Linking Genetics and Neuropathology in Parkinson's Disease

Thesis

for the degree of

Philosophiae Doctor (PhD)

by

Jon-Anders Tunold

Department of Neurology, Oslo University Hospital

Faculty of Medicine, University of Oslo

2023

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-348-0357-4

Cover: UiO.
Photo cover: Ine Eriksen, UiO.
Print production: Graphic center, University of Oslo.

«The brain is the last and grandest biological frontier, the most complex thing we have yet discovered in our universe. It contains hundreds of billions of cells interlinked through trillions of connections. The brain boggles the mind.». James D. Watson

Acknowledgements

The research included in this thesis was conducted at Oslo University Hospital, Department of Neurology, and the University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, between May 2019 and August 2023. I am sincerely thankful to the South-Eastern Regional Health Authority in Norway for funding my PhD project.

I would like to thank all study participants, whose generous contributions are instrumental in advancing our knowledge in the field of Parkinson's disease. Their willingness to share their time and personal experiences with the disease, along with their invaluable donations of blood and brain tissue, has made this research possible.

I would like to extend my utmost gratitude to my supervisor, Lasse Pihlstrøm, for your exceptional mentorship and support throughout the course of my PhD journey. Your expertise and enthusiasm for science continue to be a source of inspiration. Thank you for your trust, encouragement and belief in my abilities, which have been substantial contributions to my academic and personal growth. I am truly honored to have had the privilege of working under your supervision. I would also like to thank my co-supervisor Mathias Toft for your invaluable insights and grounded advice. Your ability to explain complex ideas in a clear and accessible manner inspires me. I would also like to extend my gratitude to my other co-supervisor Mona-Elisabeth Rootwelt-Revheim for your knowledge, enthusiasm and for the exciting collaboration with the department of nuclear medicine in the prospective study on parkinsonism in Oslo (PROSPOS) study. Our fields in medicine are interconnected, and I hope for more joint ventures in the years to come, in particular when an alpha-synuclein PET tracer becomes available.

Special thanks to all my co-authors for your knowledge and contributions to the work presented in this thesis. They are in alphabetical order Jose Bras, Dennis W. Dickson, Hanneke Geut, Rita Guerreiro, Michael G. Heckman, Sandra Pilar Henriksen, Shunsuke Koga, Nicholas B. Martin, Lasse Pihlstrøm, Owen A. Ross, Annemieke J. M. Rozemuller, Hiroaki Sekiya, Manuela M. X. Tan, Mathias Toft, Rebecca Valentino and Wilma D. J. van de Berg. I am truly honored to have had such remarkable collaborators throughout this journey.

I have been fortunate to be part of the Huntington’s disease and neurodegenerative genomics research group which has rapidly grown over the past few years. Special thanks goes to Maren

Stolp Andersen, Ingeborg Haugesag Lie, Manuela M. X. Tan, Ellen Hoven Maurtveten, Khadeeja Siddique and Juni Kristensen Folge for sharing your skills and knowledge, and for the equally stimulating scientific and non-scientific discussions at lunch. I would also like to thank all my colleagues at the dynamic research environment at the Neuroscience Research Unit, led by Tone Berge. The list of colleagues is long, but in particular I wish to mention Chiara Cappelletti, Einar Høgestøl, Kaja Nordengen, Sofie Lysholm Lian, Monisha Sharma and Zafar Iqbal. I also extend my gratitude to all of those who have contributed to enrolment and sample collection in the PROSPOS study, including Solveig Jacobsen Dalbro, Lone Holmen, Ebba Gløersen Müller, and Mona K. Beyer.

During my PhD work, I have intermittently served as a resident physician at Oslo University Hospital, Rikshospitalet. I extend my sincere gratitude to all my clinical colleagues for your generous inclusion, guidance, and for encouraging me to be involved in movement disorder research. In particular I would like to thank Sigrid Svalheim and Karolina Ryeng Skagen for making it possible to combine my passion for clinical work with research.

To my parents Betzy Marie Ellingsen Tunold and Anders Tunold. Thank you for all your support throughout my education and for providing an important foundation for my scientific undertakings. A heartfelt appreciation goes to my mother for proofreading my thesis manuscript. Special thanks to my brother Håkon Tunold for our conversations filled with laughter, which have been a great distraction. To my wife Solveig Tunold Eldhuset. I thank you for sharing the triumphs and challenges in life, including those that have accompanied my PhD project. Your patience and support have made this path more manageable. Together with our daughter Louise you bring love and happiness to our family and are a constant reminder of what truly matters in life.

