,** mg/day	400.0 (300.0, 556.3)	1.29 (1.23, 1.36)	**<0.001**	1.21 (1.11, 1.32)	**<0.001**	1.24 (1.16, 1.32)	**<0.001**	1.20 (1.12, 1.29)	**<0.001**	1.23 (1.15, 1.31)	**<0.001**	1.13 (1.03, 1.25)	**0.01**
Treatment duration, years	2.7 (2.0, 3.5)	1.41 (1.07, 1.87)	**0.02**	1.76 (1.36, 2.30)	**<0.001**	1.58 (1.30, 1.92)	**<0.001**	3.14 (2.10, 4.88)	**<0.001**	1.24 (1.02, 1.49)	**0.03**	2.17 (1.48, 3.26)	**<0.001**
Time to onset of motor complication, years													
Motor fluctuations	3.1 (1.9, 4.6)	0.51 (0.45, 0.58)	**<0.001**	0.54 (0.47, 0.62)	**<0.001**								
Dyskinesia	4.2 (2.8, 5.6)					0.35 (0.27, 0.44)	**<0.001**	0.35 (0.26, 0.44)	**<0.001**				
Dystonia	3.4 (2.1, 5.0)									0.57 (0.48, 0.67)	**<0.001**	0.53 (0.43, 0.64)	**<0.001**

Tracking Parkinson's		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 1501	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
MDS UPDRS 1, at follow-up	9.0 (6.0, 14.0)	1.47 (1.32, 1.64)	**<0.001**	1.24 (0.97, 1.59)	0.09	1.39 (1.19, 1.63)	**<0.001**	0.74 (0.48, 1.11)	0.15	1.56 (1.34, 1.81)	**<0.001**	1.63 (1.16, 2.33)	**0.006**
MDS UPDRS 1 without 1.6, at follow-up	9.0 (6.0, 13.0)	1.47 (1.33, 1.63)	**<0.001**	1.23 (0.97, 1.55)	0.09	1.33 (1.14, 1.54)	**<0.001**	0.83 (0.57, 1.19)	0.31	1.53 (1.33, 1.76)	**<0.001**	1.51 (1.13, 2.06)	**0.007**
MDS UPDRS 2, at follow-up	11.0 (6.0, 16.0)	1.46 (1.34, 1.60)	**<0.001**	1.14 (0.95, 1.37)	0.17	1.32 (1.16, 1.50)	**<0.001**	0.96 (0.72, 1.28)	0.80	1.36 (1.20, 1.53)	**<0.001**	1.03 (0.79, 1.36)	0.80
MDS UPDRS 3, at follow-up	25.0 (17.0, 35.0)	1.18 (1.12, 1.23)	**<0.001**	1.09 (1.00, 1.19)	0.05	1.07 (0.99, 1.14)	0.08	1.08 (0.94, 1.25)	0.29	1.21 (1.13, 1.29)	**<0.001**	1.05 (0.94, 1.19)	0.39
HADS anxiety at follow-up	5.0 (2.0, 9.0)	1.55 (1.35, 1.78)	**<0.001**	1.40 (1.03, 1.90)	**0.03**	1.46 (1.18, 1.81)	**<0.001**	1.22 (0.77, 1.93)	0.41	1.31 (1.07, 1.59)	**0.007**	1.05 (0.71, 1.54)	0.82
HADS depression at follow-up	5.0 (2.0, 7.0)	1.70 (1.44, 2.02)	**<0.001**	1.69 (1.15, 2.52)	**0.009**	1.61 (1.24, 2.10)	**<0.001**	1.06 (0.58, 1.90)	0.84	1.48 (1.16, 1.88)	**0.001**	0.96 (0.59, 1.56)	0.88
NMSS 7-12 at follow-up	2.0 (0.0, 6.0)	1.12 (1.05, 1.19)	**<0.001**	1.13 (0.98, 1.31)	0.10	1.05 (0.95, 1.15)	0.29	1.06 (0.85, 1.32)	0.63	1.06 (0.97, 1.15)	0.18	1.14 (0.96, 1.37)	0.14

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. UPDRS1, 2, 3, HADS anxiety, HADS depression, and NMSS 7-12 per 5-unit change, LEDD total per 100mg/day change. Abbreviations: HADS: Hospital Anxiety and Depression Scale, LD: Levodopa dose, LEDD: Levodopa equivalent daily dose, NMSS: Non-Motor Symptoms Scale

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to motor fluctuations onset, MDS UPDRS 1, 2, 3, HADS anxiety, HADS depression, and NMS 7-12 at baseline.

[3] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to dyskinesia onset, MDS UPDRS 1, 2, and history of stroke at baseline, HADS anxiety, HADS depression, and NMSS 7-12 at baseline.

[4] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, time to dystonia onset, MDS UPDRS 1, 2, 3 at baseline, HADS anxiety and HADS depression at baseline.

Table 4-3: Clinical variables including demographics and baseline characteristics in 328 patients with Parkinson's in **PPMI**.

PPMI		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 328	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
Male	215 (65.5%)	0.66 (0.38, 1.15)	0.14	0.55 (0.30, 1.01)	0.05	0.44 (0.21, 0.93)	**0.03**	0.38 (0.17, 0.84)	**0.02**	0.39 (0.17, 0.84)	**0.02**	0.23 (0.09, 0.58)	**0.002**
Female	113 (34.5%)	(reference)		(reference)		(reference)		(reference)		(reference)		(reference)	
Age at onset, years	59.5 (10.0)	0.99 (0.97, 1.02)	0.68	1.00 (0.97, 1.03)	0.89	0.98 (0.94, 1.01)	0.23	0.98 (0.94, 1.02)	0.34	0.93 (0.89, 0.97)	**<0.001**	0.92 (0.88, 0.96)	**<0.001**
Age at diagnosis, years	61.0 (9.8)	0.99 (0.97, 1.02)	0.66	1.00 (0.97, 1.03)	0.92	0.98 (0.94, 1.02)	0.28	0.98 (0.95, 1.02)	0.44	0.92 (0.89, 0.96)	**<0.001**	0.92 (0.87, 0.96)	**<0.001**
Age at study entry, years	61.5 (9.8)	0.99 (0.97, 1.02)	0.65	1.00 (0.97, 1.03)	0.92	0.98 (0.94, 1.02)	0.28	0.98 (0.95, 1.02)	0.44	0.92 (0.89, 0.96)	**<0.001**	0.92 (0.88, 0.96)	**<0.001**
Disease duration at study entry, years	0.5 (0.5)	1.32 (0.83, 2.07)	0.23	1.21 (0.72, 1.99)	0.46	1.01 (0.49, 1.85)	0.98	0.81 (0.35, 1.60)	0.58	1.34 (0.68, 2.43)	0.36	0.97 (0.46, 1.90)	0.94
Disease duration at assessment, years	3.6 (0.6)	1.34 (0.85, 2.08)	0.19	1.20 (0.72, 1.95)	0.47	1.05 (0.52, 1.89)	0.87	0.84 (0.38, 1.61)	0.63	1.32 (0.68, 2.36)	0.38	0.93 (0.43, 1.80)	0.84
BMI at study entry	27.1 (4.6)	1.03 (0.97, 1.09)	0.28	1.02 (0.95, 1.09)	0.57	1.01 (0.93, 1.09)	0.77	1.00 (0.92, 1.08)	0.98	1.11 (1.02, 1.20)	**0.01**	1.09 (1.01, 1.19)	**0.03**

PPMI		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 328	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
MDS UPDRS 1 at study entry	1.0 (0.0, 2.0)	2.98 (1.38, 6.63)	**0.006**	2.48 (1.09, 5.81)	**0.03**	2.79 (1.09, 7.12)	**0.03**	2.36 (0.87, 6.32)	0.08	3.30 (1.25, 8.69)	**0.01**	3.38 (1.07, 9.62)	**0.03**
MDS UPDRS 1 without 1.6 at study entry	1.0 (0.0, 2.0)	2.85 (1.31, 6.38)	**0.009**	2.32 (1.01, 5.47)	0.05	2.85 (1.10, 7.35)	**0.03**	2.37 (0.86, 6.46)	0.08	2.98 (1.09, 7.93)	**0.03**	2.91 (0.87, 8.55)	0.06
MDS UPDRS 2 at study entry	5.0 (3.0, 8.0)	1.80 (1.31, 2.47)	**<0.001**	1.47 (1.05, 2.07)	**0.03**	1.84 (1.22, 2.79)	**0.004**	1.55 (1.00, 2.40)	0.05	1.35 (0.87, 2.08)	0.17	0.62 (0.33, 1.13)	0.13
MDS UPDRS 3 at study entry	20.0 (14.8, 26.0)	1.03 (0.88, 1.20)	0.69	0.86 (0.71, 1.04)	0.14	1.18 (0.96, 1.44)	0.12	1.04 (0.81, 1.32)	0.77	0.96 (0.76, 1.21)	0.75	0.85 (0.64, 1.10)	0.23
STAI at study entry	62.0 (52.0, 75.0)	0.99 (0.91, 1.06)	0.75	0.87 (0.78, 0.96)	**0.008**	1.08 (0.98, 1.18)	0.10	1.01 (0.89, 1.13)	0.87	1.01 (0.91, 1.12)	0.79	0.86 (0.74, 0.99)	**0.04**
GDS at study entry	5.0 (4.0, 6.0)	0.55 (0.20, 1.45)	0.24	0.34 (0.11, 0.99)	0.05	1.73 (0.48, 5.76)	0.38	1.43 (0.36, 5.35)	0.60	1.37 (0.34, 4.97)	0.64	1.01 (0.22, 4.17)	0.99

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. UPDRS 1, 2, 3, STAI, and GDS calculated for a 5-unit change.

Abbreviations: STAI: State-Trait Anxiety Inventory, GDS: Geriatric Depression Scale, LEDD: Levodopa equivalent daily dose

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 1 and 2 at baseline.

[3] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD and MDS UPDRS 1 and 2 at baseline.

[4] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 1 at baseline, MDS UDPRS 2 and BMI at follow-up.

Table 4-4: Clinical variables 3.6 years after diagnosis in patients with Parkinson's in **PPMI**.

PPMI		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 328	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
Challenge test response, percentage change	24.2 (10.9, 40.4)	1.02 (1.00, 1.04)	**0.01**	1.02 (1.00, 1.04)	**0.02**	1.03 (1.01, 1.06)	**0.02**	1.03 (1.01, 1.06)	**0.02**	1.01 (0.99, 1.03)	0.22	1.01 (0.99, 1.04)	0.25
Challenge test dose, LEDD mg	150.0 (100.0, 250.00)	1.00 (1.00, 1.01)	0.10	1.00 (1.00, 1.00)	0.09	1.00 (1.00, 1.00)	0.65	1.00 (1.00, 1.00)	0.62	1.00 (1.00, 1.01)	**0.03**	1.00 (1.00, 1.01)	**0.02**
L-dopa only	100.0 (100.0, 200.0)	1.01 (1.00, 1.01)	**<0.001**	1.01 (1.01, 1.01)	**<0.001**	1.00 (1.00, 1.01)	0.21	1.00 (1.00, 1.01)	0.21	1.01 (1.00, 1.01)	**0.01**	1.01 (1.00, 1.01)	**0.02**
LEDD at follow-up**,** mg/day	400.0 (297.5, 610.0)	1.21 (1.12, 1.32)	**<0.001**	1.19 (1.11, 1.30)	**<0.001**	1.13 (1.05, 1.22)	**0.001**	1.12 (1.04, 1.22)	**0.003**	1.13 (1.04, 1.23)	**0.002**	1.16 (1.07, 1.28)	**<0.001**
Treatment duration, years	2.3 (1.8, 2.5)	1.23 (0.80, 1.98)	0.36	0.76 (0.46, 1.29)	0.30	1.76 (0.91, 3.85)	0.12	1.25 (0.60, 2.90)	0.57	0.72 (0.41, 1.32)	0.27	0.53 (0.27, 1.05)	0.07
Time to onset of motor complication, years													
Motor fluctuations	1.0 (0.4, 1.9)	1.39 (0.82, 2.40)	0.23	1.44 (0.83, 2.59)	0.20								
Dyskinesia	1.6 (0.8, 2.2)					0.49 (0.16, 1.25)	0.16	0.44 (0.12, 1.26)	0.16				

PPMI		Motor fluctuations (Yes/No)				Dyskinesia (Yes/No)				Dystonia (Yes/No)			
Variable	Total N = 328	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[2]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[3]	p-value	Model estimate (95% CI)[1]	p-value	Model estimate (95% CI)[4]	p-value
Dystonia	1.3 (0.5, 1.8)									1.27 (0.70, 2.37)	0.43	1.04 (0.52, 2.11)	0.90
BMI at follow-up	27.0 (5.0)	1.04 (0.98, 1.10)	0.18	1.05 (0.99, 1.11)	0.11	1.02 (0.94, 1.09)	0.66	1.02 (0.94, 1.10)	0.60	1.09 (1.01, 1.17)	**0.02**	1.10 (1.02, 1.19)	**0.01**
MDS UPDRS 1, at follow-up	1.0 (0.0, 3.0)	1.84 (1.07, 3.20)	**0.03**	0.92 (0.45, 1.79)	0.81	1.29 (0.58, 2.56)	0.50	0.56 (0.19, 1.37)	0.25	1.76 (0.79, 3.58)	0.14	0.63 (0.20, 1.70)	0.39
MDS UPDRS 1 without 1.6, at follow-up	1.0 (0.0, 3.0)	1.72 (0.98, 3.03)	0.06	0.82 (0.38, 1.63)	0.57	1.31 (0.59, 2.65)	0.47	0.59 (0.20, 1.45)	0.30	1.64 (0.70, 3.42)	0.21	0.53 (0.16, 1.51)	0.27
MDS UPDRS 2, at follow-up	8.0 (5.0, 12.0)	1.57 (1.24, 2.00)	**<0.001**	1.34 (1.00, 1.81)	0.05	1.26 (0.92, 1.71)	0.14	0.94 (0.63, 1.38)	0.77	1.55 (1.12, 2.16)	**0.008**	1.35 (0.93, 1.93)	0.10
MDS UPDRS 3, at follow-up	27.0 (18.0, 36.3)	1.06 (0.95, 1.18)	0.31	1.01 (0.90, 1.14)	0.88	0.90 (0.76, 1.05)	0.19	0.83 (0.70, 0.98)	**0.04**	0.97 (0.82, 1.13)	0.68	0.84 (0.68, 1.03)	0.11
STAI at follow-up	61.0 (51.0, 76.0)	1.01 (0.94, 1.08)	0.87	0.92 (0.84, 1.00)	0.06	1.06 (0.97, 1.16)	0.18	1.00 (0.90, 1.10)	0.94	1.05 (0.95, 1.15)	0.34	0.94 (0.83, 1.06)	0.35
GDS at follow-up	5.0 (5.0, 6.0)	1.43 (0.59, 3.42)	0.43	0.82 (0.31, 2.09)	0.68	0.95 (0.27, 3.18)	0.94	0.64 (0.18, 2.11)	0.48	1.83 (0.51, 6.39)	0.35	0.89 (0.23, 3.40)	0.87

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. UPDRS 1, 2, 3, STAI, and GDS calculated for a 5-unit change, LEDD 100mg/day change.

Abbreviations: STAI: State-Trait Anxiety Inventory, GDS: Geriatric Depression Scale, LEDD: Levodopa equivalent daily dose

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 1 and 2 at baseline.

[3] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD and MDS UPDRS 1 and 2 at baseline.

[4] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 1 at baseline, MDS UDPRS 2 and BMI at follow-up.

4.3.3 Challenge testing

A definite response to challenge testing was seen in 565 cases (60.5%) in **Tracking Parkinson's**. Using 24.5% as the cut-off for defining definite responders and limited responders showed a bell-shaped curve (Figure 4-2). The median change in MDS UPDRS 3 from pre- to post-challenge L-dopa dose was 41.9% (IQR 33.3-52.9) in those with a definite response, versus 11.3% (IQR 3.8-17.9) in those with a limited response. Patients who showed a definite L-dopa response were younger (65.0 years, SD 9.2) compared to those with a limited response (67.8 years, SD 8.5, $p<0.001$). Those with a definite response had a lower MDS UPDRS 3 score at study entry (19.0, IQR 14.0-28.0) than those with a limited response (22.0, 15.0-32.0; $p<0.001$) (Figure 4-3).

The demographic and phenotypic characteristics of the cases who undertook the L-dopa challenge test in Tracking Parkinson's are shown in Table 4-5 to Table 4-6. The median LEDD at the time of the challenge test was higher in those with a definite response (450.0mg, IQR 300.0-600.0) compared to those with a limited response (400.0mg, IQR 300.0-519.5; $p=0.002$). There was no significant difference in the prescription of antiparkinsonian medication between definite and limited responders.

Further, those with a definite response showed a smaller increase in MDS UPDRS 3 from study entry to challenge test (an increase of median 3.0 points, IQR -3.0 to 9.0) compared to an increase of 6 points (-1 to 14) in those with a limited response ($p<0.001$). Accordingly, definite responders had a lower MDS UPDRS 3 score at challenge testing of median 23.0 (15.0-32.0) compared to limited responders (30.0, 20.0-41.0; $p<0.001$).

There were no statistical differences in disease duration, gender, motor subtype, non-motor scores, depression, or autonomic scores between those with a definite versus a limited L-dopa response.

Considering comorbid vascular disease and vascular risk factors, only 48.8% of the 86 patients with a history of diabetes showed a definite L-dopa response. In patients with a previous history of stroke (n = 43), 62.8% showed a definite L-dopa response. Similarly, in patients with a vascular risk, 57% of the 467 patients had a definite response. However, these observations were not statistically significant after adjusting for age, gender, and disease duration. There was also no significant difference between the definite and limited responder groups for body mass index. There was no

association of a higher or lower L-dopa dose with a definite or limited response at challenge testing (Table 4-9).

The demographic and phenotypic characteristics of the cases who undertook the L-dopa challenge test in the **PPMI** study are shown in Table 4-7 to Table 4-8. A definite response to challenge testing was seen in 88 cases (49.2%). The median change in MDS UPDRS 3 from pre- to post-challenge L-dopa dose was 40.7% (IQR 32.2-53.8) in those with a definite response, versus 11.1% (1.2-18.2) in those with a limited response. There were no significant differences in the demographics of both responding groups. However, definite responders had a slightly higher total challenge dose (OR: 1.00, 95% CI: 0.99-1.00, p=0.03), whereas limited responders had a higher MDS UPDRS 3 at challenge (1.16, 1.00-1.35, p=0.05). Additionally, limited responders had a slightly lower LEDD (0.91, 0.83-0.99, p=0.04), and were associated with a PIGD motor subtype (3.37, 1.63-7.25, p=0.001).

The LEDD at the time of the challenge test was higher in those with a definite response (571.2 mg, IQR 400.0-732.8) compared to those with a limited response (480.0 mg, IQR 300.0-675.0; p=0.004). There was no significant difference in the prescription of antiparkinsonian medication between definite and limited responders.

There were no statistical differences in disease duration, gender, depression, or autonomic scores between those with a definite versus a limited L-dopa response.

PPMI did not record specific data on patients with a history of diabetes. In patients with a comorbid cardiovascular condition including hypertension, 45% of the 109 patients had a definite response. However, these observations were not statistically significant after adjusting for age, gender, and disease duration. There was also no significant difference between the definite and limited responder groups for body mass index. The L-dopa dose used in the acute challenge test was not significantly different between definite and limited responders (Table 4-8). There was no evidence of an association of a higher or lower total challenge test dose with the challenge test response (Table 4-10).

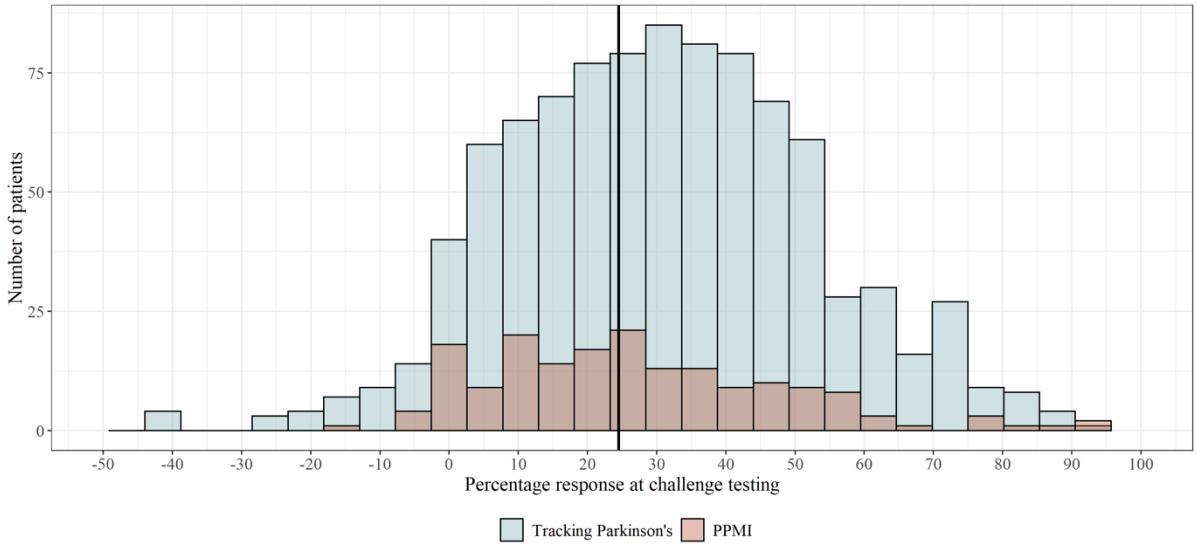

Figure 4-2: Histogram of percentage changes in MDS UPDRS 3 in Tracking Parkinson's and PPMI.

Both studies reported substantial proportions of patients on both sides of the 24.5% cut-off mark (black line), resulting in a bell-shaped distribution of challenge test responses. Two outlying percentage responses were omitted from this figure but not from analysis. Outliers were one case in Tracking with -114.3%, and one case with -76.2% in PPMI.

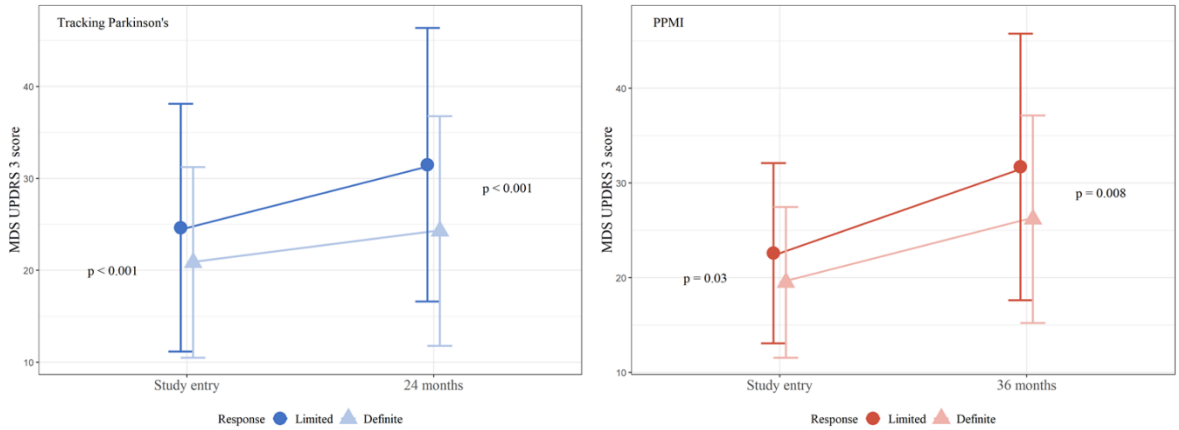

Figure 4-3: Change in MDS UPDRS 3 scores from study entry to time of challenge test in definite and limited responders.

In both cohort studies, definite responders have a significantly lower UPDRS 3 score at both, study entry and challenge testing, suggesting a better motor function in definite responders compared to more limited responding patients.

Table 4-5: Clinical variables including response to L-dopa challenge test in 934 patients with Parkinson's in the **Tracking Parkinson's** study.

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Male	629 (67.3%)	375 (66.4%)	254 (68.8%)	1.08 (0.81, 1.44)	0.60	0.96 (0.69, 1.33)	0.80
Female	305 (32.7%)	190 (33.6%)	115 (31.2%)	(reference)		(reference)	
Age at onset, years	64.2 (9.6)	63.0 (10.0)	66.1 (8.7)	1.04 (1.02, 1.05)	**<0.001**	1.03 (1.02, 1.05)	**<0.001**
Age at diagnosis, years	66.0 (9.0)	65.0 (9.2)	67.8 (8.5)	1.04 (1.02, 1.05)	**<0.001**	1.03 (1.01, 1.05)	**<0.001**
Age at study entry, years	69.1 (9.0)	68.0 (9.1)	70.8 (8.5)	1.04 (1.02, 1.05)	**<0.001**	1.03 (1.01, 1.05)	**<0.001**
Disease duration at study entry, years	1.4 (0.9)	1.4 (0.9)	1.4 (0.9)	1.04 (0.89, 1.20)	0.64	1.09 (0.91, 1.30)	0.34
Disease duration at assessment, years	2.9 (0.9)	2.9 (0.9)	2.9 (0.9)	1.03 (0.89, 1.19)	0.71	1.07 (0.90, 1.28)	0.42
BMI at study entry	26.6 (24.2, 29.5)	26.5 (24.2, 29.7)	26.7 (24.2, 29.1)	0.99 (0.97, 1.02)	0.69	0.99 (0.95, 1.02)	0.45
MDS UPDRS 1 at study entry	9.0 (5.0, 12.0)	9.0 (5.0, 13.0)	9.0 (6.0, 12.0)	0.91 (0.80, 1.04)	0.19	0.85 (0.72, 1.00)	0.05

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
MDS UPDRS 1 without 1.6 at study entry	9.0 (5.0, 12.0)	9.0 (5.0, 13.0)	9.0 (5.0, 11.0)	0.92 (0.81, 1.05)	0.21	0.85 (0.73, 1.00)	0.05
MDS UPDRS 2 at study entry	9.0 (5.0, 13.0)	9.0 (5.0, 13.0)	9.0 (5.0, 14.0)	1.00 (0.90, 1.13)	0.93	0.88 (0.76, 1.01)	0.08
MDS UPDRS 3 at study entry	20.0 (14.0, 29.0)	19.0 (14.0, 28.0)	22.0 (15.0, 32.0)	1.13 (1.06, 1.20)	**<0.001**	1.19 (1.11, 1.28)	**<0.001**
HADS anxiety at study entry	4.0 (2.0, 8.0)	4.0 (2.0, 8.0)	5.0 (2.0, 8.0)	1.05 (0.90, 1.23)	0.53	0.92 (0.75, 1.11)	0.37
HADS depression at study entry	4.0 (2.0, 7.0)	4.0 (2.0, 7.0)	4.0 (2.0, 6.0)	0.98 (0.80, 1.20)	0.85	0.87 (0.68, 1.11)	0.27
NMSS at study entry	2.0 (0.0, 5.8)	2.0 (0.0, 6.0)	2.0 (0.0, 5.0)	1.02 (0.93, 1.11)	0.71	0.94 (0.84, 1.06)	0.34
History of stroke at study entry							
Yes	43 (4.6%)	27 (4.8%)	16 (4.4%)	0.80 (0.41, 1.51)	0.50	0.58 (0.25, 1.32)	0.20
No	886 (95.4%)	535 (95.2%)	351 (95.6%)	(reference)			

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
History of diabetes at study entry							
Yes	86 (9.3%)	42 (7.5%)	44 (12.0%)	1.50 (0.96, 2.37)	0.08	1.10 (0.65, 1.86)	0.72
No	842 (90.7%)	519 (92.5%)	323 (88.0%)				
Vascular risk at study entry							
Yes	467 (50.5%)	266 (47.6%)	201 (55.1%)	1.17 (0.89, 1.54)	0.27	1.11 (0.81, 1.52)	0.53
No	457 (49.5%)	293 (52.4%)	164 (44.9%)				

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. MDS UPDRS 3 is calculated for a 5-unit change, and LEDD for 100mg/day change.

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 3 at baseline, and change in MDS UPDRS 3 from baseline to challenge.

Table 4-6: Clinical variables of the challenge test cohort at the 2-year time point of testing in **Tracking Parkinson's**.

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Challenge test response, percentage change	30.5 (15.0, 45.4)	41.9 (33.3, 52.9)	11.3 (3.9, 17.9)	NA	NA	NA	NA
L-dopa challenge dose, LD mg/day	100.0 (100.0, 125.0)	100.0 (100.0, 125.0)	100.0 (100.0, 100.0)	1.00 (1.00, 1.00)	0.55	1.00 (1.00, 1.00)	0.88
LEDD at challenge**,** mg/day	400.0 (300.0, 575.00)	450.0 (300.0, 600.0)	400.0 (300.0, 519.5)	0.91 (0.85, 0.97)	**0.004**	0.89 (0.83, 0.96)	**0.002**
Treatment duration, years	2.7 (2.0, 3.5)	2.7 (2.0, 3.5)	2.5 (1.9, 3.4)	0.69 (0.50, 0.96)	**0.03**	0.98 (0.82, 1.18)	0.86
MDS UPDRS 1, at challenge	10.0 (7.0, 14.0)	10.0 (6.0, 14.00)	10.0 (7.0, 13.0)	1.01 (0.89, 1.15)	0.85	0.90 (0.77, 1.05)	0.18
MDS UPDRS 1 without 1.6, at challenge	9.0 (6.0, 13.0)	10.0 (6.0, 13.0)	9.0 (6.0, 13.0)	0.99 (0.88, 1.11)	0.86	0.87 (0.75, 1.01)	0.06
MDS UPDRS 2, at challenge	11.0 (6.0, 16.0)	11.0 (6.0, 15.0)	11.0 (6.0, 16.0)	1.07 (0.96, 1.18)	0.21	0.91 (0.79, 1.04)	0.17
MDS UPDRS 3, at challenge	25.0 (17.0, 36.0)	23.0 (15.0, 32.0)	30.0 (20.0, 41.0)	1.20 (1.14, 1.27)	**<0.001**	1.19 (1.11, 1.28)	**<0.001**

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Change in MDS UPDRS 3 from study entry to challenge	4.0 (-2.0, 11.0)	3.0 (-3.0, 9.0)	6.0 (-1.0, 14.0)	1.03 (1.02, 1.05)	**<0.001**	1.04 (1.03, 1.06)	**<0.001**
Motor subtype							
Tremor-dominant	306 (36.7%)	190 (37.3%)	116 (35.8%)	(reference)		(reference)	
PIGD	427 (51.2%)	258 (50.6%)	169 (52.2%)	1.00 (0.73, 1.36)	0.99	1.11 (0.78, 1.58)	0.56
Indeterminate	101 (12.1%)	62 (12.2%)	39 (12.0%)				
Hoehn & Yahr score							
0-1	289 (31.3%)	193 (34.6%)	96 (26.2%)	(reference)		(reference)	
2-2.5	523 (56.7%)	317 (56.9%)	206 (56.3%)	1.24 (0.92, 1.69)	0.16	0.83 (0.57, 1.21)	0.33
3+	111 (12.0%)	47 (8.44%)	64 (17.5%)				
HADS anxiety at challenge	5.0 (2.0, 9.0)	5.0 (2.0, 9.0)	5.0 (2.0, 8.0)	1.01 (0.86, 1.19)	0.89	0.89 (0.72, 1.08)	0.24

Tracking Parkinson's		Challenge test response		Logistic regression model estimates			
Variable	Total N=934	Definite N=565 (60.5%)	Limited N=369 (39.5%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
HADS depression at challenge	5.0 (2.0, 7.0)	5.0 (2.0, 7.0)	5.0 (2.0, 8.0)	1.14 (0.94, 1.39)	0.18	0.99 (0.78, 1.25)	0.91
NMSS at challenge	2.0 (0.0, 6.0)	2.0 (0.0, 6.0)	2.0 (0.0, 6.0)	1.00 (0.92, 1.08)	0.93	0.94 (0.85, 1.05)	0.29

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. MDS UPDRS 3 is calculated for a 5-unit change, and LEDD for 100mg/day change.

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: LEDD, MDS UPDRS 3 at baseline, and change in MDS UPDRS 3 from baseline to challenge.

Table 4-7: Clinical variables including response to L-dopa challenge test in 179 patients with Parkinson's in the **PPMI** study.

PPMI		Challenge test response		Logistic regression model estimates			
Variable	Total N = 179	Definite N=88 (49.2%)	Limited N=91 (50.8%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Male	125 (69.8%)	59 (67.0%)	66 (72.5%)	1.23 (0.64, 2.36)	0.53	1.16 (0.58, 2.34)	0.67
Female	54 (30.2%)	29 (33.0%)	25 (27.5%)	(reference)		(reference)	
Age at onset, years	60.7 (9.8)	59.6 (9.1)	61.8 (10.3)	1.02 (0.99, 1.05)	0.18	1.02 (0.98, 1.05)	0.31
Age at diagnosis, years	62.1 (9.7)	61.1 (9.1)	63.1 (10.2)	1.02 (0.99, 1.05)	0.21	1.02 (0.98, 1.05)	0.37
Age at study entry, years	62.6 (9.8)	61.6 (9.2)	63.6 (10.2)	1.02 (0.99, 1.05)	0.21	1.02 (0.98, 1.05)	0.36
Disease duration at study entry, years	0.5 (0.5)	0.5 (0.5)	0.5 (0.5)	1.14 (0.61, 2.19)	0.67	0.90 (0.44, 1.83)	0.76
Disease duration at assessment, years	3.5 (0.5)	3.5 (0.5)	3.5 (0.5)	1.05 (0.57, 1.94)	0.88	0.83 (0.42, 1.65)	0.60
BMI at study entry	27.0 (24.3, 30.0)	26.3 (24.6, 29.4)	26.2 (23.8, 29.3)	1.00 (0.93, 1.07)	0.94	0.99 (0.92, 1.06)	0.79

PPMI		Challenge test response		Logistic regression model estimates			
Variable	Total N = 179	Definite N=88 (49.2%)	Limited N=91 (50.8%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
MDS UPDRS 1 at study entry	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.17 (0.50, 2.78)	0.72	0.82 (0.31, 2.04)	0.67
MDS UPDRS 1 without 1.6 at study entry	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.17 (0.50, 2.81)	0.72	0.82 (0.31, 2.07)	0.68
MDS UPDRS 2 at study entry	5.0 (3.0, 8.0)	5.5 (3.0, 8.0)	5.0 (3.0, 8.0)	0.99 (0.69, 1.42)	0.97	0.66 (0.42, 1.00)	0.06
MDS UPDRS 3 at study entry	20 (14.5, 26.5)	19.0 (14.0, 23.25)	22.0 (15.0, 29.0)	1.21 (1.01, 1.45)	**0.04**	1.17 (0.97, 1.42)	0.10
STAI at study entry	64.0 (52.0, 76.8)	63.0 (52.75, 75.0)	64.0 (52.0, 77.0)	1.02 (0.94, 1.11)	0.65	0.98 (0.89, 1.07)	0.61
GDS at study entry	5.0 (4.0, 6.0)	5.0 (4.0, 6.0)	5.0 (4.0, 6.0)	0.92 (0.34, 2.54)	0.88	1.11 (0.37, 3.31)	0.85

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. MDS UPDRS 3 is calculated for a 5-unit change, and LEDD for 100mg/day change.

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: MDS UPDRS 3 at baseline, motor subtype and MDS UPDRS 2 at challenge.

Table 4-8: Clinical variables of the challenge test cohort at the 3 year time point of testing in **PPMI**.