Oslo, August 2023
Jon-Anders Tunold

Table of Contents

Abbreviations

α-synuclein	Alpha-synuclein
$A\beta_{1-42}$	42 amino acid isoform of amyloid-beta
AD	Alzheimer's disease
ALAS1	5'-aminolevulinate synthase 1
Amyloid-β	Amyloid-beta
APOE	Apolipoprotein E
ATP10B	ATPase phospholipid transporting 10B
ATP13A2	ATPase cation transporting 13A2
ATP6V0A1	ATPase H+ transporting V0 subunit a1
BIN1	Bridging integrator 1
BST1	Bone marrow stromal cell antigen 1
CBD	Corticobasal degeneration
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHCHD2	Coiled-coil-helix-coiled-coil-helix domain containing 2
COMT	Catechol-O-methyltransferase
COQ7	Coenzyme Q7, hydroxylase
CSF	Cerebrospinal fluid
CTSB	Cathepsin B
DaT-SPECT	Dopamine transport single-photon emission computed tomography
DLB	Dementia with Lewy bodies
DNAJC13	DnaJ heat shock protein family (Hsp40) member C13
DNAJC6	DnaJ heat shock protein family (Hsp40) member C6
EIF4G1	Eukaryotic translation initiation factor 4 gamma 1
FBXO7	F-box protein 7
FTD	Frontotemporal dementia
GAK	Cyclin G-associated kinase
GALC	Galactosylceramidase
GBA1	Glucosylceramidase beta 1
GIGYF2	GRB10 interacting GYF protein 2
GRN	Granulin precursor
GUSB	Glucuronidase beta
GWAS	Genome-wide association study

HTRA2	HtrA serine peptidase 2
LD	Linkage disequilibrium
LRP10	LDL receptor related protein 10
LRRK2	Leucine rich repeat kinase 2
MAPT	Microtubule associated protein tau
MCCC1	Methylcrotonyl-CoA carboxylase subunit 1
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
MSA	Multisystem atrophy
NEU1	Neuraminidase 1
NFT	Neurofibrillary tangle
NOD2	Nucleotide binding oligomerization domain containing 2
OR	Odds ratio
P-tau	Phosphorylated tau at threonine 181
PARK7	Parkinsonism associated deglycase (DJ-1)
PD	Parkinson’s disease
PDD	Parkinson’s disease dementia
PET	Positron emission tomography
PINK1	PTEN induced kinase 1
PLA2G6	Phospholipase A2 group VI
PPMI	Parkinson’s Progression Marker Initiative
PRKN	Parkin RBR E3 ubiquitin protein ligase
PRS	Polygenic risk score
PSP	Progressive supranuclear palsy
RAB29	RAB29, member RAS oncogene family
RBD	Rapid eye-movement sleep behavior disorder
RIMS2	Regulating synaptic membrane exocytosis 2
SCARB2	Scavenger receptor class B member 2
SH3GL2	SH3 domain containing GRB2 like 2, endophilin A1
SNCA	Synuclein alpha
SNP	Single nucleotide polymorphism
SORL1	Sortilin related receptor 1
SYNJ1	Synaptojanin 1
T-tau	Total tau

TMEM175	Transmembrane protein 175
TMEM230	Transmembrane protein 230
UCHL1	Ubiquitin C-terminal hydrolase L1
VAMP4	Vesicle associated membrane protein 4
VPS13C	Vacuolar sorting protein 13 homolog C
VPS35	VPS35 retromer complex component
WES	Whole exome sequencing
WGS	Whole genome sequencing

Thesis summary

Parkinson's disease is a neurodegenerative disorder that becomes more prevalent with age. Diagnosis is based on the presence of motor symptoms such as bradykinesia, rigidity and resting tremor. Patients also develop non-motor symptoms, including cognitive impairment and dementia. Significant heterogeneity exists in the presentation and temporal onset of symptoms for each individual. While the precise cause of Parkinson's disease remains unknown, the loss of dopaminergic nerve cells in the substantia nigra contributes to the development of motor symptoms. The administration of dopaminergic medications is a crucial part of managing these symptoms. Furthermore, individuals with Parkinson's disease develop deposits of the protein alpha-synuclein, which aggregate in Lewy bodies inside neurons. Many individuals, in particular those who develop dementia, also exhibit deposits of the proteins amyloid-beta and tau, commonly associated with Alzheimer's disease. Despite alleviating medication, there is currently no treatment available to stop or slow the disease progression.

In the past three decades, genetic risk factors for Parkinson's disease have been identified. Studies on families with multiple affected members have uncovered rare, monogenic forms of the disease. Additionally, large-scale population studies called genome-wide association studies (GWAS) have identified common genetic risk factors for Parkinson’s disease. Genetic risk factors can also influence the disease course by lowering the age at disease onset or elevate the risk for symptoms such as dementia. Moreover, genetic risk factors have provided insights into the molecular mechanisms underlying the development of Parkinson's disease, potentially opening avenues for future disease-modifying treatments.

This thesis presents results from three studies building on previously identified genetic risk factors to gain a deeper understanding of how they contribute to Parkinson's disease. The first study investigated the association between risk variants in the *APOE* and *MAPT* genes and the development of dementia. Our findings suggest that both of these variants contribute to an earlier onset of dementia in individuals with Parkinson's disease. In the second study, we explored the relationship between polygenic risk scores, reflecting the cumulative effect of genetic risk factors, and the three common protein aggregates seen in Parkinson's disease. Notably, we found that the polygenic risk score reflecting lysosomal functions was associated with increased Lewy pathology in patients with low levels of amyloid-β and tau deposits. Lysosomes are cellular compartments responsible for breakdown and recycling of various molecules, including alpha-synuclein, and

genetic alterations linked to lysosomal functions have previously been identified as risk factors for Parkinson's disease. In the third study, we examined the association between the lysosomal polygenic risk score and the development of cognitive impairment in Parkinson's patients. We discovered that a higher lysosomal polygenic risk score was associated with an earlier development of cognitive impairment in patients with a low risk of amyloid-beta and tau deposits.