PPMI		Challenge test response		Logistic regression model estimates			
Variable	Total N = 179	Definite N=88 (49.2%)	Limited N=91 (50.8%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Challenge test response, percentage change	24.2 (10.9, 40.4)	40.7 (32.2, 53.8)	11.1 (1.2, 18.2)	NA	NA	NA	NA
Challenge test dose, LEDD mg	150.0 (100.0, 250.0)	180.0 (100.0, 250.0)	150.0 (100.0, 250.0)	1.00 (1.00, 1.00)	0.10	1.00 (0.99, 1.00)	**0.03**
L-dopa only	100.0 (100.0, 200.0)	140.0 (100.0, 200.0)	100.0 (100.0, 187.5)	0.99 (0.99, 1.00)	0.27	1.00 (0.99, 1.00)	0.14
LEDD at challenge**,** mg/day	500.0 (300.0, 709.5)	571.2 (400.0, 732.75)	480.0 (300, 675.0)	0.96 (0.88, 1.03)	0.23	0.91 (0.83, 0.99)	**0.04**
Treatment duration, years	2.4 (1.9, 2.5)	2.4 (1.9, 2.5)	2.4 (1.9, 2.5)	0.94 (0.56, 1.57)	0.81	0.80 (0.46, 1.37)	0.42
MDS UPDRS 1, at challenge	1.0 (1.0, 3.0)	2.0 (1.0, 3.3)	1.0 (0.0, 3.0)	0.70 (0.35, 1.41)	0.32	0.35 (0.15, 0.81)	**0.02**
MDS UPDRS 1 without 1.6, at challenge	1.0 (0.5, 3.0)	1.0 (1.0, 3.0)	1.0 (0.0, 3.0)	0.72 (0.35, 1.48)	0.38	0.38 (0.15, 0.89)	**0.03**
MDS UPDRS 2, at challenge	8.0 (5.0, 11.0)	7.0 (4.0, 10.3)	9.0 (5.0, 13.5)	1.42 (1.07, 1.91)	**0.02**	1.25 (0.92, 1.72)	0.15
MDS UPDRS 3, at challenge	28.0 (19.5, 38.0)	25.0 (17.75, 35.0)	31.0 (22.0, 40.0)	1.18 (1.04, 1.35)	**0.01**	1.16 (1.00, 1.35)	**0.05**

PPMI		Challenge test response		Logistic regression model estimates			
Variable	Total N = 179	Definite N=88 (49.2%)	Limited N=91 (50.8%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
Change in MDS UPDRS 3	7.0	6.0	8.0	1.02	0.22	1.01	0.49
from study entry to challenge	(0.0, 15.0)	(-2.0, 12.3)	(1.5, 16.5)	(0.99, 1.04)		(0.98, 1.04)	
Motor subtype							
Tremor-dominant	107	64	43	(reference)		(reference)	
	(59.8%)	(72.7%)	(47.3%)				
PIGD	53	15	38	3.87	**<0.001**	3.37	**0.001**
	(29.6%)	(17.0%)	(41.8%)	(1.91, 8.15)		(1.63, 7.25)	
Indeterminate	19	9	10				
	(10.6%)	(10.2%)	(11.0%)				
Hoehn & Yahr score							
0-1	29	16	13	(reference)			
	(16.2%)	(18.2%)	(14.3%)				
2-2.5	133	67	66	1.12	0.79	0.91	0.84
	(74.3%)	(76.1%)	(72.5%)	(0.49, 2.57)		(0.37, 2.24)	
3+	17	5	12				
	(9.5%)	(5.7%)	(13.2%)				
STAI at challenge	61.0	60.0	64	1.04	0.33	0.99	0.75
	(50.0, 77.0)	(49.0, 76.5)	(51.5, 76.5)	0.96, 1.13		(0.90, 1.08)	

PPMI		Challenge test response		Logistic regression model estimates			
Variable	Total N = 179	Definite N=88 (49.2%)	Limited N=91 (50.8%)	Model estimate (95% CI) [1]	p-value	Model estimate (95% CI) [2]	p-value
GDS at challenge	5.0 (5.0, 6.0)	5.0 (5.0, 6.0)	5.0 (5.0, 6.0)	1.36 0.49, 3.83	0.55	0.70 (0.21, 2.31)	0.56
Cardiovascular condition							
Yes	109 (60.9%)	49 (55.7%)	60 (65.9%)	1.38 (0.73, 2.63)	0.32	1.45 (0.73, 2.90)	0.29
No	70 (39.1%)	39 (44.3%)	31 (34.1%)	(reference)		(reference)	

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables. MDS UPDRS 3 is calculated for a 5-unit change, and LEDD for 100mg/day change.

[1] Adjusted for gender, age at diagnosis, disease duration.

[2] Adjusted for gender, age at diagnosis, disease duration, and additionally: MDS UPDRS 3 at baseline, motor subtype and MDS UPDRS 2 at challenge.

Table 4-9: L-dopa response according to dose in **Tracking Parkinson's**.

	All cases n=925		Definite n=562		Limited n=363		Logistic regression output	
L-dopa dose used in challenge	Number of cases (%)	Percentage change in MDS UPDRS 3	Number of cases (%)	Percentage change in MDS UPDRS 3	Number of cases (%)	Percentage change in MDS UPDRS 3	Model estimate (95% CI)[1]	p-value
>50-100mg	144 (15.6%)	30.1 (16.1, 44.6)	87 (15.5%)	40.0 (33.3, 50.0)	57 (15.7%)	10.5 (3.5, 17.9)	(reference)	(reference)
>100-150mg	579 (62.6%)	30.4 (14.3, 45.3)	347 (61.7%)	42.1 (33.3, 53.1)	232 (63.9%)	11.5 (4.4, 17.1)	0.99 (0.68, 1.45)	0.96
Over 150mg	202 (21.8%)	31.5 (18.8, 47.5)	128 (22.8%)	42.0 (33.3, 52.6)	74 (20.4%)	11.5 (3.7, 19.4)	0.83 (0.53, 1.31)	0.42

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables.

[1] Adjusted for gender, age at diagnosis, disease duration.

Table 4-10: L-dopa response according to dose in **PPMI.**

	All cases n=143		Definite n=88		Limited n=91		Statistical output χ^2
L-dopa dose used in challenge	Number of cases (%)	Percentage change in MDS UPDRS 3	Number of cases (%)	Percentage change in MDS UPDRS 3	Number of cases (%)	Percentage change in MDS UPDRS 3	p-value
50mg	1 (0.7%)	45.5 (45.5, 45.5)	1 (1.3%)	45.5 (45.5, 45.5)	0 (0.0%)	NA	0.41
>50-100mg	11 (7.7%)	7.5 (3.4, 26.5)	4 (5.2%)	37.5 (27.3, 47.8)	7 (10.6%)	6.9 (0.0, 7.3)	
>100-150mg	66 (46.2%)	26.0 (17.5, 39.7)	34 (44.2%)	39.4 (33.5, 53.8)	32 (48.5%)	16.8 (5.7, 20.9)	
Over 150mg	65 (45.5%)	31.0 (17.1, 43.8)	38 (49.4%)	41.0 (32.8, 53.3)	27 (40.9%)	14.3 (9.3, 17.8)	

Chi-square statistical test

4.4 Discussion

Two large cohorts of prospectively recruited recent-onset PD patients indicate that motor complications and challenge test responses are connected via the patient's motor function: better motor function is associated with the development of dyskinesia, which is also associated with a definite (rather than limited) challenge test response.

4.4.1 Motor complications

Gender seems to play a role in the development of motor complications. In the present analysis, female gender was associated with motor fluctuations, dyskinesia, and off dystonia. The literature mainly associates motor fluctuations with females (Ouma et al., 2017, Yoritaka et al., 2013), whereas some other large cohort studies did not find an association (Kadastik-Eerme et al., 2017, Kelly et al., 2019). For example, dyskinesia is often associated with female gender (Yoritaka et al., 2013, Scott et al., 2016) but this has not been a universal finding (Kelly et al., 2019). In the current analysis, an association of worse dystonia with female gender was found, which has not been reported before. This could be attributable to the larger sample size in Tracking Parkinson's, but there could be a change over time as other reports cover a longer disease duration than we have presently in the two cohorts studied.

A study showed that younger patients are significantly more likely to develop dyskinesia and motor fluctuations than older patients (Kelly et al., 2019), however, we found a negative association of age with dystonia in PPMI, i.e. younger patients were significantly more likely to develop dystonia than older patients. This association was not replicated for either wearing-off, dyskinesia or dystonia in the Tracking Parkinson's study. An explanation could be the rarity of studies reporting dystonia, causing smaller studies to be too underpowered to detect a true significant difference.

A longer **disease duration**, however, was strongly associated with all three complications, particularly dyskinesia, which is an entirely consistent finding across studies, while noting that some reports relate motor complications to the duration of treatment, rather than the duration of the disease, so that direct comparisons are not always feasible (Ahlskog and Muenter, 2001, Aquino and Fox, 2015, Blanchet et al., 1996). Other variations between the current findings and prior reports is the limited inclusion of cases observed from early in their disease course, and the reports from patients participating in randomised clinical drug trials which prescribed treatment regimens typically maximising a single drug class before adding further agents (Hauser

et al., 2007, Koller et al., 1999), that differs from the more usual combined drug class approach used in the clinical non-interventional setting (Kelly et al., 2019, Kim et al., 2020).

Off dystonia was the only motor complication associated with a higher BMI at baseline and at the time of the challenge test. Lower BMI has been associated with increased dyskinesia e.g. in the Oxford Discovery cohort, but they did not analyse dystonia findings in relation to BMI (Kelly et al., 2019). These observations are consistent, considering dystonia as a manifestation of lower dopamine availability in patients with higher body mass, and dyskinesia as largely a feature of higher dopamine levels which are more likely to be reached in patients with lower body mass. The Oxford study report included data of up to 10 years of disease duration, which is longer than the Tracking Parkinson's study, and we can expect that an association of dyskinesia with lower BMI will emerge with continued observations in the Tracking Parkinson's study.

While the main focus of the present analysis was in relation to motor features, it is now recognised that several **non-motor features** can fluctuate as part of the 'on-off' cycle in response to medication in Parkinson's disease (Poewe, 2008). A lower **MDS UPDRS 1** score (fewer non-motor complications) was found in dyskinetic cases, while off dystonia and wearing-off were associated with a higher MDS UPDRS 1 (worse non-motor features). These results replicate findings from the Oxford discovery data, an inception cohort of 734 patients with a follow-up period of 10 years (Kelly et al., 2019). The MDS UPDRS 1 score is derived from several domains (cognitive and neuropsychiatric) which might suggest that symptoms such as anxiety are the most important in this relationship with motor fluctuations (Kelly et al., 2019). Other non-motor variables like the **HADS** for anxiety and depression have also shown an association with motor fluctuations. A cross-sectional study of 250 PD patients supports this finding, as patients with motor fluctuations experienced higher frequencies of generalised anxiety disorders, independent of their motor state (Leentjens et al., 2012). Depression was also more present in fluctuators (Leentjens et al., 2012), suggesting that depression and anxiety could be related to 'wearing-off' of L-dopa or a brief abstinence syndrome (Cantello et al., 1986, Vazquez et al., 1993). However, a higher frequency during 'off' periods would be expected to support this hypothesis but other explanations need to be explored. This again underlines the greater impact and disability caused by motor fluctuations on daily activities. The assessment and comparison of the MDS

UPDRS 4 item from chapter 3.2 has shown a similar result but has now been confirmed in a regression analysis using the non-motor score.

However, the MDS UPDRS 1 score also includes a question about the dopamine dysregulation, which might also be relevant to the relationships observed between motor and non-motor features. The exclusion of this item from the analysis, showed similar results to the total MDS UPDRS 1 score. In the PPMI study, both variables were not significantly associated with motor fluctuations, but they were strongly associated with dystonia in the Tracking Parkinson's study at the follow-up visit. This indicates that dopamine dysregulation potentially induced by excess medication intake, contributes to the findings and is a factor that would be worth exploring in future analyses.

Patients with dyskinesia have overall better motor scores. A lower MDS UPDRS 3 was found in dyskinetic patients, which again feeds into the narrative of dyskinesia being less impactful than motor fluctuations for the patient. The motor score was also better in association with dyskinesia in the Oxford discovery data, even though only showing borderline significance (Kelly et al., 2019).

A longer **treatment duration** was associated with motor fluctuations and dyskinesia, but no association was found for dystonia. Only Tracking Parkinson's, and not PPMI, showed these associations. This might be explained by the differing entry criteria, requiring cases to be drug naïve for PPMI and therefore being at an earlier disease stage at baseline, and the 20% higher prevalence of patients with prescribed L-dopa in Tracking. The studies also show a slightly higher dose of antiparkinsonian medication, of around 50mg levodopa equivalent daily (LEDD 446mg for Tracking and 501mg in PPMI), however, it is important to note that the disease duration in the PPMI cohort was 1 year longer, which could explain the higher dose in this study despite the treatment duration in PPMI being shorter. As already shown in multiple studies, a higher LEDD was always strongly associated with motor complication development.

Motor complications, especially fluctuations and dyskinesia were associated with a greater degree of motor improvement at challenge testing in the PPMI study. This is not a new observation, as the ELLDOPA study showed a mean percentage improvement exceeding 40% in dyskinetic patients at 9 weeks of study duration (Hauser et al., 2009), and another longitudinal study of 34 patients, showed that motor fluctuators had a better response to L-dopa within the first 5 years (Clissold et al., 2006). However, the

replication of this observation in much larger patient numbers does help to make this a more impactful finding.

4.4.2 Challenge testing

There is a substantial variation in the degree of motor response to L-dopa. A dichotomous approach for the analysis of challenge test responses according to the 24.5% threshold (Merello et al., 2002) is an established method, but it is important to note that it splits the bell-shaped response curve into two halves, rather than identifying responders and non-responders as distinct subgroups.

These results are in line with variation in L-dopa responsiveness seen in clinical trials and pathological case series (Fahn et al., 2004, Hauser et al., 2009, Hughes et al., 1992). The ELLDOPA study showed an average improvement in UPDRS 3 of 27.4% (SD 30.6) at 9 weeks, and 26.2% (SD 36.4) at 24 weeks in 260 L-dopa treated patients (Fahn et al., 2004, Hauser et al., 2009). Because of the known differences between the UPDRS 3 and MDS UPDRS Part 3 scores (Merello et al., 2011), the comparable percentage improvements in the ELLDOPA study using the MDS UPDRS 3 would give improvements of 33.6% (SD 37.5) at 9 weeks and 32.1% (SD 44.6) at 24 weeks. Variation in the degree of L-dopa response has also been reported in pathologically confirmed PD (Hughes et al., 1992). The L-dopa response was available in 69 out of 76 confirmed PD cases and was graded as definite in 29%, good in 39%, limited in 13%, and nil-to-poor in 4% (Hughes et al., 1992).

It can be concluded that the degree of motor improvement in response to L-dopa is subject to significant variation, although the reasons for this are unclear. Therefore, it was important to better define what factors may be crucial in determining the level of responsiveness.

Firstly, the examination of demographic and disease-related features found that older patients showed a less robust response to L-dopa, which has been observed previously (Wickremaratchi et al., 2009). This did not confirm lower L-dopa response rates in male and postural instability patients as previously reported (Hauser et al., 2009). However, the results identified a significant relationship between L-dopa responsiveness and baseline motor scores, as well as rates of motor progression at challenge testing. These findings are of clinical significance: patients with lower responsiveness to L-dopa have higher motor scores (i.e. worse motor function) and a faster motor progression. A relationship between increasing age and progression of disability has been observed previously and in part explained by L-dopa non-responsive motor symptoms (Velseboer et al., 2013). There was evidence for a difference in prescribed antiparkinsonian medication in the PPMI cohort, with more limited responders taking amantadine

compared to definite responders (p=0.04), and L-dopa being more prescribed amongst definite responders (p=0.02) when adjusting for gender, age at diagnosis and disease duration.

Secondly, based on previous findings within the Tracking Parkinson's study that patients with vascular disease or an increased vascular risk factor show higher motor scores, more cognitive problems (Malek et al., 2016), and their association with age, the relationship between comorbid vascular disease and L-dopa response was analysed in this chapter. However, we did not find any evidence for an association of challenge test response and vascular disease or risk factors, suggesting that vascular comorbidity is not an independent driver of an L-dopa response.

There are several other potential explanations for variation in L-dopa responsiveness. The dose of L-dopa used to assess responsiveness is clearly important and is another source of variation in the Tracking Parkinson's study and others. As adopted in the PPMI study (Parkinson Progression Marker, 2011), the patient's standard morning L-dopa dose in the acute challenge test was also used in Tracking, which may underestimate the response to L-dopa in some cases. However, we reasoned that this relatively lower dose was appropriate, as it would be better tolerated by patients. The results showed that the dose used in Tracking challenge tests was lower in limited responders compared to limited responders in PPMI, bearing in mind that PPMI used both dopamine agonists and L-dopa (according to study documentation) at challenge testing. The LEDD at the time of the challenge test was significantly lower (around 50mg per day in Tracking, and 70mg per day in PPMI) in patients with a limited treatment response compared to those with a definite response, while there was no difference in LEDD between the groups at study entry. This raises the possibility that some patients are under-dosed despite worsening motor severity. Other factors may influence the drug dose given to some patients, such as neuropsychiatric features (Lawton et al., 2018). These findings suggest that clinicians are more likely to increase dopamine replacement therapy doses when there is a stronger L-dopa response.

The calculation of L-dopa responsiveness has potential limitations and may not reflect the true L-dopa responsiveness, particularly in patients that are under-dosed. While a more standardized dose could increase the measured responsiveness, research practice has evolved away from using higher challenge test doses. Further analysis in the PPMI cohort will partially correct for this and allow a better exploration of this 'dose effect' as

doses increase over time, given that the PPMI protocol involves repeated challenge test assessments.

Acute challenge tests with higher L-dopa doses were reported in prior studies. For example, a unit dose of 250mg L-dopa resulted in a positive response (i.e. a reduction in motor score equivalent to the threshold used in this chapter) in 39 of 55 early PD cases (70.9%) (Merello et al., 2002). An earlier systematic review of dopaminergic challenge tests in PD included two studies using acute L-dopa challenges, calculated that 69% of de novo PD and 76% of established PD exceeded this same threshold of response. Combined, this results in using 250mg L-dopa in 45 cases, 200mg L-dopa in one case, and 100mg L-dopa in 21 cases (Clarke and Davies, 2000). The direct comparison with our findings is difficult, as those previous studies used a mixture of tasks and degrees of improvement to assess the response (e.g. walking speed, tapping tasks, and 15-20% improvement in motor scores) and only 1 of the 4 studies assessed the acute L-dopa challenge response using UPDRS 3 scores (Clarke and Davies, 2000).

An escalating L-dopa challenge test dose was assessed in few reports: in one study 16 out of 22 cases (72.7%) responded at 100mg L-dopa, which increased by one case (to 77.2%) when the L-dopa challenge dose was increased to 200mg (D'Costa et al., 1995). Further support of the impact of the L-dopa dose comes from the ELLDOPA study (Fahn et al., 2004). Considering those cases with an improvement of more than 10% in motor score after 24 weeks of L-dopa treatment compared to baseline, this accounted for 76.5% of cases prescribed 300mg L-dopa per day, compared to 89% of cases prescribed 600mg L-dopa per day (Hauser et al., 2009). The other major consideration in L-dopa responsiveness relates to known pharmacokinetic variability among patients. Variations in body weight (Warren Olanow et al., 2013), gut absorption (Mukherjee et al., 2016) and gender (LeWitt, 2015) were all reported as potential contributors to response variations. The known higher bioavailability of L-dopa in postmenopausal women (LeWitt, 2015) did not, however, translate to gender differences in either cohort analysed in this chapter.

There are several other biological mechanisms that influence pharmacokinetics and pharmacodynamics of L-dopa at the cellular level. Higher levels of erythrocyte catechol-O-methyltransferase (which metabolises L-dopa) may impair the L-dopa response (Reilly et al., 1980), and genetic variants of this (Sampaio et al., 2018). Other enzymes, including dopa decarboxylase (Devos et al., 2014), and monoamine oxidase

type B (Sampaio et al., 2018) are emerging as contributors to later L-dopa associated motor fluctuations. It is likely that genetics influence the pharmacodynamic and pharmacokinetic processes of L-dopa, which can therefore also influence the motor response to the drug, even in early stages of the disease.

The results of these detailed genetic analyses (including data about motor complications from our ongoing observations) are being reported by other members of the research team.

Chapter 5: Dopaminergic depletion at symptom onset and treatment impact

5.1 Introduction

The development of motor dysfunction is strongly associated with the level of dopaminergic depletion in the Parkinson's brain: the more dopamine is lost in the brain, the more must be restored with dopamine replacement therapy. Classical pathological studies suggested that the dopaminergic loss at the time of clinical presentation was around 70% (i.e. 30% residual activity), but those studies were based on small numbers of cases and depended on a backwards projection from time of death to time of clinical diagnosis (Fearnley and Lees, 1991).

Modern imaging techniques allow measurement of dopaminergic loss in the living brain, in larger numbers of patients, and with repeated observations. The aim of this chapter is to analyse imaging data from the PPMI study, to calculate what degree of dopamine deficiency is present in very early Parkinson's. Firstly, levels of dopamine deficiency at the onset of motor symptoms will be analysed and compared to healthy controls. Secondly, these readouts will be evaluated as to how they translate into clinical features, with a focus on the very earliest motor presentation of hemi-parkinsonism, where symptoms present entirely on one side of the body, allowing an examination of the pre-motor hemisphere.

5.1.1 Measuring dopaminergic activity by functional imaging

The dorsal striatum is a structure in the centre of the brain, comprising the putamen and caudate. A loss of dopamine in this area of the brain induces parkinsonian symptoms (Ehringer and Hornykiewicz, 1960), however for a long time dopamine levels in the brain could only be assessed at post-mortem, such as by using immunostaining methods. Advancing age causes some degree of neuronal loss in the striatum; early studies reported a reduction of dopamine of 36-48% (Hirai, 1968, Mann et al., 1984, McGeer et al., 1977) which was also quantified in a biochemical study as a 13% loss of dopamine in the caudate per decade (Riederer and Wuketich, 1976). This age-related loss does not explain the dramatic loss seen in Parkinson's, where classic pathological studies found a depletion of 50% of nigral neurons and 80% of striatal dopamine before clinical signs appear (Marsden, 1990). Depletion of around 70% of dopaminergic neurons until the onset of motor function impairment is a currently often quoted general concept. However, the pathological study on which this is based, used cell body counts in a 'regional semi-quantitative' study in only 20 PD cases with a disease duration from 1.5 to 38 years, and 36 control cases. By backwards

calculation, it was estimated that the preclinical (premotor) period was an average of 4.7 years, during which time there was slowly progressive neuronal loss before the threshold was reached for motor symptom onset (Fearnley and Lees, 1991). However, the overall small sample size, assumptions made about a steady rate of progression, and a very limited number of cases that were early in their disease course, raises questions about the validity of the conclusions.

Today, imaging methods can visualise the loss of dopamine much more accurately *in vivo.* Magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and positron emission tomography (PET) are often used to detect structural, functional, and molecular changes in the PD brain. Using SPECT and PET imaging methods, studies found that there is a depletion of 10% of dopaminergic neurons per year (Hilker et al., 2005, Marek et al., 2001).

The most widely applied SPECT imaging method in Parkinson's uses a tracer called FP-CIT which uses the ioflupane (^{123}I) isotope (trade name DaTSCAN) that binds presynaptically to dopamine transporters which are most dense in the putamen and caudate. The amount of tracer uptake visible in the SPECT images, in comparison to background activity in areas lacking specific dopamine transporters, allows semi-quantitative calculations of dopamine activity in the striatum.

Since its approval by the European Medicines Agency in 2000 and the US Food and Drug Administration (FDA) in 2011, SPECT imaging with DaTSCAN™ has been an important tool to differentiate PD cases from other forms of parkinsonism and tremor without a dopaminergic deficit (Hauser and Grosset, 2012, Benamer et al., 2000, Contrafatto et al., 2012). This improves diagnostic accuracy in cases with clinical uncertainty and can direct more appropriate treatment plans. In the research setting, it is now often used as a confirmatory test for the presence of dopamine deficiency, thereby increasing study power by the exclusion of more benign movement disorders such as dystonic tremor and drug-induced parkinsonism.

A retrospective survey of physicians treating 125 patients showed that DaTSCAN was requested in 63% due to ambiguous clinical presentation of the patients, in 46% because patients did not respond to treatment, and in <1% because patients were planned for participation in a clinical trial (Seifert and Wiener, 2013). Another study showed that 131 subjects with clinically uncertain parkinsonian syndrome (CUPS) undergoing DaTSCAN

imaging had significantly more changes in clinical management after 12 weeks compared to 136 cases without a scan (Kupsch et al., 2013), and this effect was still shown at 1-year follow-up (Kupsch et al., 2012). However, its use remains limited in clinical practice to cases of diagnostic uncertainty, for reasons of cost and the small quantity of radiation exposure.

Age at symptom onset but also disease duration have an impact on striatal uptake ratios. A recent study using PPMI data, compared early and mild/late-onset PD and showed that both age groups have a similar rate of decline of a derived 9% per annum in dopaminergic denervation (Koros et al., 2020). Other SPECT studies found that older age at onset is associated with greater impairment of striatal binding (Pagano et al., 2016) and that an increased disease duration is associated with a decrease in putamen uptake contralateral to the affected body side (Badoud et al., 2016).

Disease stages and motor subtypes, as well as the symptomatic body sides, are associated with the number of striatal dopamine transporters. A large SPECT with DaTSCAN study with 301 PD cases and 110 controls showed an association of increased disease stage with decreased ipsilateral (unaffected) caudate uptake (Badoud et al., 2016).

Patients with an H&Y score of 2 had a significantly lower uptake in the ipsilateral putamen, and a significantly lower uptake ratio in the ipsilateral caudate, compared to H&Y 1 (Sanjari Moghaddam et al., 2018). A significantly higher striatal uptake ratio was found in tremor-dominant presenting PD cases compared to akinetic-rigid and mixed type in a study including 67 PD cases (Spiegel et al., 2007).

5.1.2 Hemi-Parkinson's as a marker of presymptomatic disease.

Parkinson's is well recognised as a disease typified by the onset of motor symptoms on one side (unilateral) which then later progresses into an asymmetric presentation in around 87% of patients (Barrett et al., 2011), where one body side is more affected than the other (asymmetric), the remaining small proportion having a more symmetric pattern.

Pathologically, it has been established that patients with a clinically asymmetrical disease onset show significantly asymmetric degeneration in the substantia nigra with greater neuronal loss contralateral to the initially affected bodyside (Kempster et al., 1989). An MRI study also showed significant differences between symptomatic and asymptomatic hemispheres in early-onset hemi-PD, supporting these other observations of an asymmetric degeneration of the nigrostriatal pathway (Wang et al., 2015). This was confirmed by several studies using F-dopa, and DAT tracers with SPECT and PET imaging methods in hemi-PD. These studies showed a reduction in tracer uptake of around 50% in the affected putamen and a reduction of 25% to 35% in the unaffected putamen (Guttman et al., 1997, Sawle et al., 1994, Morrish et al., 1995, Rakshi et al., 1999).

These studies suggest that there is a threshold of striatal degeneration of around 50% and that once the initially asymptomatic putamen reaches this, the patient will develop bilateral symptoms. Within 3 to 6 years, most patients will progress clinically from unilateral to bilateral involvement, often retaining an asymmetric pattern where the onset side is worse. The reason for this lateralisation is not yet fully understood.

One suggested explanation is that there is more effective compensation from the less affected side, which increases dopamine synthesis and release from the surviving neurons (Boulet et al., 2008, Brotchie and Fitzer-Attas, 2009, Meissner et al., 2003, Ungerstedt, 1971). Another study suggested it might be related to handedness, as most PD patients are right-handed and 72% of right-handed patients had lower uptake ratios in the left posterior putamen, and 28% had a marked reduction in the right posterior putamen (Scherfler et al., 2012). This study concluded that the asymmetric degeneration in the putamen is not random and acknowledges that given the 28% with a reduction in the right putamen this was only a partial explanation of the observation. There are additional observations regarding cortical changes that are disease-related (Bruck et al., 2004, Mak et al., 2014) and may be a marker of disease progression (Jubault et al., 2011).

Several studies support the idea of an extensive presymptomatic phase in PD, where dopaminergic degeneration progresses but clinical motor impairments have yet to present. Hemi-Parkinson's patients are in an early stage of disease progression and are evidence for this assumption, as they show a bilateral striatal degeneration, yet they have neither signs nor symptoms in one side of the body (Marek et al., 1996, Tissingh et al., 1998).

The length of the presymptomatic phase derived from imaging studies varies greatly between studies, from 2 to 8 years (de la Fuente-Fernandez et al., 2011, Hilker et al., 2005, Morrish et al., 1998, Nurmi et al., 2001). However, there are some difficulties with these calculations, for reasons cited earlier. In addition, some normal individuals with dopamine activity at the lower end of the normal range, and patients with early Parkinson's with a presumed decline from high normal ranges to levels that are abnormal for them, but overlapping with healthy individuals, are observed in many studies (Benamer et al., 2003, Booij et al., 1997).

The PPMI study allows the investigation of SPECT DaTSCAN uptake ratios in an early-onset and drug-naïve PD cohort with a comprehensive dataset, to shed light on differences and similarities between PD cases and healthy controls but also to analyse how much dopamine is lost before motor symptoms manifest clinically, by examining cases very early in their clinical course.

5.2 Materials and Methods

Data

Data were analysed from the Parkinson's Progression Markers Initiative (PPMI). The study was introduced in section 1.3. Data were obtained from https://www.ppmi-info.org/ (download: 03/2018) (Parkinson Progression Marker, 2011). Patients with a reported change in diagnosis such as other forms of parkinsonism were excluded from the analysis.

Data preparation

Patients enrolled with the recent onset cohort in PPMI had a confirmed dopamine transporter deficit via SPECT imaging (using DaTSCAN™ or in a small number of cases VMAT-2 PET) at screening (-45 days before study enrolment) and had repeat scans at 12, 24, 48, and 60 months of study duration. Subjects from the healthy control cohort had no first-degree relatives with idiopathic PD and did not take drugs interfering with tracers used at SPECT imaging. Healthy controls were scanned for DAT once, at screening.

Data acquisition

The target dose for subjects was 185 MBq or 5.0 mCi of DaTSCAN™. The dose range for injection was 111 to 185 MBq or 3.0 to 5.0 mCi of DaTSCAN™. Subjects were imaged 4 ± 0.5 hours after tracer injection, so that wash-out of the tracer was consistent between patients.

According to PPMI documentation, SPECT raw projection data from all imaging centres was imported to a HERMES (Hermes Medical Solutions, Stockholm, Sweden) system for iterative (HOSEM) reconstruction. The reconstructed HOSEM files were then transferred to the PMOD (PMOD Technologies, Zurich, Switzerland) for subsequent processing. The images were attenuation corrected using the Chang 0 method and then filtered with a Gaussian 3D 6.0mm filter and normalised to the Montreal Neurologic Institute (MNI) space. A single slice image was generated from multiple axial slices, cantered around the slice with the highest striatal uptake. Regions of interest (ROI) were placed on the left and right caudate, and left and right putamen, using the occipital cortex as reference tissue. Count densities for each region were extracted and used to calculate striatal binding ratios (SBRs) for each of the 4 regions. SBR was calculated as $(\frac{target\ region}{reference\ region}) - 1$.

Analysis in this chapter

For the comparison of dopamine transporter readings in early motor Parkinson's with healthy controls, we used the earliest scan of PD participants, restricting to cases with a disease duration of less than or equal to 1 year at the time of scanning. The analysis of hemi-Parkinson's cases was based on patients with a Hoehn & Yahr score of 1 or 1.5 (i.e. unilateral, or unilateral and axial), hereafter referred to as grade 1. Long-term data analysis was limited to up to 6 years of disease duration, due to missing data at later time points.

Uptake ratios and percentage change

To give a better indication of the variation in tracer uptake across regions, caudate and putamen were grouped into better and worse side, rather than left and right. In the comparison of PD cases with healthy controls, the percentage change in dopaminergic loss was derived for PD cases, using healthy control values as 100% in the formula $100 - (\left(\frac{HC-PD}{HC}\right) x\ 100)$. Taking the uptake readings from PD and healthy controls *together*, each region was grouped into quartiles from Q1-Q4 (low to high), using the 25th, 50th, and 75th percentile cut-offs as follows: Q1 if the uptake value was <25th percentile of the cut-off, Q2 if uptake value was ≥25th and <50th, Q3 if uptake was ≥50th and <75th, and Q4 if the value was ≥75th percentile value.

Linear regression to assess the latency period

Longitudinal data were used for patients with a completed SPECT scan and MDS UPDRS 3 scoring at any time point. Univariate linear regression models were fitted for the association of the predictor variables MDS UPDRS 3 and symptom duration with the outcome of the regional striatal uptake ratios. An equation derived from these regression models (y=mx+b) was then used to calculate the preclinical symptom duration until the regression line meets the mean uptake value for healthy controls.

Statistical analysis

To test for differences in striatal DaTSCAN binding ratios in healthy controls vs early Parkinson's, logistic regression models were fitted for the binary outcome variables of cohort affiliation. All models were adjusted for gender, and age at scan. Multicollinearity was considered and models were adjusted accordingly. Predictor variables were demographics, linear binding ratios, and ordinal uptake quartiles from lowest (1) to highest (4).

For the correlation of clinical disease severity and dopamine activity, a logistic regression model was fitted for the binary outcome of Hoehn & Yahr scoring with 1(unilateral) and 2 (bilateral). All models were adjusted for gender, age at diagnosis, and disease duration. Predictor variables were demographics, clinical scores for motor and non-motor severity, and striatal binding ratios.

Challenge test responses, clinical scorings, and prescribed LEDD were taken from the latest reported challenge test visit. Challenge test responses were dichotomised using the ≥24.5% cut-off as described in chapter 4.2. Logistic regression models were fitted, using the binary challenge test response as outcome, and striatal uptake ratios as predictor variables. Linear regression models were fitted, using LEDD at the latest challenge test as outcome, and striatal uptake ratios as predictor variables. All models were adjusted for age at diagnosis, gender, and disease duration.

Model estimates for logistic regression were reported as p-value, odds ratio and 95% confidence intervals. Estimates for linear regression models were reported as beta coefficient, standard error, and multiple R-squared. For a clearer interpretation of the results, odds ratios and confidence intervals were adjusted for a 5-unit increase for MDS UPDRS scores and a 100mg increase for LEDD.

Normality of residuals was tested to detect any outliers or influential points. All p-values were 2-tailed, and hypothesis testing was conducted at 5% statistical significance. All data were processed using RStudio version 1.3.959.

5.3 Results

PPMI enrolled a total of 423 recent-onset PD cases and 196 healthy controls. 418 of those 423 (98.8%) had SPECT imaging data available, and 364 of those (86.1% of 423) had data at less than or equal to 1-year disease duration.

The mean age at enrolment in the PD cohort was 61.3 (9.7) years, and at scanning was 61.2 (9.7) years, giving a mean disease duration of 0.3 (0.3) years at the time the scan was conducted. The cohort consisted of 126 (34.6%) women. 352 PD (98.9% of 356) cases had H&Y scoring available for the specified time interval. 111 (31.5% of 352) were grouped into H&Y 1 with unilateral involvement, and 241 (68.5% of 352) cases were H&Y 2 with bilateral involvement (Table 5-1).