These three studies delve into the genetic influence on protein accumulation and cognitive decline in Parkinson's disease, in the intersection between clinical neurology, neuropathology and genetics. Gaining a deeper understanding of genetic risk factors for Parkinson’s disease is anticipated to play a pivotal role in developing future disease-modifying treatments and selecting patients who would benefit the most from such interventions.

Sammendrag på norsk

Parkinsons sykdom er en nevrodegenerativ sykdom hvor forekomsten øker med alderen. Diagnosen stilles på bakgrunn av de motoriske symptomene bradykinesi, rigiditet og hviletremor. Pasientene utvikler også ikke-motoriske symptomer som blant annet kognitiv svekkelse og demens. Sykdommen preges av stor grad i variasjon av hvilke symptomer som rammer og når de inntreffer hos den enkelte. Den underliggende årsaken til Parkinsons sykdom er ukjent, men sentralt for utvikling av de motoriske symptomene er tap av dopaminerge nerveceller i et område av hjernen som kalles substantia nigra, og tilførsel av dopaminerge legemidler er derfor en viktig del av symptombehandlingen av Parkinsons sykdom. Videre har pasienter med Parkinsons sykdom avleiringer av proteinet alfa-synuklein, som hoper seg opp inne i nerveceller i Lewylegemer. Mange, og spesielt de som utvikler demens, har også avleiring av proteinene amyloid-beta og tau, som er vanlige proteinavleiringer ved Alzheimers sykdom. Til tross for at man har legemidler som demper symptomene, finnes det ingen behandling som kan bremse eller stoppe utviklingen av sykdommen.

De siste tiårene har man påvist genetiske risikofaktorer for Parkinsons sykdom. Undersøkelser av familier hvor mange slektninger er rammet har avdekket sjeldne arvelige former for Parkinsons sykdom. Videre har store populasjonsstudier, kalt genomvide assosiasjonsstudier, avdekket vanlige genetiske risikofaktorer som øker sannsynligheten for å utvikle sykdommen. Genetiske risikofaktorer kan også bidra til å senke debutalderen eller øke risiko for utvikling av enkelte symptomer som f.eks. demens. Kartlegging av genetiske risikofaktorer har også gitt et innblikk i de underliggende molekylære mekanismene som bidrar til utvikling av Parkinsons sykdom. Disse mekanismene kan være potensielle angrepspunkt for fremtidig sykdomsmodifiserende behandling.

I denne avhandlingen presenteres resultater fra tre studier hvor vi bygger videre på tidligere identifiserte genetiske risikofaktorer for å forsøke å forstå bedre hvordan disse bidrar til Parkinsons sykdom. I den første studien undersøkte vi sammenhengen mellom risikovarianter i genene *APOE* og *MAPT* og utvikling av demens. Våre resultater gir holdepunkter for at begge disse variantene bidrar til tidligere utvikling av demens hos pasienter med Parkinsons sykdom. I den andre studien undersøkte vi sammenhengen mellom polygene risikoskårer som reflekterer summen av genetiske risikofaktorer og de tre vanligste proteinavleiringene ved Parkinsons sykdom. Hovedfunnet i denne studien var at summen av genetiske risikofaktorer for Parkinsons sykdom involvert i lysosomale funksjoner (lysosomal polygen risikoskår) var forbundet med større utbredelse av Lewylegemer i

hjernen hos pasienter som hadde lite amyloid-beta- og tau-avleiringer. Lysosomer er cellenes nedbrytings og gjenvinningsstasjon, og kan bla. bryte ned alfa-synuklein. Forandringer i gener knyttet til lysosomale funksjoner har tidligere blitt identifisert som risikofaktorer for Parkinsons sykdom. I den tredje studien undersøkte vi sammenhengen mellom den lysosomale polygene risikoskåren og utvikling av kognitiv svekkelse hos pasienter med Parkinsons sykdom. Vi fant at en høyere lysosomale polygen risikoskår var forbundet med tidligere utvikling av kognitiv svekkelse hos pasienter som hadde lav risiko for utvikling av amyloid-beta- og tau-avleiringer.

De tre studiene utforsker genetisk påvirkning knyttet til proteinavleiring og kognitiv svekkelse ved Parkinsons sykdom, i skjæringspunktet mellom klinisk nevrologi, nevropatologi og genetikk. En dypere forståelse av genetiske risikofaktorer for Parkinsons sykdom er forventet å spille en sentral rolle i utvikling av fremtidig sykdomsmodifiserende behandling og utvelgelse av pasienter som vil ha mest nytte av slike legemidler.

List of publications

Paper 1 (Published)

Tunold JA, Geut H, Rozemuller JMA, Henriksen SP, Toft M, van de Berg WDJ, Pihlstrøm L. APOE and MAPT Are Associated With Dementia in Neuropathologically Confirmed Parkinson's Disease. Front Neurol. 2021; doi: 10.3389/fneur.2021.631145

Paper 2 (Published)

Tunold JA, Tan MMX, Koga S, Geut H, Rozemuller AJM, Valentino R, Sekiya H, Martin NB, Heckman MG, Bras J, Guerreiro R, Dickson DW, Toft M, van de Berg WDJ, Ross OA, Pihlstrøm L. Lysosomal polygenic risk is associated with the severity of neuropathology in Lewy body disease. Brain. 2023; doi: 10.1093/brain/awad183

Paper 3 (Published after submission of the thesis. Presented here as a manuscript)

Tunold JA*, Tan MMX*, Toft M, Ross OA, van de Berg WDJ, Pihlstrøm L. Lysosomal polygenic burden is associated with cognitive progression in Parkinson's disease in patients with low risk of Alzheimer co-pathology.