Among the healthy controls, 2 cases were on prescribed antiparkinsonian medication without evident explanation and were therefore excluded from analysis, and 3 did not have any SPECT imaging data, resulting in 191 (97.4% of 196) control cases with adequate quality SPECT imaging data available (Figure 5-1). Among the healthy controls were 68 (35.6%) women. The mean age of the controls at enrolment was 60.6 (11.3) years and mean age at the time of imaging was 60.7 (11.3) years.

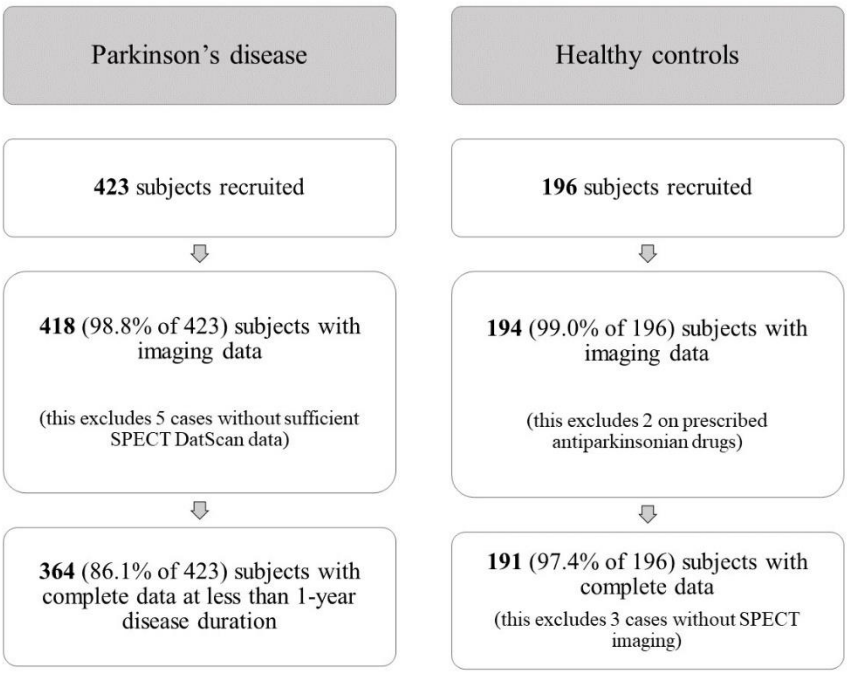

Figure 5-1: Flow-chart outlining the cohort selection process for both, subjects with Parkinson's and healthy controls.

5.3.1 Dopamine transporter readings in early motor Parkinson's compared to controls

A direct comparison of uptake ratios in the striatal regions caudate and putamen, comparing Parkinson's and healthy controls showed significantly reduced values in PD, but there was overlap between the two groups (Figure 5-2).

Comparison of striatal uptake levels in Parkinson's with healthy controls

In the caudate region, considering the side with the higher reading, PD patients had a significantly lower uptake value of 2.18 (0.58) vs 3.11 (0.64) in healthy controls (OR: 0.07, 95% CI: 0.04-0.1, p<0.001). This translates to 70.1% of the normal uptake level (defining healthy controls as 100%). The highest proportion of PD patients was in the lowest uptake quartile with 135 (37.1%), followed by 117 (32.1%) in the second lowest quartile. Healthy controls were predominantly in the highest uptake quartile with 103 (53.9%), however, 2 cases (1.1%) were in the lowest and 23 (12.0%) were in the second lowest quartile.

Considering the caudate side with the worse reading, the uptake in Parkinson's was 1.81 (0.53) and significantly lower compared to healthy controls with 2.88 (0.60) (0.03, 0.01-0.05, p<0.001). This difference translates into an uptake level in Parkinson's of 62.8% of the normal uptake. Most PD cases were in the lowest uptake quartile 135 (37.1%), followed by 132 (36.3%) in the second lowest quartile. 1 (0.5%) of healthy controls had caudate readings in the lowest quartile for uptake, and 9 (4.7%) were in the second lowest quartile, with the majority of healthy controls being in the highest uptake quartile.

In the putamen, considering the side with the higher reading, the binding ratio of 1.00 (0.37) in Parkinson's was significantly lower compared to healthy controls at 2.27 (0.57) (0.003, 0.001-0.008, p<0.001). PD patients had a 44.1% uptake level compared to 100% in healthy controls. 138 (37.9%) PD cases were in the lowest uptake quartile, followed by 134 (36.8%) in the second lowest quartile. Most healthy controls were in the highest quartile (131, 68.6%) but 3 (1.6%) cases had uptake values in the second lowest quartile.

In the worse side of the putamen, cases with PD had an uptake ratio of 0.68 (0.27), compared to a significantly higher uptake of 2.04 (0.54) in healthy controls (0.0005, 0.00008-0.002, p<0.001). This calculated as an uptake level of 33.3% in PD, compared to 100% in healthy controls. Again, the majority of PD cases had very low readings: 139 (38.2%) in the lowest quartile and 138 (37.9%) in the second lowest quartile. The difference to healthy controls

became even clearer, with no healthy cases being in the two lowest quartiles, and 136 (71.2%) in the highest uptake quartile.

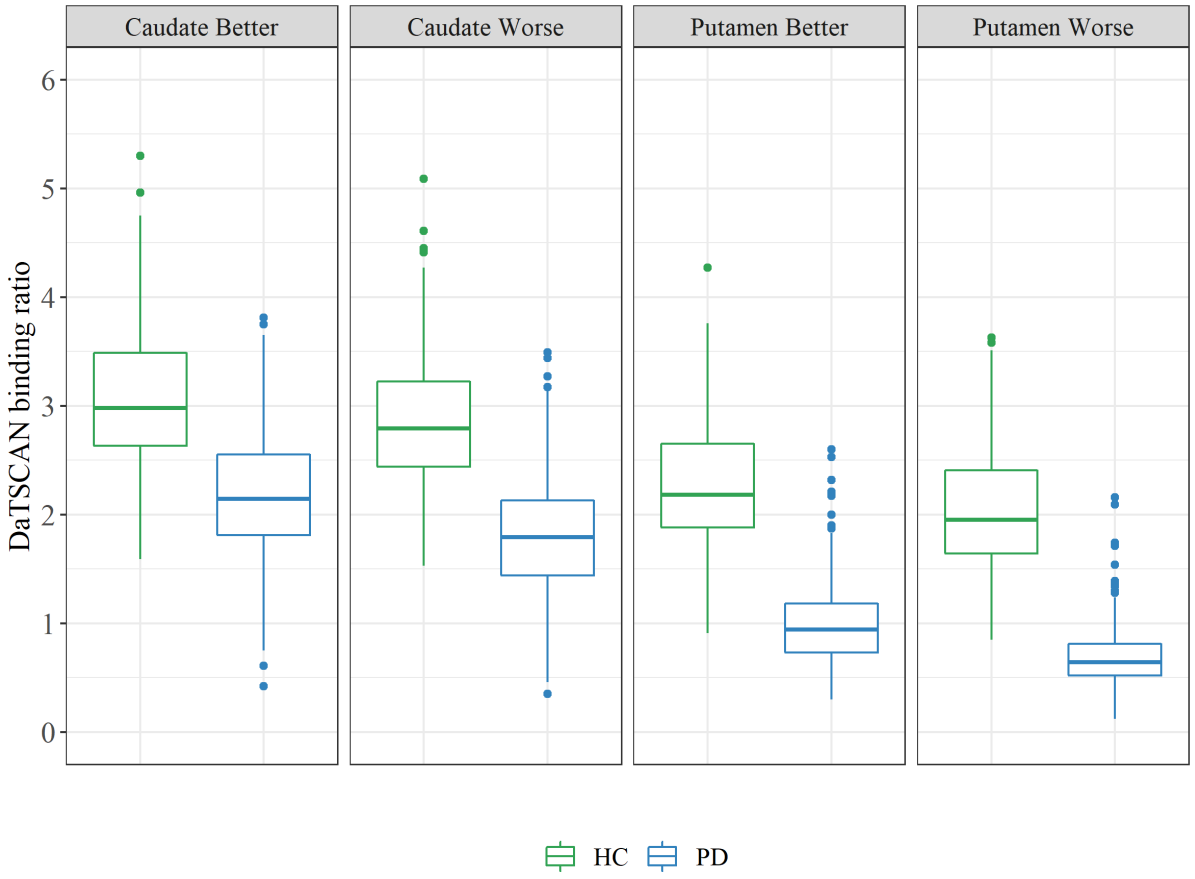

Figure 5-2: Comparison of tracer uptake in the different brain regions for healthy controls and Parkinson's subjects.
Tracer uptake and consequently the presence of dopamine transporters is greater in healthy controls compared to early motor Parkinson's. Parkinson's cases showed a particularly high number of cases with high or low values, represented by dots (outliers) above or below the boxes. Data are median and interquartile range (box), minimum and maximum (whiskers).

Table 5-1: Demographics and uptake ratios for 364 Parkinson's cases at less than or equal to 1 year disease duration and 191 healthy controls at their earliest scan.

Variable	Parkinson's cohort	Healthy controls	Model estimate (95% CI)	p-value
Total n	364 (86.1% of 423)	191 (97.4% of 196)		
Gender				
Female	126 (34.6%)	68 (35.6%)		(ref)
Male	238 (65.4%)	123 (64.4%)	1.04 (0.72, 1.50)	0.8
Age at enrolment	61.3 (9.7)	60.6 (11.3)	1.01 (0.99, 1.02)	0.50
Age at scan	61.2 (9.7)	60.7 (11.3)	1.00 (0.99, 1.02)	0.60
Caudate better	2.18 (0.58)	3.11 (0.64)	0.07 (0.04, 0.1)	**<0.001**
Uptake level	70.1%	100%		
Quartiles			Logistic regression	
1 (<1.98)	135 (37.1%)	2 (1.1%)		(ref)
2 (<2.44)	117 (32.1%)	23 (12.0%)	0.07 (0.01, 0.3)	**<0.001**
3 (<2.94)	73 (20.1%)	63 (33.0%)		

4 (≥2.94)	39 (10.7%)	103 (53.9%)		
Caudate worse	1.81 (0.53)	2.88 (0.60)	0.03 (0.01, 0.05)	**<0.001**
Uptake level	62.8%	100%		
Quartiles			Logistic regression	
1 (<1.63)	135 (37.1%)	1 (0.5%)		(ref)
2 (<2.11)	132 (36.3%)	9 (4.7%)	0.1 (0.006, 0.6)	**0.03**
3 (<2.65)	73 (20.1%)	65 (34.0%)		
4 (≥2.65)	24 (6.6%)	116 (60.7%)		
Putamen better	1.00 (0.37)	2.27 (0.57)	0.003 (0.001, 0.008)	**<0.001**
Uptake level	44.1%	100%		
Quartiles			Chi-square	
1 (<0.84)	138 (37.9%)	0 (0.0%)	9.8	**<0.001**
2 (<1.18)	134 (36.8%)	3 (1.6%)	9.1	**<0.001**
3 (<1.96)	84 (23.1%)	57 (29.8%)	1.7	0.66
4 (≥1.96)	8 (2.2%)	131 (68.6%)	17.1	**<0.001**

Putamen worse	0.68 (0.27)	2.04 (0.54)	0.0005 (0.00008, 0.002)	**<0.001**
Uptake level	33.3%	100%		
Quartiles			Chi-square	
1 (<0.58)	139 (38.2%)	0 (0.0%)	9.9	**<0.001**
2 (<0.82)	138 (37.9%)	0 (0.0%)	9.8	**<0.001**
3 (<1.73)	84 (23.1%)	55 (28.8%)	1.5	1.00
4 (≥1.73)	3 (0.8%)	136 (71.2%)	18.2	**<0.001**
Dose injected	33.7 (64.1)	32.3 (61.9)	1.00 (1.00, 1.00)	0.81

Data are mean (SD) for continuous and n (%) for categorical variables. Data were corrected for age at scan and gender.

Comparison of lateralised hemisphere uptake ratios in Parkinson's with the degree of uptake in healthy controls

As an alternative comparison to the above approach, comparison of symptomatic and asymptomatic hemispheres in early PD was made. For the PD cases, this used the uptake values ipsilateral to the affected bodyside, and for controls, this used higher side readings. Again, results showed significant differences for each side of putamen and caudate, but there was overlap between PD and controls (Figure 5-3).

In the ipsilateral (asymptomatic) caudate, PD patients had a significantly lower binding ratio of 2.16 (0.57) compared to the better-side caudate uptake of 3.11 (0.64) in healthy controls (OR: 0.07, 95% CI: 0.05-0.10, $p<0.001$). In the contralateral (symptomatic) caudate, Parkinson's patients had a significantly lower uptake of 1.83 (0.54) compared to the worse-side uptake ratio of 2.88 (0.60) in healthy controls (0.04, 0.02-0.06, $p<0.001$).

In the ipsilateral (asymptomatic) putamen, PD cases had an uptake of 0.98 (0.38), which was significantly lower than the better-side uptake in healthy controls of 2.27 (0.57) (0.005, 0.002-0.01, $p <0.001$). In the contralateral (symptomatic) putamen, PD again had significantly lower binding ratios of 0.70 (0.27) compared to healthy controls at 2.04 (0.54) (0.0009, 0.0003-0.003, $p<0.001$).

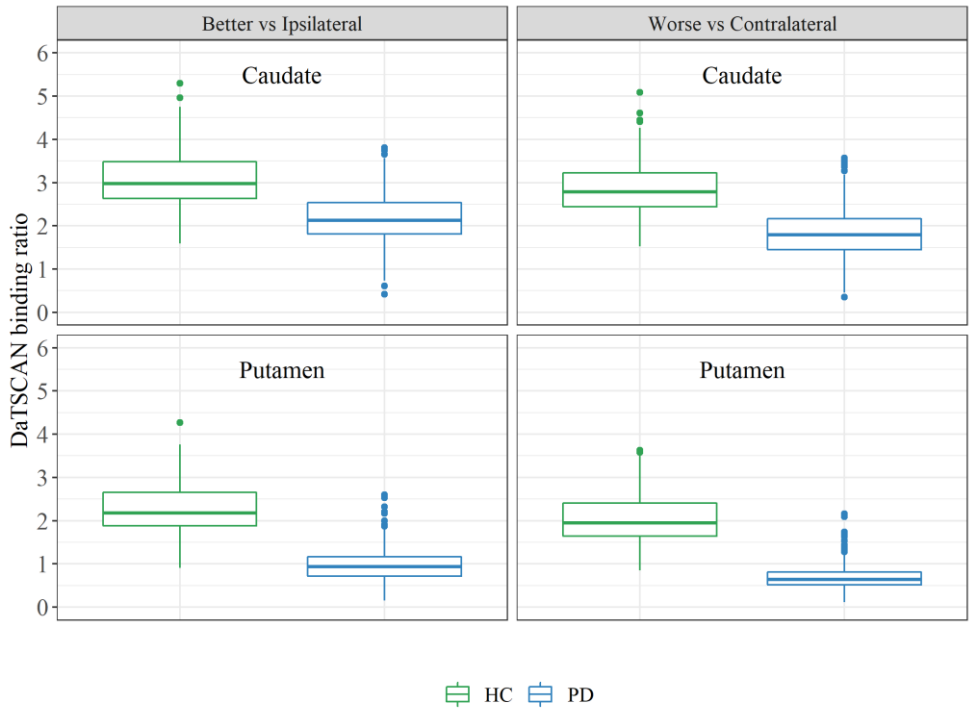

Figure 5-3: Comparison of better-side and worse-side striatal uptake ratios in healthy controls against ipsilateral (asymptomatic) and contralateral (symptomatic) uptake ratios in cases with Parkinson's.
In all striatal regions, PD had a lower binding ratio than HC ($p<0.001$). Better vs ipsilateral but also worse vs contralateral values showed overlap between healthy controls and PD, especially in the caudate.

Deriving the latency period from dopamine transporter readings

Correlating the striatal uptake ratios of all four regions with the MDS UDPRS 3 score of all patients with a recorded SPECT scan from baseline to 5 years follow-up, showed a statistically significant negative relationship between the two variables: with decreasing tracer uptake, patients had higher (worse) MDS UPDRS 3 scores in all four striatal regions ($p<0.001$). After appropriate adjustment of the linear regression models for age at diagnosis, gender, and disease duration, the results remained statistically significant, with linear regression explaining around 13% of variation in all regions (Multiple R-squared).

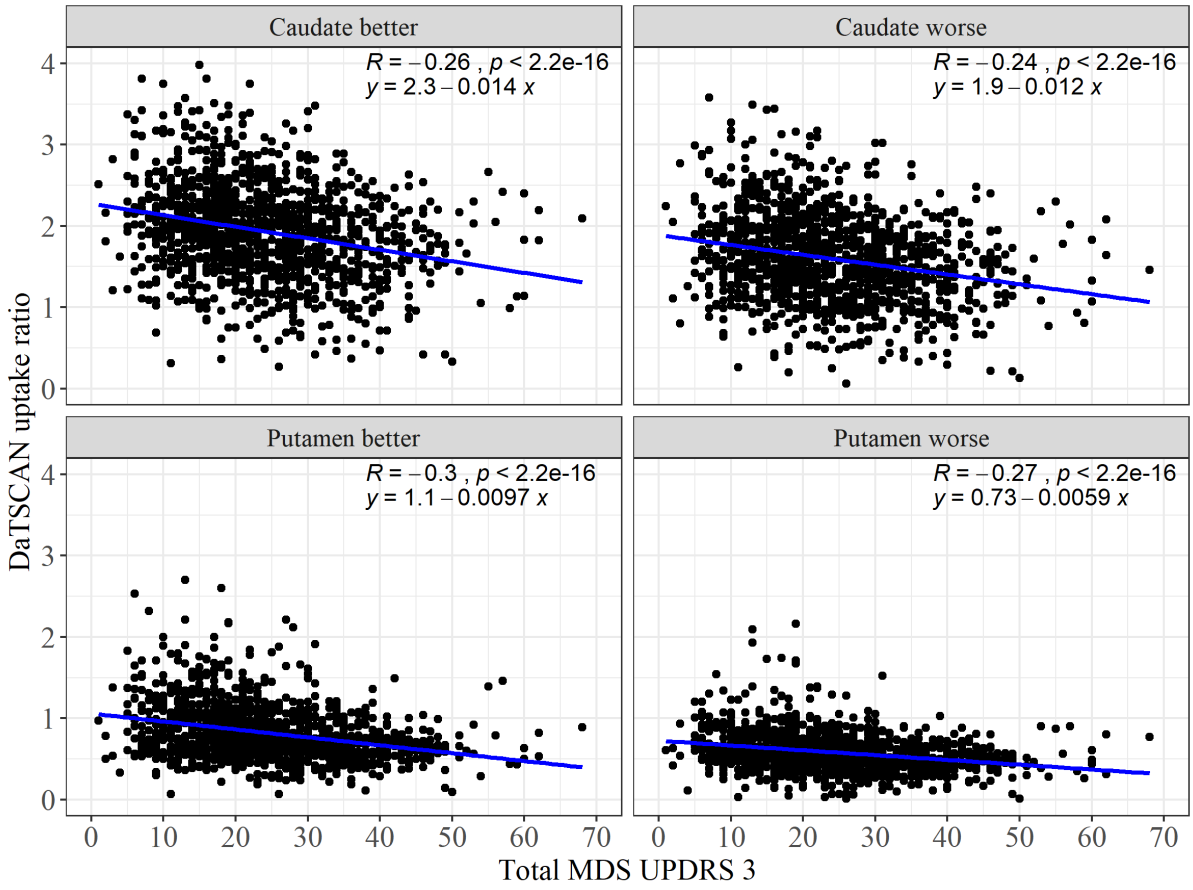

Figure 5-4: Relationship between striatal DaTSCAN uptake and the clinical severity measured with MDS UPDRS 3.
All striatal regions show a significant negative correlation with the MDS UDPRS 3 score measured at any time point within 5 years follow-up: the lower the uptake value, the worse the motor uptake. Slope of the fitted linear regression line is steeper in the caudate compared to the putamen, which is caused by the generally lower uptake ratios in the putamen.

Assessing the relationship between better and worse striatal degeneration with symptom duration showed a statistically significant relationship in all four regions: with increasing symptom duration, loss of dopaminergic transporters worsens ($p<0.001$, Figure 5-5).

The resulting linear regression equations allowed the calculation of the preclinical phase in the better-side caudate as 9.5 years (Table 5-2). Using the linear regression model at annual symptom duration time points, a mean annual decrease of 5.0% could be derived. The worse caudate reading predicted a longer preclinical phase of 11.5 years, and a mean annual decline of 5.1% was derived.

The preclinical phase in the putamen was much longer compared to the caudate, while noting that a large proportion of uptake ratios were very low (see Discussion regarding the potential impact) (Table 5-3). In the better putamen, the latent phase was calculated as 19.2 years before symptom onset and the mean annual decline was 8.2%. The worse side of the caudate was calculated with a preclinical phase of 36.2 years with a mean annual decline of 6.1%.

Table 5-2: Linear regression model from longitudinal observations translated into preclinical phase and annual decline.

	Caudate better	Caudate worse	Putamen better	Putamen worse
Equation (lm)	y=2.2-0.096*x	y=1.9-0.085*x	y=1-0.066*x	y=0.7-0.037*x
Uptake in healthy controls, mean	3.11 (0.64)	2.88 (0.60)	2.27 (0.60)	2.04 (0.57)
Preclinical phase, years	9.5	11.5	19.2	36.2
Annual decline, mean	5.0%	5.1%	8.2%	6.1%
0	2.20	1.90	1.00	0.70
1	2.10 (-4.5%)	1.82 (-4.2%)	0.93 (-7.0%)	0.66 (-5.7%)
2	2.00 (-4.8%)	1.73 (-5.0%)	0.87 (-6.5%)	0.63 (-4.5%)
3	1.91 (-4.5%)	1.65 (-4.6%)	0.80 (-8.0%)	0.59 (-6.3%)
4	1.82 (-4.7%)	1.56 (-5.5%)	0.74 (-7.5%)	0.55 (-6.8%)
5	1.72 (-5.5%)	1.48 (-5.1%)	0.67 (-9.5%)	0.52 (-5.5%)
6	1.62 (-5.8%)	1.39 (-6.1%)	0.60 (-10.4%)	0.48 (-7.7%)

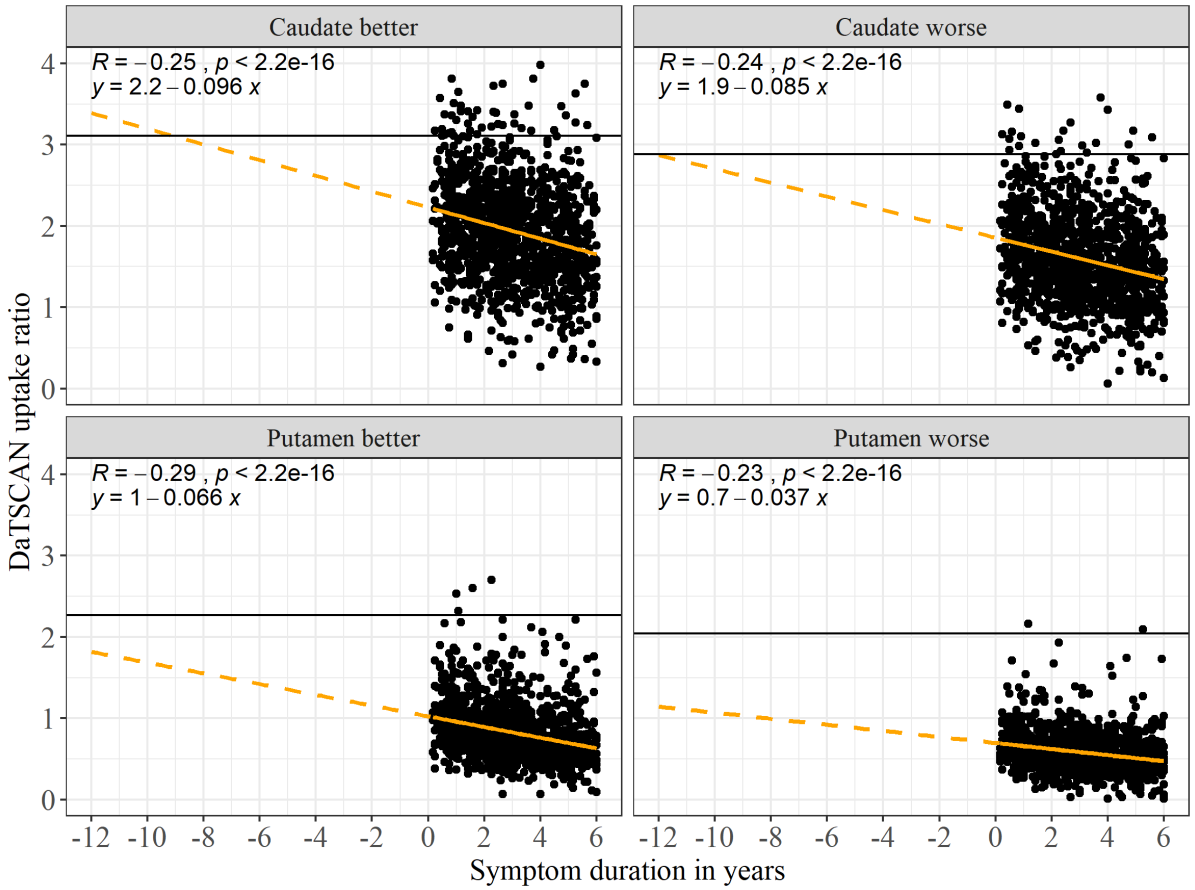

Figure 5-5: Deriving the preclinical period according to dopamine transporter readings. Observed uptake ratios were plotted against the symptom duration in years (0-6 years), and a statistically significant association of a decrease of striatal uptake over time was found ($p<0.001$). The linear regression line was drawn backwards to a minimum of -12 years, to find the time point where the predictive linear regression line (orange dashed line) meets the mean uptake value of healthy controls (black horizontal line) in the according area. Only in the caudate, both lines meet within the plotted time frame, resulting in a preclinical phase of around 9-11 years before symptom onset. The putamen slope was shallower, resulting in a preclinical phase of 19-36 years before symptom onset. It is important to note, that binding ratios in the putamen have a much smaller variance, compared to the caudate, which results in a more flattened slope of the regression line.

5.3.2 Relationship between dopamine activity and clinical severity

The PD cohort was grouped into 111 (31.5%) cases with a H&Y stage 1 (unilateral involvement), and 241 (68.5%) with H&Y stage 2 (bilateral involvement) (Table 5-3).

Patients with a clinically unilateral presentation were significantly younger at diagnosis, at 57.9 (9.9) years, compared with 62.4 (9.2) years at diagnosis for the bilateral group (OR: 1.05, 95% CI: 1.03-1.08, p<0.001). Although the average disease duration was the same between unilateral and bilateral cases, the unilateral cases had a slightly smaller SD, and hence a narrower spread for disease duration: unilateral cases 0.3 months (0.2) compared to bilateral cases 0.3 (0.3) (3.06, 1.17-8.51, p=0.03).

In the unilateral group, 50 (45.0%) showed symptoms on the left body side and 61 (55.0%) on the right side. In the bilateral group, 112 (46.5%) had symptoms predominantly on the left body side, 125 (51.9%) predominantly on the right side, and 4 (1.7%) cases were affected equally on the left and right body side.

Considering clinical scores, patients in the H&Y 1 group had a lower MDS UPDRS 1 of median 1.0 (0.0-2.0) (3.27, 1.43-8.17, p=0.01), MDS UPDRS 2 of median 3.0 (2.0-6.0) (1.83, 1.31-2.63, p<0.001), and MDS UPDRS 3 of 12.0 (9.0-16.0) (3.62, 2.74-4.97, p<0.001) at the time of SPECT imaging, compared to patients with a H&Y of 2 who had a median MDS UPDRS 1 of 1.0 (0.0-2.0), MDS UPDRS 2 of 5.0 (3.0-8.0) and a median MDS UPDRS 3 of 22.0 (18.0-28.0).

There were no significant differences in motor subtype between the two H&Y stages. 66 (59.5%) of H&Y 1 patients had a tremor-dominant subtype, compared to 172 (72.0%) in the H&Y 2 group. 28 (25.2%) vs 41 (17.2%) were PIGD, and 26 (10.9%) were indeterminate.

Comparing better, worse, contralateral, and ipsilateral uptake ratios between the two H&Y stages, there were no significant differences. However, in comparison to unilateral cases, bilateral PD patients had a slightly lower uptake in the better putamen side (0.45, 0.24-0.83, p=0.01), which was also seen when using data for the ipsilateral side (0.44, 0.23-0.80, p=0.008). A comparison of worse and contralateral sides showed no significant differences.

There was no statistically significant association of handedness with H&Y staging. 102 (91.9%) of unilateral cases were right-handed vs 214 (88.8%) in the bilateral group. 6 (5.4%) vs 21 (8.7%) were left-handed, and 3 (2.7%) vs 6 (2.5%) were categorised as mixed.

Overall comparison of ipsilateral and contralateral striatal uptake

After establishing differences between the H&Y groups within the same striatal regions, a comparison of the ipsilateral (unaffected) with the contralateral (affected) striatal hemisphere was conducted (Figure 5-6). In both regions, caudate and putamen, the ipsilateral side had a significantly higher binding ratio compared to the contralateral side.

In the caudate, the overall ipsilateral uptake was 2.16 (0.57) and was significantly higher compared to the contralateral side at 1.83 (0.54) (OR: 2.95, 95% CI: 2.20-3.97, p<0.001). Cases in H&Y 1 had a mean ipsilateral caudate tracer uptake of 2.26 (0.62) which was significantly higher than the uptake in the contralateral side of 1.90 (0.56) (2.88, 1.80-4.77, p-value<0.001). In the H&Y 2 group, the ipsilateral caudate uptake was 2.11 (0.54), compared to a significantly lower uptake in the contralateral side of 1.80 (0.53) (3.01, 2.09-4.42, p<0.001). Using the combined mean uptake in the caudate of healthy controls of 2.99 (0.63) as 100%, the ipsilateral caudate in Parkinson's had an overall uptake of 72.2%, and 75.6% in H&Y 1 and 70.6% in H&Y 2 cases (Table 5-3). In the contralateral caudate, the total uptake was 61.2%, 63.5% in H&Y 1, and 60.2% in cases with H&Y 2.

In the putamen, the overall ipsilateral uptake was 0.98 (0.38) and was significantly higher compared to the 0.70 (0.27) in the contralateral putamen (17.8, 9.9-33.2, p<0.001). In the H&Y 1 group, the mean ipsilateral uptake was 1.08 (0.40) and significantly higher compared to the contralateral side at 0.74 (0.24) (37.2, 12.8-123.3, p<0.001). In patients with H&Y 2, the ipsilateral side had a mean uptake of 0.93 (0.36) which was higher than the contralateral side with 0.68 (0.29) (13.9, 6.2-29.7, p<0.001). Overall, the ipsilateral putamen uptake was 45.4% of the combined mean uptake in healthy controls of 2.16 (0.56) which was defined as 100%. The uptake was 50.0% in the H&Y 1 group, and 43.1% in cases with H&Y 2. In the contralateral putamen, the total uptake was 32.4%, 34.3% in H&Y 1, and 31.5% in the H&Y 2 group.

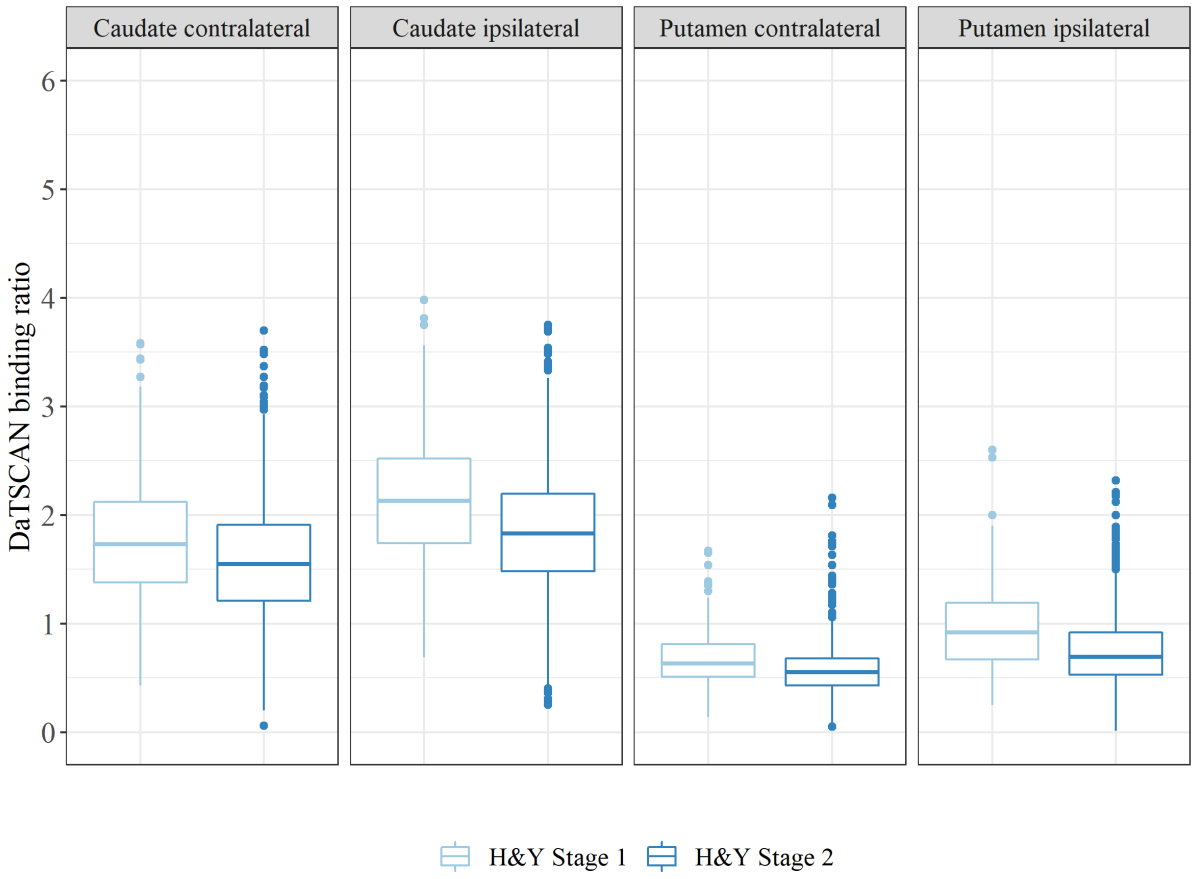

Figure 5-6: DaTSCAN binding ratios in the contralateral and ipsilateral striatal regions, grouped by disease severity (H&Y).

Cases with H&Y 1 (unilateral) had higher uptake compared to H&Y 2 (bilateral), which was statistically significant in the ipsilateral putamen. The difference is more dramatic in the caudate area compared to the putamen. There was a significantly higher uptake ratio in the ipsilateral striatal region, compared to the contralateral region ($p<0.001$). This held true in the comparison between the H&Y groups, and also overall in the Parkinson's cohort.

Table 5-3: Demographic data and striatal binding ratios for 111 cases with H&Y I and 241 cases with H&Y II at a disease duration of less than or equal to 1 year.