*Shared first authorship

Additional publications

Pihlstrøm L, Shireby G, Geut H, Henriksen SP, Rozemuller AJM, Tunold JA, Hannon E, Francis P, Thomas AJ, Love S, Mill J, van de Berg WDJ and Toft M. Epigenome-wide association study of human frontal cortex identifies differential methylation in Lewy body pathology. Nat Commun. 2022;13(1):4932.

Introduction

1.1 A brief history of Parkinson's disease

Throughout history, several civilizations have observed and recognized features of Parkinson's disease (PD). In the Indian tradition of Ayurveda, the plant *Mucuna Pruriens*, now known to contain the dopamine precursor levodopa, has even been used for treatment of symptoms resembling PD(1). In 1817, James Parkinson provided the first modern description of PD as a neurological condition when he published his monograph "Essay on the shaking palsy"(2). Parkinson's text portrayed six individuals exhibiting characteristic disease features of what he termed "shaking palsy" or "paralysis agitans", by the characteristic tremor displayed by patients. The term Parkinson's disease was first introduced by the renowned neurologist Jean-Martin Charcot in the 1870s, who in addition to tremor identified bradykinesia and rigidity as cardinal symptoms(3). Advances in the neuropathological underpinnings of PD were made in the late 19th and early 20th century. Early evidence that PD originated from lesions in the substantia nigra came from a case-description of a young man suffering from tuberculosis and signs of left sided unilateral parkinsonism. At autopsy, a tubercle in the right substantia nigra was identified, matching the left sided symptoms(4). In 1912, the German-American neurologist Fritz Heinrich Lewy first described eosinophilic intraneuronal inclusions in the dorsal nucleus of the vagus and other brainstem nuclei in patients with PD(5). These inclusions were in 1919 further shown to locate to the substantia nigra and named Lewy bodies (corps de Lewy) by Konstantin Nikolaevitch Trétiakoff(6). Trétiakoff also described depigmentation of the substantia nigra, now known to be a result of loss of neurons containing neuromelanin. The combination of depigmentation of the substantia nigra and Lewy bodies in the brains of affected patients still remain the main histopathological features of PD.

The search for a link between the substantia nigra and PD intensified in the last half of the 20th century. Arvid Carlsson and colleagues demonstrated that the dopamine precursor 3,4-dihydroxyphenylalanine (dopa) was able to reverse the effects of reserpine in animals – which causes pharmacologically induced PD(7). Further, they found that dopamine was depleted in the brains of research animals when administering reserpine, and repleted upon administering dopa, suggesting that the motor symptoms of PD were related to dopamine(8). Subsequent studies by Oleh Hornykiewicz and colleagues demonstrated that patients with PD in fact had profound loss of dopamine in the striatum(9) and the substantia nigra(10), leading to the recognition of the nigrostriatal pathway(11). Concurrently, clinical trials with intravenous administration of the

dopamine precursor levodopa to patients with PD showed striking yet short-lived improvement of motor symptoms(12). In the late 1960s George Cotzias and colleagues demonstrated a prolonged effect of oral levodopa when administered with a peripheral carboxylase inhibitor (carbidopa), which inhibited extracerebral metabolization of levodopa to dopamine, allowing a smaller dose of levodopa to be effective and less peripheral adverse effects of dopamine(13). This combination known as levodopa-carbidopa quickly became the gold standard for symptomatic therapy of Parkinson's disease and is still the main pharmacological therapy for most patients. In 1970, Cotzias and colleagues also discovered the therapeutic effectiveness of the dopamine receptor agonist apomorphine for symptomatic treatment of PD(14). This marked the introduction of the second dopaminergic replacement therapy that continues to be utilized in the management of PD.

1.2 Current concepts of Parkinson's disease

1.2.1 Epidemiology

PD is the second most common neurodegenerative disorder after Alzheimer's disease (AD)(15). PD may affect all age groups, but a sharp increase in incidence and prevalence is seen after the age of 60(16, 17) (Figure 1). In European populations the overall prevalence is 1.8 % above the age of 65 and 3 % above the age of 80(16, 18). Moreover, men are more susceptible to PD than women, with a male-to-female ratio of approximately 2:3(19, 20). The Global Burden of Disease study found PD to be the fastest growing neurological condition with a doubling in global prevalence from 1990 to 2015, reaching 6.2 million affected people(21). As the incidence increases with age, the global population is aging and life expectancy is increasing, a doubling in prevalence is projected to happen again within the next generation(22). This projection presents a formidable public health challenge known as the "Parkinson Pandemic"(22), demanding the concerted efforts from researchers, clinicians and policymakers to understand the underlying cause and find effective interventions for PD.

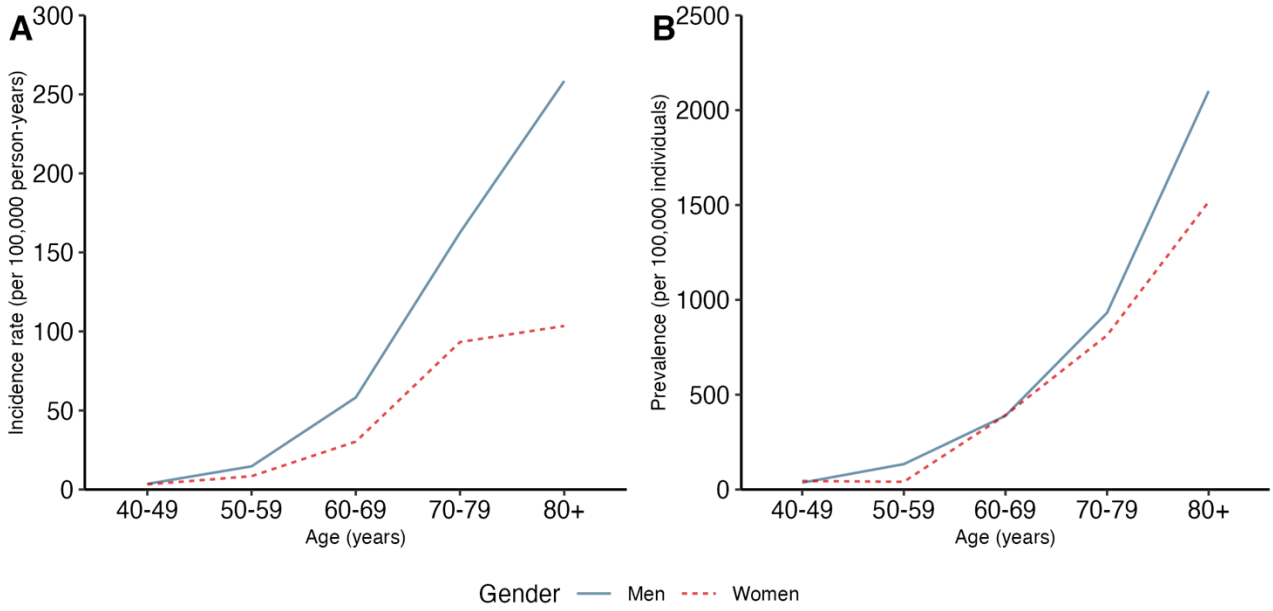

Figure 1: *A) Incidence, and B) prevalence for PD stratified by gender. Crude data has been plotted from two meta-analyses on incidence and prevalence of PD(16, 17). Figure adapted from Poewe W et al., Parkinson Disease, 2017(15), Macmillan Publishers Limited, part of Springer Nature, reproduced with permission of SNCSC.*

1.2.2 Symptoms

Affected individuals typically experience an insidious onset and gradual deterioration in functioning, leading to increasing disability over time. However, individuals with PD exhibit a high degree of heterogeneity due to the wide variation in the combination of symptoms experienced and the timing of their occurrence. The three cardinal motor symptoms of PD are bradykinesia (slowness of movement and decrement in amplitude or speed), rest tremor (involuntary rhythmic and oscillatory movement in a body part at rest) and rigidity (velocity-independent resistance to passive movement), collectively known as parkinsonism(23). With disease-progression, motor symptoms become more troublesome to manage, as many patients develop motor complications such as motor fluctuations and dyskinesia which are associated with long-term levodopa therapy(15). Moreover, patients may develop axial deformities, postural instability and eventually falls (Figure 2). Additionally, a range of less visible non-motor symptoms are a major source of disease-related disability(24). These include sensory disturbances, autonomic dysfunction, sleep disorders and neuropsychiatric features(25) (Figure 2). Some of these features such as dream enactment during rapid eye-movement (REM) sleep (REM sleep behavior disorder (RBD)), hyposmia (reduced sense of smell), depression and constipation may precede the onset of motor symptoms by many years(26). Consequently, the initial stages of PD pose a challenge for detection,

as the individual non-motor symptoms are non-specific for the disease. The burden of non-motor symptom often increases as the disease progresses. With limited treatment options available, non-motor symptoms often predominate as the disease advances, severely affecting health-related quality of life and function(25).

Cognitive impairment is recognized as one of the most debilitating non-motor symptoms of PD, ranging from mild cognitive impairment (MCI) to PD dementia (PDD). MCI may be regarded as an intermediate phase between normal cognition and dementia where the cognitive impairment does not significantly interfere with daily activities and thus not sufficient to meet diagnostic criteria for dementia. Already at diagnosis ~20 % of patients may have developed MCI, with a high conversion rate to dementia within the following years(27). In PDD, cognition is more severely affected, with a marked impact on quality of life, overall survival, increased caregiver burden and health care costs(28, 29). PDD is common and may affect as many as ~80 % of patients long term(30). However, the time to dementia onset is highly variable with some patients developing dementia within the first few years after PD diagnosis, while others remain dementia free for decades(28). As treatment options remain limited, identifying risk factors contributing to early development of dementia is a current focus of research with the aim to better inform individual prognosis but also increase our understanding of the biological and molecular basis of PDD, and facilitate the detection of potential therapeutic targets.