	Total	H&Y 1	H&Y 2	Model estimate (95% CI)	p-value
Number of Patients	352 (100%)	111 (31.5%)	241 (68.5%)		
Gender					
Female	122 (34.7%)	44 (39.6%)	78 (32.4%)	(ref)	
Male	230 (65.3%)	67 (60.4%)	163 (67.6%)	1.32 (0.82, 2.14)	0.25
Age at diagnosis	60.9 (9.7)	57.9 (9.9)	62.4 (9.2)	1.05 (1.03, 1.08)	**<0.001**
Age at enrolment	61.3 (9.7)	58.2 (9.8)	62.8 (9.2)	1.05 (1.03, 1.08)	**<0.001**
Age at scan	61.2 (9.7)	58.2 (9.8)	62.7 (9.2)	1.05 (1.03, 1.08)	**<0.001**
Disease duration	0.3 (0.3)	0.3 (0.2)	0.3 (0.3)	3.06 (1.17, 8.51)	**0.03**

	Total	H&Y 1	H&Y 2	Model estimate (95% CI)	p-value
Dominant symptomatic side					
Left	112 (31.8%)	0 (0.0%)	112 (46.5%)		
Right	125 (35.5%)	0 (0.0%)	125 (51.9%)		
Left = Right	4 (1.1%)	0 (0.0%)	4 (1.7%)		
Unilateral	111 (31.5%)	111 (100%) Left: 50 (45.0%) Right: 61 (55.0%)	0 (0.0%)		
MDS UPDRS 1 at scan	1.0 (0.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	3.27 (1.43, 8.17)	**0.01**
MDS UPDRS 2 at scan	5.0 (3.0-8.0)	3.0 (2.0-6.0)	5.0 (3.0-8.0)	1.83 (1.31, 2.63)	**<0.001**
MDS UPDRS 3 at scan	19.0 (13.0-26.0)	12.0 (9.0-16.0)	22.0 (18.0-28.0)	3.62 (2.74, 4.97)	**<0.001**
Motor subtype					
Indeterminate	43 (12.3)	17 (15.3)	26 (10.9)		(ref)

	Total	H&Y 1	H&Y 2	Model estimate (95% CI)	p-value
PIGD	69 (19.7)	28 (25.2)	41 (17.2)	0.88 (0.38, 1.97)	0.75
Tremor-dominant	238 (68.0)	66 (59.5)	172 (72.0)		
Caudate uptake ratio					
Better	2.18 (0.58)	2.28 (0.62)	2.13 (0.55)	0.79 (0.52, 1.19)	0.27
Worse	1.81 (0.52)	1.88 (0.56)	1.77 (0.50)	0.76 (0.48, 1.18)	0.22
Contralateral	1.83 (0.54)	1.90 (0.56)	1.80 (0.53)	0.82 (0.53, 1.26)	0.37
Uptake level (of healthy controls)	61.2%	63.5%	60.2%		
Ipsilateral	2.16 (0.57)	2.26 (0.62)	2.11 (0.54)	0.74 (0.48, 1.12)	0.16
Uptake level (of healthy controls)	72.2%	75.6%	70.6%		

	Total	H&Y 1	H&Y 2	Model estimate (95% CI)	p-value
Putamen uptake ratio					
Better	1.00 (0.37)	1.10 (0.39)	0.95 (0.35)	0.45 (0.24, 0.83)	**0.01**
Worse	0.68 (0.26)	0.72 (0.23)	0.66 (0.28)	0.42 (0.17, 0.99)	0.05
Contralateral	0.70 (0.27)	0.74 (0.24)	0.68 (0.29)	0.48 (0.20, 1.10)	0.08
Uptake level (of healthy controls)	32.4%	34.3%	31.5%		
Ipsilateral	0.98 (0.38)	1.08 (0.40)	0.93 (0.36)	0.44 (0.23, 0.80)	**0.008**
Uptake level (of healthy controls)	45.4%	50.0%	43.1%		
Handedness					
Left	27 (7.7%)	6 (5.4%)	21 (8.7%)	1.23 (0.69, 2.36)	0.51
Right	316 (89.8%)	102 (91.9%)	214 (88.8%)		

	Total	H&Y 1	H&Y 2	Model estimate (95% CI)	p-value
Mixed	9 (2.6%)	3 (2.7%)	6 (2.5%)		

Data are mean (SD) or median (IQR) for continuous, and n (%) for categorical. MDS UPDRS scores adjusted for a 5-unit increase.

5.3.3 Treatment impact in Parkinson's

193 (54.8% of 352) underwent challenge testing for dopamine replacement therapy at a median disease duration of 4.3 years (IQR 1.2-4.5). The median off treatment score at challenge was 30 (22.0-38.0) and the median on score was 19.0 (12.0-30.0), which translated to a median improvement of 27.8% (14.3-45.2). This percentage change was dichotomised into 112 (58.0%) definite and 81 (42.0%) limited responders using the ≥24.5% cut-off. The median challenge test dose given was 150.0 (100.0-245.0) mg (Table 5-4).

There was no association of early striatal uptake ratios with long-term challenge test response to dopamine replacement therapy.

In the better-side caudate, definite responders had a mean uptake of 2.19 (0.53) and limited responders had an uptake of 2.11 (0.53) (OR: 0.87, 95% CI: 0.48-1.54, p=0.63). In the worse-side caudate, definite responders had an uptake of 1.77 (0.49) which was not significantly lower than limited responders with 1.76 (0.48) (1.07, 0.58-1.96, p=0.83) (Table 5-5).

The better-side putamen in definite responders had an uptake of 0.98 (0.36), which was not significantly different from limited responders with 0.93 (0.31) (0.85, 0.34-2.03, p=0.72). In the worse-side putamen, definite responders showed binding values of 0.67 (0.23) and limited responders had 0.63 (0.22) (0.49, 0.13-1.75, p=0.28) (Table 5-5).

The latest recorded median prescribed LEDD was 540.0 (357.0-775.0) mg. At the last recorded challenge test, most patients were on L-dopa treatment (83.4%), while 46.1% were on dopamine agonists, 43.0% were on MAO-B inhibitors, 12.4% on amantadine, 4.7% on COMT inhibitors, and 2.1% were on anticholinergics (Table 5-4).

Patients were prescribed higher LEDD at the time of their latest challenge test when their putaminal uptake ratios were lower. This was true for both, the better side (B: -224.2, Std. Error: 83.0, Multiple R-Squared 0.08, p=0.008) and worse side (-316.5, 121.9, 0.08, p=0.01), patients were prescribed higher LEDD at their latest challenge test. Both sides of the caudate did not hold any statistically significant association (p>0.05) (Table 5-5).

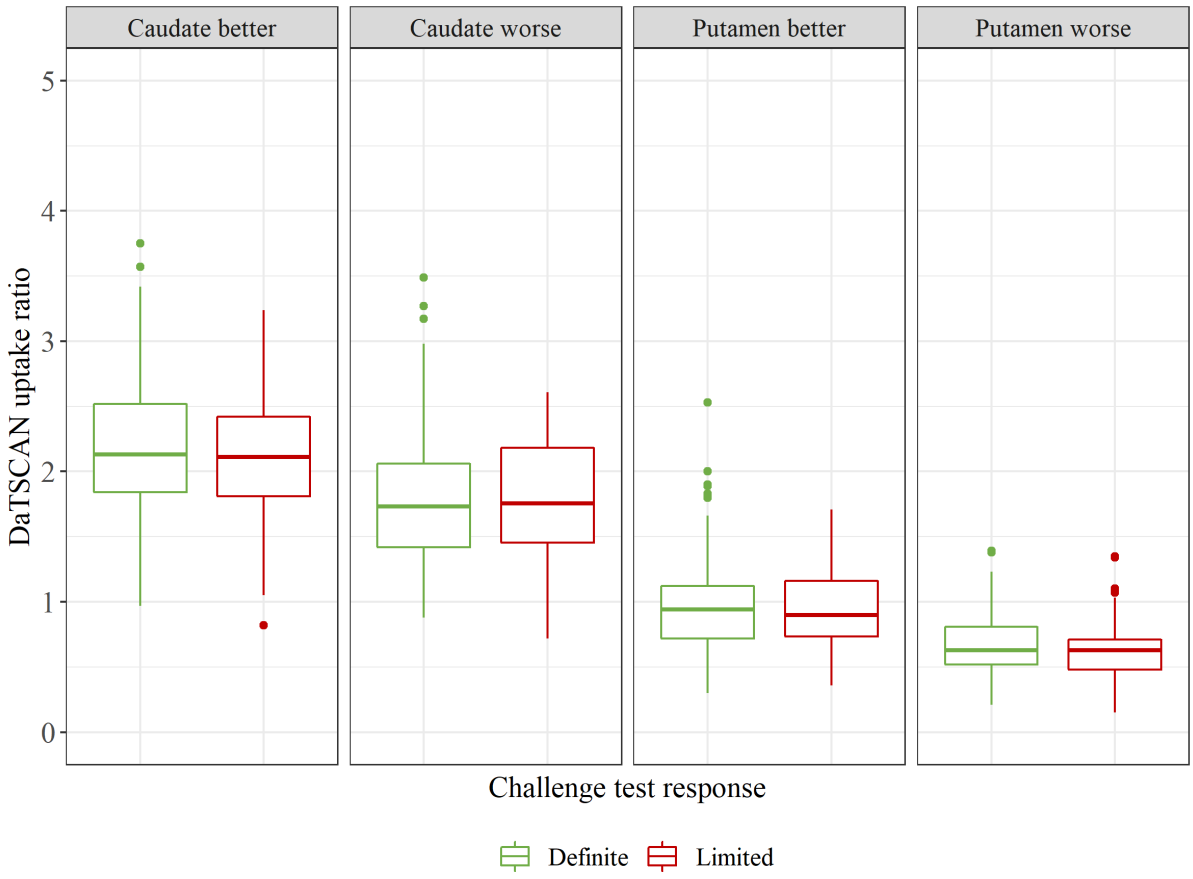

Figure 5-7: Striatal uptake ratios according to long-term response to dopamine replacement therapy challenge testing in 193 patients.
112 (58%) of patients had a definite response (≥24.5% percentage change at challenge), and 81 (42%) were limited. Striatal uptake ratios were not significantly different between the definite and limited groups ($p>0.05$).

Table 5-4: Summary statistics of cases with baseline DaTSCAN imaging and clinical information at their last recorded challenge test.

Variable		Total
Number of Patients		193 (54.8% of 352)
Disease duration at challenge		4.3 (1.2, 4.5)
Percentage improvement at challenge		27.8 (14.3, 45.2)
Response		
	Definite	112 (58.0%)
	Limited	81 (42.0%)
MDS UPDRS 3 off score at challenge		30.0 (22.0, 38.0)
MDS UPDRS 3 on score at challenge		19.0 (12.0, 30.0)
Challenge test dose		150.0 (100.0, 245.0)
Prescribed drug		
	Anticholinergic (Yes)	4 (2.1%)
	Amantadine (Yes)	24 (12.4%)
	COMT-I (Yes)	9 (4.7%)
	Dopamine agonist (Yes)	89 (46.1%)
	L-dopa (Yes)	161 (83.4%)
	MAO-B (Yes)	83 (43.0%)
LEDD mg		540.0 (357.0, 775.0)

Data are mean (SD) or median (IQR) for continuous and n (%) for categorical variables.

Table 5-5: Correlation of striatal uptake ratios with last challenge test response and prescribed LEDD in 193 patients with Parkinson's.

	Challenge test response Summary		**Logistic regression output Challenge test response**		**Linear regression output LEDD mg**			
	Definite	Limited	Model estimate (95% CI)	p-value	B	Std. Error	Multiple R^2	p-value
Caudate better	2.19 (0.53)	2.11 (0.53)	0.87 (0.48, 1.54)	0.63	-48.2	55.4	0.05	0.39
Caudate worse	1.77 (0.49)	1.76 (0.48)	1.07 (0.58, 1.96)	0.83	-90.5	58.6	0.06	0.12
Putamen better	0.98 (0.36)	0.93 (0.31)	0.85 (0.34, 2.03)	0.72	-224.2	83.0	0.08	**0.008**
Putamen worse	0.67 (0.23)	0.63 (0.23)	0.49 (0.13, 1.75)	0.28	-316.5	121.9	0.08	**0.01**

All models adjusted for age at diagnosis, gender, and disease duration at the last recorded challenge test response. Linear regression reports unstandardised regression slope, standard error, and multiple R-squared.

5.4 Discussion

In this chapter, the residual availability of dopamine transporters in very early Parkinson's was examined to define the extent of neuronal degeneration at clinical presentation, and, by focussing on early unilateral disease to determine the dopamine levels in the clinically unaffected side of the brain.

The availability of striatal dopamine transporters in early-stage Parkinson's was significantly lower compared to healthy controls, which is expected given earlier observations, while noting that most previous studies had much smaller sample sizes, particularly for the early disease stage of interest (Benamer et al., 2003, Booij et al., 1997).

Neuropathological studies calculated, by backward projection in 20 patients examined at autopsy, that patients with Parkinson's would have a neuronal loss based on cell counts of 48% in the whole of the caudal substantia nigra (but higher at 68% in the lateral ventral tier of the substantia nigra) at the time of clinical onset of the disease (Fearnley and Lees, 1991). However, an 80% loss of striatal dopamine activity at symptom onset is often mentioned (Dauer and Przedborski, 2003, Fearnley and Lees, 1991). One proposed explanation for the greater loss of dopamine activity compared to the number of surviving nigral neurones is that the remaining dopaminergic neurons are performing poorly. An alternative explanation is that the 80% loss mentioned (which is not referenced) is incorrect (Dauer and Przedborski, 2003). In the PPMI data, in 352 cases within one year of diagnosis, at worst there was 67.6% loss in the contralateral putamen, and at best 27.8% loss in the ipsilateral caudate. These findings present a major challenge to the long-held concept of 80% loss of dopamine activity at the time of symptom onset.

These findings can be considered in comparison to prior studies which are now summarised. PET studies showed that at 1.7 years disease duration, 12 PD patients had 27-45% residual activity in the putamen and 71% in the caudate, compared to 11 healthy controls (Nurmi et al., 2003). At 2 years symptom duration, another study showed a putaminal activity of 42% in the putamen and 76% in the caudate in 8 early PD cases, compared to 7 healthy controls (Nurmi et al., 2000). Another study showed a 50% activity in the contralateral putamen at 2.3 years of disease duration in 11 patients vs 10 controls (Guttman et al., 1997). Using SPECT imaging, at 2.4 years of disease duration, a residual binding activity of 58% was found in the striatum in 24 PD patients

(Pirker et al., 2002). At 3.6 years, 8 PD patients had a residual 50% tracer uptake in the whole striatum (Chouker et al., 2001).

The results from these imaging studies, despite their different scanner and tracer use, indicate that the putamen is overall more affected than the caudate and that the lateralization of striatal regions not just anatomically but also in accordance to the affected and unaffected body side is present. The PPMI study found lower uptakes in the putamen compared to the caudate, however, the better side of the putamen had a residual activity of over 40%, which is higher compared to the studies highlighted above. The same applies to the better caudate, which has a much higher uptake compared to the worse side.

Reasons for differences between these studies include technical and selection factors. Different tracers used can impact the interpretation of results. The metabolism of PET tracers mimics the conversion of L-dopa to dopamine but also the presynaptic storage of dopamine. In Parkinson's, the dopa decarboxylase is upregulated in an effort to increase the amount of dopamine in the brain, which also increases dopamine synthesis (Lee et al., 2000, Zigmond et al., 1990, Ribeiro et al., 2002). Due to this up-regulation, more dopamine is released into the synaptic cleft, potentially resulting in an overestimate of available dopamine especially by the PET tracer ^{18}F-dopa (Ribeiro et al., 2002).

In contrast, SPECT tracers follow a different pathway, as they bind to presynaptic dopamine transporters that are largely on the surface of the neurone (while ^{18}F-dopa is largely taken up intraneuronally). In the event of a shortage of dopamine in the synaptic cleft such as in PD, dopamine reuptake into the presynaptic neurone is down-regulated, to preserve the amount of available dopamine in the cleft (Zigmond et al., 1990, Brooks, 2004, Lee et al., 2000). As a result, there are fewer tracer binding events than usual. The use of tracers specifically binding to dopamine transporters can therefore result in an underestimate of dopamine transporter availability.

In conclusion, the results of these pathological and clinical imaging studies indicate that early disease PD patients still have a large number of neurons left and suggest that there remains considerable scope for neuroprotection in clinical trials.

Lateralization in accordance with the symptomatic side

A long-established feature of Parkinson's, that there is significant lateralization of features, was examined in detail in the imaging findings. The lateralization of striatal regions into contra- and ipsilateral showed this pattern in almost all cases.

The specific analysis of the dopaminergic uptake in hemi-Parkinson's cases is important, as this represents presymptomatic disease in one brain hemisphere. Despite the unilateral symptoms, bilateral depletion was shown in both striatal regions with an ipsilateral uptake of 50% in the putamen and 76% uptake in the caudate, which were significantly higher compared to the contralateral sides. This defines a threshold above the one at which symptoms are yet to emerge, and presumably reflects backup neuronal capacity along with compensatory adaptive mechanisms.

A more advanced depletion in the contralateral side was also found in other SPECT studies using the DaTSCAN tracer, reporting a reduction of 34-41% uptake in the contralateral caudate, and 22-28% in the ipsilateral caudate. The uptake in the contralateral putamen was 42-58% compared to 33-44% in the ipsilateral putamen (Filippi et al., 2005, Marek et al., 1996). All studies reported very similar uptake ratios compared to the analysis of this chapter, however, the putaminal depletion in the PPMI study was always slightly greater compared to these studies. Differences may be explained by effects of increasing age and longer disease duration at SPECT imaging, and technical factors in the delineation of brain areas, the area used as the marker of background activity, and the calculation of uptake ratios. Another factor could be the smaller sample sizes used in the other studies compared to 111 hemi-Parkinson's cases in PPMI.

Patient demographics and clinical severity

Another important finding from these studies is the overall decline with increasing disease severity and symptom duration. In the PPMI study, imaging findings correlated partially with clinical disease stage: striatal uptake ratios were significantly lower in H&Y 2 cases, compared to H&Y 1 cases, being only for the ipsilateral (less affected) caudate.