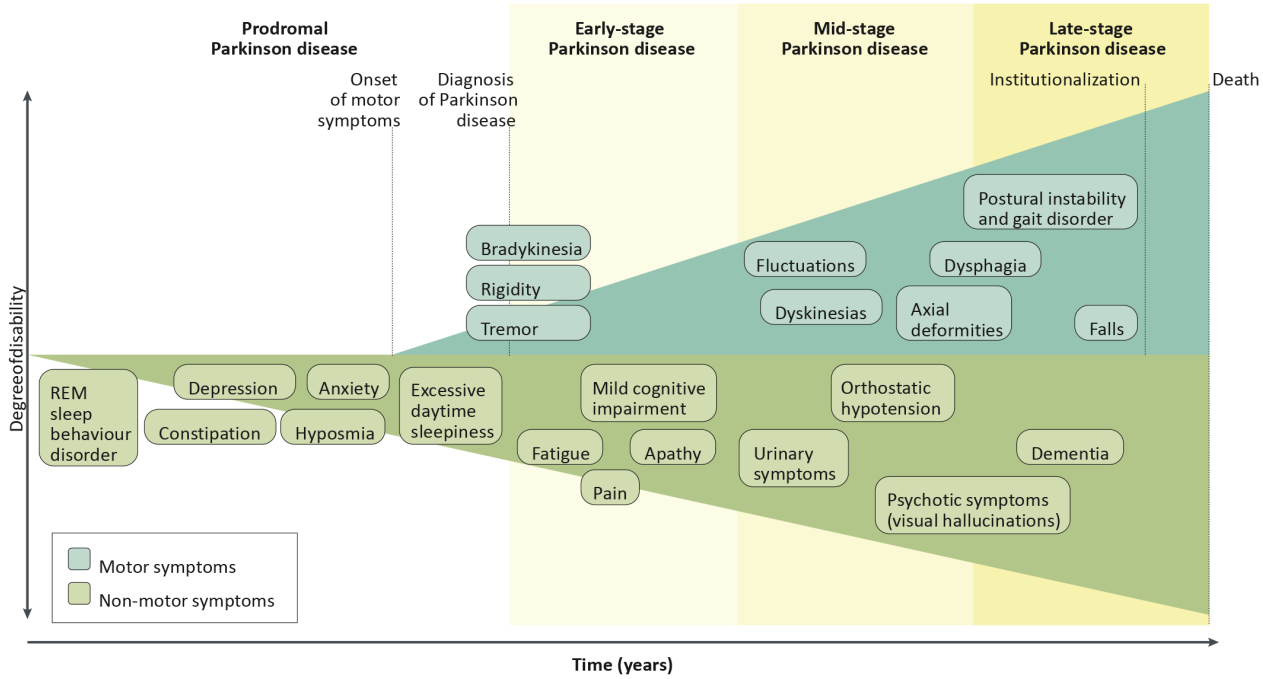

***Figure 2**: Motor and non-motor symptoms of PD. Figure from Poewe W et al., Parkinson Disease, 2017(15), Macmillan Publishers Limited, part of Springer Nature, reproduced with permission of SNCSC.*

1.2.3 Treatment

Current treatment of PD is symptomatic, aiming to alleviate motor and non-motor symptoms. Pharmacological treatment of motor symptoms is primarily dopaminergic, seeking to replace the action of dopamine in the depleted striatum. Oral treatment with agents such as levodopa, in combination with a decarboxylase inhibitor to prevent peripheral metabolism of levodopa, and dopamine agonists are the mainstay of pharmacotherapy for most patients, and may control motor symptoms for years. Monoamine oxidase B (MAO-B)-inhibitors, blocking the degradation of dopamine, may also be used as mono therapy in the early stages of disease for patients with less troublesome motor symptoms or in adjunct with levodopa or dopamine agonists. Current evidence supports to initiate treatment with levodopa due to its superior effect on motor symptoms compared to dopamine agonists and MAO-B inhibitors, and the impulse control disorders frequently associated with dopamine agonists(31). As the disease progresses, fluctuations in the response to pharmacotherapy often develops, known as motor fluctuations. Moreover patients may develop dyskinesias which are involuntary movements often occurring at peak medication concentration(32). At this stage of disease, more frequent administration of lower doses of levodopa and combination of dopaminergic agents is often necessary. Adjunctive treatment with agents such

as catechol-O-methyltransferase (COMT) inhibitors which block the enzyme responsible for metabolizing levodopa, may prolong the effect of levodopa. Nevertheless, some patients do not achieve optimal symptom control of motor symptoms with oral medication. For these patients, more advanced treatment options with pump-delivered therapies, deep brain stimulation (DBS) or magnetic resonance imaging (MRI)-guided focused ultrasound (MRgFUS) may be considered. Pump-delivered therapies include continuous subcutaneous administration of the dopamine agonist apomorphine or intrajejunal administration of levodopa with or without a COMT-inhibitor(33-35). DBS is a well-established treatment with long-term benefits for selected patients with motor complications, dyskinesias or tremor refractory to medical therapy(36). The procedure includes unilateral, or more commonly bilateral surgical placement of leads to the subthalamic nucleus (STN) or the globus pallidus internus (GPi). More recently, MRgFUS has emerged as a less invasive treatment option for patients with suboptimal control of tremor. The procedure uses highly focused ultrasound to produce a lesion in the ventral intermediate nucleus (VIM), STN or GPi, yet the optimal target is yet to be determined(37).