In H&Y 1 cases, other clinical SPECT imaging studies using DaTSCAN reported a contralateral caudate uptake ratio of 1.5-1.9 and an ipsilateral uptake of 1.7-2.3 (Happe et al., 2007, Sanjari Moghaddam et al., 2018, Booij et al., 1998). In the contralateral

putamen, the uptake ratio was 0.7-1.1 and 1.0-1.6 in the ipsilateral side (Happe et al., 2007, Sanjari Moghaddam et al., 2018, Booij et al., 1998). In the H&Y 2 group, contralateral caudate uptake was 1.5-1.8 with 1.6-2.1 in the ipsilateral side. In the putamen, ratios were 0.7-0.9 in the contralateral side and 0.9-1.2 in the ipsilateral side (Happe et al., 2007, Sanjari Moghaddam et al., 2018). The results from the studies are entirely consistent with the findings in this chapter. Similar methods were applied across all of these studies, e.g. using the occipital lobe as the reference tissue for uptake ratio and being in early disease with a disease duration of up to 2.8 years.

Clinical scores

Even within the selected series of cases with a disease duration of 1 year or less, patients with a H&Y 2 stage had a significantly longer disease duration than H&Y 1 cases. There are few other studies that allow a detailed comparison of demographic features in relation to clinical imaging results, however, a recent study including 124 H&Y 1 cases with a mean 2.3 years disease duration compared to 164 H&Y 2 cases with a mean 2.8 years disease duration did not show statistical significance for the disease duration between the 2 groups (Sanjari Moghaddam et al., 2018).

Patients with a H&Y score of 2 were also older than H&Y 1 cases at diagnosis, study enrolment, and scan. The same study referred to above showed that 164 H&Y 2 cases with a mean age of 63.9 were significantly older compared to 124 patients with H&Y 1 score (Sanjari Moghaddam et al., 2018). Another study with only 21 Parkinson's cases did not find an age difference between the two H&Y groups (Happe et al., 2007).

The clinical MDS UPDRS motor score (MDS UPDRS 3) is often compared to dopamine transporter tracer uptake in PD, as dopaminergic depletion directly affects motor function and can be physically measured. Other clinical scores like MDS UPDRS 1 and 2 are rarely tested for, as they also assess other features such as cognitive function, mood disorders and sleep disturbances in UPDRS 1, and less prominent small motor function impairments observed in daily living such as speech, eating, and handwriting in UPDRS 2. Per definition, it is expected that patients with a higher H&Y score also have a higher MDS UPDRS 3 score, as the H&Y score is inherently related to the MDS UPDRS 3 score (Goetz et al., 2008). However, in this early disease cohort with a short disease duration, already two-thirds of patients have progressed to H&Y 2 and show significantly higher MDS UPDRS 1, 2, and 3 scores, compared to the H&Y 1 group.

Most imaging studies did not assess clinical severities and their association with the MDS UPDRS 1 score, however, one study included sleep scales in their analysis and did not find a significantly different score of the Epworth Sleepiness Scale (ESS), Parkinson's disease sleep scale (PDSS), and self-rating depression scale (SDS) among the nine H&Y 1 cases and twelve H&Y 12 cases (Happe et al., 2007).

The higher UPDRS 2 score in the H&Y 2 group was also shown in another study with a mean UPDRS 2 score of 6.0 (4.2) in H&Y 2 which was significantly higher compared to H&Y 1 patients with a score of 0.4 (1.0) (Sanjari Moghaddam et al., 2018). It is important to note that this study used a basic Mann-Whitney U-test without adjustments, whereas the results in this chapter are based on a logistic regression model, accounting for differences in age, gender, and disease duration.

Two other studies also found H&Y 2 patients with a UPDRS 3 score of 20.4 (8.8) and 16.9 (10.9) which was again significantly higher than H&Y 1 with 1.2 (2.1) and 8.8 (8.6) (Sanjari Moghaddam et al., 2018, Happe et al., 2007). Both studies therefore reported lower UPDRS 3 scores for both groups than shown in the analysis of this chapter. Reasons for this discrepancy could be partially due to the smaller sample size of nine cases with H&Y 1 and twelve with H&Y 2 in one study (Happe et al., 2007) but also a different disease duration 2.5 years longer in the same study compared to PPMI (Happe et al., 2007).

In the total Parkinson's cohort assessed in this chapter, a higher striatal uptake ratio was associated with a better motor function. This finding was also shown for the putamen in another study, using the UPDRS motor score and correlating it with the putaminal uptake ratio using SPECT imaging with ^{123}I-FP-CIT (Benamer et al., 2000).

Impact on long-term treatment

Early striatal uptake ratios did not correlate with long-term responsiveness to dopaminergic therapy as measured with challenge testing. Another study showed an association, using the L-dopa challenge test response (based on UPDRS) as a continuous outcome variable and a striatal asymmetry index (SAI) instead of the uptake ratio. The study showed that higher percentage changes at challenge test were associated with a higher SAI (Contrafatto et al., 2011). However, this study also used the UPDRS and patients were drug naïve until 3 days prior to the challenge test, where they received 250mg of L-dopa instead of the standard morning dose of L-dopa and dopamine agonists in PPMI.

A lower uptake in the putamen in early disease was found to be significantly associated with a higher dose of dopaminergic therapy at the last recorded challenge test visit. This finding is in keeping with another study, showing that patients in the group with the visually graded lowest dopamine uptake in the striatum required higher medication doses at 3 years follow-up (Nissen et al., 2014).

5.4.1 The latency period from dopamine depletion to initial motor onset

Parkinson's has an extensive preclinical period, where the disease progresses in the brain but has not yet manifested physically. Especially the bilateral depletion in unilaterally presenting PD gives rise to the question of how long degeneration progresses until it physically shows. The early identification of Parkinson's is of great importance in the hope of treating the disease early-on which could slow progression. Additionally, preclinical Parkinson's can shed light onto how and why dopaminergic neurons degenerate and can be useful for the investigation of neuroprotection, a commonly discussed factor in the progression of the disease and drug development (Lang, 2006, Tolosa et al., 2007, Waldmeier et al., 2006).

Many studies investigated the time period from the onset of non-motor symptoms until the manifestation of motor symptoms, referred to as the prodromal phase. Hyposmia was shown to have a prodromal period of 22 years (Fereshtehnejad et al., 2019), 20 years or more for constipation (Savica et al., 2009) and also 20 years for molecular markers like the accumulation of alpha-synuclein in the colon (Hilton et al., 2014, Stokholm et al., 2016).

In pathological studies, a preclinical duration of 4-5 years until motor symptoms emerge was suggested (Fearnley and Lees, 1991, Greffard et al., 2006). In clinical imaging studies, the preclinical period was estimated by extrapolating data from fitted regression lines. In PET and SPECT studies, the preclinical period was estimated between 3 and 7 years (Chouker et al., 2001, Hilker et al., 2005, Morrish et al., 1998, Vingerhoets et al., 1994). One study specifically derived the preclinical period for the anterior putamen at 4.6 years, and 6.5 years for the posterior putamen (Nurmi et al., 2001).

A preclinical period of 10 years in the caudate and 20-40 years in the putamen, as suggested by the analysis in this chapter, is more in keeping with the studies examining specific clinical features than in comparison to other imaging studies using similar backward calculations. The PPMI study is unusual in performing repeated imaging over a 6-year period, while most earlier studies use just 2 or 3 scans over a shorter time duration, from which the estimate of the preclinical period is made.

Some studies do not use regression methods but instead derived an annual mean change to calculate the preclinical duration (Morrish et al., 1998). A linear dopaminergic depletion is the most commonly shown pattern (Chouker et al., 2001, Marek et al., 2001, Parkinson Study, 2002, Pirker et al., 2003), which in this chapter was also the best

fit for the data – but still only explained around 10% of the variation in the data. A linear backwards projection assumes that the progression rate, and the data, follow the same pattern at the earlier time points. This is likely to be flawed, especially considering the more affected putamen. In support of this, a study of 28 post-mortem Parkinson's cases showed that 4 years after diagnosis there was a complete loss of staining in the dorsal putamen but that the depletion of melanized nigral neurons was slower (Kordower et al., 2013). The very low uptake ratios with a high proportion of values near zero means that linear regression cannot project realistic time periods. Hence, the preclinical phase derived from the caudate is more likely to be true, yet the method needs to account for varying patterns of depletion in the preclinical phase.

The neuronal decline per year to estimate the progression of the disease can also be compared between the present analysis and other work. Numbers vary across studies as well as imaging techniques. Overall, an annual reduction of 12.5% was reported in PET studies (Morrish et al., 1998) and 6-11.2% striatal decline using SPECT (Chouker et al., 2001, Marek et al., 2001, Pirker et al., 2002, Pirker et al., 2003, Winogrodzka et al., 2003). Separated according to region, an annual 13.1% decline in the putamen and 12.5% in the caudate was reported using PET (Nurmi et al., 2000) and 8% in the putamen and 4% in the caudate with SPECT imaging (Winogrodzka et al., 2003).

In the analysis in this chapter, the caudate had a 5-5.1% reduction per year with the putamen ranging at 6-8%, which lies within the range of other SPECT studies conducted. The differences between results from PET and SPECT studies could have multiple reasons. The maximum number of participants in the studies used for comparison is 50 (Winogrodzka et al., 2003), and some studies recruited only around 10 patients (Morrish et al., 1998, Nurmi et al., 2003, Pirker et al., 2002). Another explanation could be the time interval between scans, from which this annual reduction was derived. The majority of studies had an interval from first to last scan of 1 to 2 years (Chouker et al., 2001, Morrish et al., 1998, Nurmi et al., 2003, Pirker et al., 2002, Winogrodzka et al., 2003), and only one study had an interval of 5 years (Pirker et al., 2003), which is therefore closest to the total observation period in the PPMI study of 6 years.

5.4.2 Case definition in PPMI compared to diagnostic criteria for PD, and other cohort studies

According to the MDS criteria, parkinsonism is defined as the presence of bradykinesia in combination with either rest tremor, rigidity or both – so bradykinesia is a definite requirement for a diagnosis (Hughes et al., 1992, Postuma et al., 2015). Other early disease studies (Filippi et al., 2005, Happe et al., 2007) or investigative trials (Parkinson Study, 2002) follow this definition for their cohort selection, but the PPMI study has a unique position among other studies: it allowed patients to enter the study with asymmetric resting tremor and without bradykinesia. This was a deliberate strategy to include very early cases and obtain confirmation of dopamine deficiency (and exclude cases with normal imaging from the PD cohort). This looser clinical definition of the disease allows the inclusion of early PD stages with lower H&Y scores. It is therefore ideal for long-term monitoring of disease development.

Looser definitions, however, also raise the question of the diagnostic accuracy in this subset. Especially early disease Parkinson's is difficult to differentiate from other forms of parkinsonism with a dopaminergic deficit. All patients showed a depletion of dopamine transporter on their SPECT scan, although some patients showed uptake ratios that overlapped with those of healthy controls. Examination of the serial findings on SPECT imaging would be helpful to confirm that these cases had a progressive decline in dopaminergic activity compatible with PD, but this was beyond the scope of the present analysis.

In conclusion, despite an extensive preclinical period, a large proportion of dopaminergic transporters remain active in the early stages of the disease. A differentiation of striatal regions according to symptomatic onset is informative about the extent of neuronal loss in comparison to clinical features. Identifying patients early in their disease progression is feasible using relaxed clinical criteria supported by imaging. These methods can be implemented in the search for patients to test neuroprotective drugs.

Chapter 6: General Discussion

This thesis investigated the composition of L-dopa responsiveness and which factors contribute to it. It has shown that for the assessment of L-dopa responsiveness, a simple subjective estimate of motor improvement over time is not sufficient and that it is rather a multifaceted feature that should be assessed carefully. This complexity has implications in clinical practice, clinical trials, and the diagnostic criteria for Parkinson's disease.

6.1 Clinical Practice

The most important finding of this thesis is the great variability of L-dopa responsiveness: A response to L-dopa that is less than excellent is compatible with a Parkinson's disease diagnosis, as was shown in chapter 2 and chapter 4 using different analysis approaches. In chapter 2, a systematic review of published studies found that 10-12% of pathologically confirmed Parkinson's cases had little to no response to L-dopa, whereas in chapter 4 almost half of the patients responded in what was designated a limited way to their challenge tests, in two large scale clinical cohorts. This 'middle ground' in a large proportion of cases with Parkinson's, between an excellent response to L-dopa (which is a supportive diagnostic criterion) (Postuma et al., 2015) and a completely absent response (that points to an alternative diagnosis) helps to explain one aspect of diagnostic difficulty in assessing the parkinsonian patient.

In clinical practice, L-dopa responsiveness is generally subjectively assessed by the patient and the clinician. The difficulty with this approach is that symptoms vary between patients, both in their nature and their dominance in everyday life. For example, the patient with a tremor-dominant Parkinson's disease pattern may report a lack of improvement with L-dopa, as may the patient with rigidity as a dominant feature but who is untroubled by it. A specific percentage change on challenge testing is beyond usual resources in clinical practice but from a clinical perspective, a dichotomised approach into limited versus definite responsiveness to reflect the responses below or above a certain threshold as previously established (Merello et al., 2011, Postuma et al., 2015) and applied in Chapter 4, would be a useful method. This changes the concept of an excellent response representing Parkinson's disease, and a suboptimal response indicating an alternative diagnosis.

Even though dichotomization is the most commonly used approach in clinical research and greatly improves the interpretability of results, it comes with great downsides: loss

of information and consequent reduction of statistical power to examine the relationship between the L-dopa responsiveness and other variables (MacCallum et al., 2002). The use of a dichotomization in this thesis can be justified because the cut-off is not simply the median of the data but it is informed by specific observations made in Parkinson's disease (Merello et al., 2011). The resultant split of the challenge test results in both clinical cohorts into approximately two halves as shown in chapter 4 is a dramatic result given this large proportion of patients with a poorer response to the drug.

One consequence of this dichotomization is that patients with a definite L-dopa response have a greater risk of motor complications (which are also a supportive criterion for the diagnosis of Parkinson's).

Motor complications are the main reasons why L-dopa treatment is sometimes delayed in early disease Parkinson's. The balancing act between achieving motor improvement at the cost of manifestation of motor complications is an everyday challenge in clinical practice. Chapter 3 has shown that a study with an L-dopa delaying strategy compared to a study with the early introduction of L-dopa into the treatment schedule both show the same prevalence of motor complications over a 6-year period. Therefore, the analysis of chapter 3 showed that patients with an early introduction of L-dopa are not at a disadvantage, but longer-term follow-up is required in these large-scale comparative studies before drawing a final conclusion about the early introduction of L-dopa into the treatment schedule in early disease cases.

6.2 Clinical Trials

The assessment of L-dopa responsiveness by challenge testing in clinical practice is rare. It is however a standard method in the assessment for eligibility of deep-brain stimulation. Generally, patients who show an >30% improvement are more likely to benefit from DBS (Lang et al., 2006, Rodriguez et al., 2007). However, L-dopa responsiveness has also been assessed as an inclusion criterion in clinical trials for more advanced therapies in fluctuating patients e.g. inhaled L-dopa (LeWitt et al., 2016), inhaled (Grosset et al., 2013) or injected Apomorphine (Dewey et al., 2001). Extension of the challenge test approach to clinical trials in earlier disease stages is supported by the findings in this thesis.

The core focus of Parkinson's treatment is the replacement of the neurotransmitter dopamine. Based on this thesis, we now know there is a wide variation of responsiveness to the L-dopa that is used as a source of dopamine. Chapter 5 has shown

the extensive amount of residual dopaminergic activity in different regions of the striatum at the onset of the disease. Preventing these neurons from further degeneration is a current major focus of research for early disease Parkinson's. Another approach would be to focus on depleted neurotransmitters other than dopamine.

The neurotransmitter serotonin (Hou et al., 2012, Sun et al., 2015) and the hormone noradrenaline (Greenfield and Bosanquet, 1953) are known to be deficient in Parkinson's and are involved in overcoming the functional deficit in the dopamine deficient brain (Brotchie and Fitzer-Attas, 2009). This suggests that focussing on additional pathways simultaneously could lead to new promising treatments, especially within the aim of resolving both motor and non-motor symptoms.

Neuroprotection is a frequently discussed topic, as developing a treatment that could halt the depletion of dopamine would be a huge achievement. Numerous studies attempted to develop a neuroprotective drug without success, which may have been contributed by treating Parkinson's as a single entity, rather than selecting or considering sub-populations. Most recent trials follow methodology from classic studies (Group, 1989, Olanow et al., 1995) and do not include any assessment of L-dopa responsiveness.

After extensive research in clinical studies (Marshall et al., 2009), DaTSCAN has become a diagnostic adjunct in clinical practice, and a screening tool for more recent clinical trials in early disease. Based on the work of this thesis, L-dopa responsiveness should become an additional standard inclusion criterion for clinical trials. This could help subgrouping patients into more versus less responsive, and inclusion of this variable could help assessing the response to the drug treatment in question.

At certain stages of Parkinson's, there is little to no presynaptic dopaminergic neuronal activity left, which is likely to mean that dopaminergic activity is beyond repair. The degree of uptake as measured with DaTSCAN could be used as a covariate in the analysis of clinical studies. Analysing L-dopa responsiveness alongside the degree of residual dopaminergic activity could help to define the effects of new drugs and improve statistical power in a drug development programme.

In conclusion, the large variation in the degree of L-dopa responsiveness in Parkinson's has significant implications both for clinical practice including differential diagnosis of parkinsonism. L-dopa responsiveness as a supportive diagnostic criterion remains an important factor for this differentiation, however, a more limited response should not

necessarily point to an alternative diagnosis. In clinical research that aims to identify new and improved treatments, assessment of L-dopa responsiveness could be used as a grouping variable for studies including agents that target chemical pathways other than dopamine, and agents which offer the hope of neuroprotection to prevent or reverse a progressive disease process.

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