In contrast to the motor symptoms, the majority of non-motor symptoms respond poorly to dopaminergic therapy. However, deficits in other neurotransmitters including acetylcholine, serotonin and norepinephrine/noradrenaline have also been implicated in PD and are now recognized to be responsible for a range of non-motor symptoms(25). In general, pharmacotherapy used to treat similar symptoms in the non-PD population are chosen. For instance, cholinesterase inhibitors may have beneficial effects on cognitive symptoms in patients with dementia, while selective serotonin reuptake inhibitors (SSRI) may be useful for treating depression(38). Moreover, mineralcorticoids (florinef) and medication targeting adrenergic receptors such as midrodine and droxidopa may be used to treat orthostatic hypotension, and phosphodiesterase inhibitors (sildenafil) may be efficacious for treatment of erectile dysfunction(38). For a more comprehensive review of treatment of motor and non-motor symptoms, please refer to(31, 32, 38).

As evident from the preceding discussion, current treatment of PD often involves combining various classes of pharmacological agents, and for some patients supplemented with invasive treatments to treat the individual motor and non-motor symptoms. However, there are currently no available disease-modifying therapies that either slow or halt the disease progression. Gaining a deeper understanding of the mechanisms underlying the disease is of key importance to identify potential drug targets and develop disease modifying therapies. Additionally, the discovery of biomarkers with the potential to identify individuals at risk for disease is crucial, as it would enable

the initiation of treatment at an early stage, before neurodegeneration has become profound. If these endeavors succeed, they hold promise for improved outcomes and quality of life for individuals living with PD.

1.2.4 Clinical diagnostic criteria for Parkinson's disease

Still today the diagnosis of PD remains a clinical diagnosis based on patient history and physical examination. However, the diagnosis remains challenging as the clinical features of PD can overlap with various types of secondary parkinsonism and other neurodegenerative disorders. In particular distinguishing PD from atypical parkinsonian disorders in the early stages of disease poses a challenge, even for experienced neurologists(39). Atypical parkinsonism encompasses several neurodegenerative diseases such as multisystem atrophy (MSA), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), where parkinsonism is a prominent clinical feature, but the full range of symptoms, progression and underlying pathology differ from PD. Additionally, PD share a range of features with dementia with Lewy bodies (DLB), with timing of dementia being the major clinical distinction between the two conditions.

There are currently no biomarkers in clinical utility to sufficiently discriminate between PD and related neurodegenerative disorders in the earliest phases of disease. However, Dopamine Transport (DaT) imaging assessed by brain single-photon emission computed tomography (SPECT) may be used to evaluate the density of presynaptic dopaminergic terminals in the striatum, as a surrogate of neurodegeneration of the substantia nigra pars compacta. Although abnormal DaT imaging alone is not conclusive for a PD diagnosis, normal DaT binding is considered an exclusion criterion for PD(23). Magnetic resonance imaging (MRI) may also aid in the diagnosis of PD, and importantly reveal structural changes to distinguish between PD and atypical parkinsonism(40). Moreover, Flourine-18 fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) may detect patterns of altered glucose metabolism, supportive of atypical parkinsonism(41).

Clinical diagnostic criteria aim to minimize the diagnostic error. The current diagnostic criteria for PD, the International Parkinson and Movement Disorder Society (MDS) clinical diagnostic criteria for PD, were published in 2015 and require a two-step process of PD diagnosis. The first step is to establish the presence of parkinsonism, defined as bradykinesia in combination with rest tremor or rigidity(23). In the second step positive features (supportive criteria) that argue for the diagnosis of PD, and negative features (absolute exclusion criteria and red flags) that argue against PD are

assessed. Then positive and negative features are weighted to determine whether the parkinsonism is attributable to PD with two levels of diagnostic certainty i.e., clinically established PD or clinically probable PD. Nevertheless, clinicopathological studies have shown that the diagnostic error rate is high, in particular in the early disease stages where the full range of symptoms have not developed(39). Thus, the gold standard for a definitive diagnosis remains post-mortem identification of neuropathological hallmark changes in the brain.

1.2.5 Clinical diagnostic criteria for dementia with Lewy bodies

Traditionally, clinical distinction between PDD and DLB is based on the temporal onset of dementia relative to parkinsonism. Dementia presenting before or within one year of parkinsonism onset is diagnosed as DLB, while dementia developing in the setting of establish PD as PDD. However, in the most recent clinical diagnostic criteria for PD, dementia predating the onset of parkinsonism was removed as an exclusion criterion for PD, diluting the distinction between PD and DLB(23). The most recent revision of the diagnostic criteria for DLB recommends maintaining the distinction between PDD and DLB based on the onset of dementia relative to parkinsonism in clinical practice(42). However, the guideline also recognize DLB as one of the phenotypes within the broader spectrum of Lewy body disease (LBD)(42).

DLB is a progressive dementia sufficient to interfere with daily activities. The core features of the disease are fluctuations in cognition, visual hallucinations, RBD and parkinsonism. While parkinsonism is not necessary for a diagnosis of DLB, it will eventually develop in 85 % of patients(42). Additional supportive criteria are postural instability with repeated falls, severe autonomic dysfunction with constipation, orthostatic hypotension or urinary incontinence, hyposmia, apathy, anxiety and depression, which overlaps with the non-motor symptoms of PD(42). Similar to PD, no biomarkers are yet sufficient to diagnose DLB, but DaT-SPECT imaging may be used to demonstrate reduced DaT uptake in the striatum which serves as an important distinguishing factor between DLB and AD. The diagnostic error rated for DLB are even higher than for PD, with the most frequent misdiagnosis being AD(43).

1.3 Neuropathology

The loss of dopaminergic neurons in the substantia nigra pars compacta with subsequent depletion of dopamine in the striatum is a central neuropathological feature of PD. The resultant

dopaminergic deficiency is associated with the motor symptoms of PD, in particular bradykinesia and rigidity(44). It has been estimated that by the time motor symptoms have developed, 50 % of dopaminergic neurons in the substantia nigra pars compacta have been lost and striatal dopamine has been reduced by up to 80 %(45). Consequently, the neurodegeneration is already advanced at the time of clinical diagnosis, thus limiting the effectiveness of potential disease modifying therapies at this stage.

Aggregation of misfolded proteins and formation of inclusion bodies is a common feature of neurodegenerative diseases such as PD, atypical parkinsonian disorders, DLB and AD. Whereas alpha-synuclein (α-synuclein) is the most commonly aggregated protein in PD and DLB, amyloid-beta (amyloid-β) and tau are characteristic for AD. These proteins undergo a polymerization process where soluble monomers form oligomers that ultimately may aggregate into large insoluble fibrils. Although still a matter of debate(46), the aggregates or their precursors are believed to result in deleterious consequences for the cells harboring these accumulated proteins(47). While each neurodegenerative disorder displays the accumulation of distinct protein aggregates, the co-occurrence of pathologies is common, pointing to pathogenic links between these diseases. Further, the presence of overlapping protein aggregates adds complexity to the diagnosis and treatment, and raises the question of which is most important to the disease. The three most common protein pathologies found in PD are discussed below.

1.3.1 Lewy pathology

In PD, vulnerable neurons develop inclusions in perikarya called Lewy bodies (LB) and within their neuronal processes known as Lewy neurites (LN)(5, 6, 48). Collectively, Lewy bodies and Lewy neurites are known as Lewy pathology (LP). Because Lewy pathology is a shared feature between PD and DLB, they are together referred to as Lewy body disease (LBD). The discovery of α-synuclein as a major component of Lewy bodies represented a significant milestone in PD research, and has since served as a key marker for the disease(49). Additionally, a diverse range of other proteins including ubiquitin and tau, lipids and distorted mitochondria and lysosomes have been identified in these inclusions(50, 51), providing insights into mechanisms underlying the formation of Lewy pathology.

In PD, Lewy pathology anatomically extends beyond the substantia nigra, and can be found in the olfactory bulb, dorsal motor nucleus of the vagus nerve, the lower raphe nuclei and locus coeruleus.

In the later stages of disease, Lewy pathology can also be detected in the amygdala, hippocampus, thalamus and the neocortex(52, 53). These observations imply that the distribution of Lewy pathology follows a non-random pattern, displaying predilection for certain subcortical and cortical regions. Moreover, the degenerative process in PD is not only confined to the central nervous system (CNS). In addition, Lewy pathology has been found in the peripheral autonomous nervous system, and organs innervated by the latter, including the gastrointestinal tract, the heart, kidneys, urogenital system and skin(54). The involvement of the peripheral autonomous nervous system is believed to explain the high prevalence of autonomic symptoms in PD. In particular involvement of the enteric nervous system (ENS) of the gastrointestinal tract has been hypothesized to be an early event, preceding the involvement of the CNS(55).

Several neuropathological staging schemes for Lewy pathology have been developed(52, 56, 57). Braak and co-workers hypothesized that Lewy pathology progresses in a stereotypical pattern starting in the enteric nervous system and the olfactory bulb, known as the dual-hit-hypothesis(52, 58). Lewy pathology then spreads within the central nervous system through six stages in caudal to rostral direction, roughly aligning with the clinical symptoms of the disease(52) (Figure 3). Each stage represents affection of new regions and worsening of the pathology in previous regions. Importantly, according to the Braak staging, Lewy pathology is first encountered in the substantia nigra at stage 3, corresponding to the onset of motor symptoms. Further, this implies that the early non-motor symptoms are primarily caused by Lewy pathology in the enteric nervous system, olfactory bulb and lower brainstem. Consequently, the extent of neuropathology is well progressed at the time of diagnosis. While the caudal to rostral spread of Lewy pathology between interconnected brain regions may not be universal(59), subsequent studies have confirmed that the criteria are applicable for most cases(60). Although not all clinical symptoms may align with proposed distribution of Lewy pathology(61), in particular dementia is associated with widespread Lewy pathology. Most PD patients with dementia exhibit neocortical Lewy pathology, corresponding to Braak LP stage 5-6(62-64). However, dementia may also occur in the absence of neocortical Lewy pathology in a proportion of cases(65), suggesting that other features in addition to neocortical Lewy pathology contribute to development of dementia.

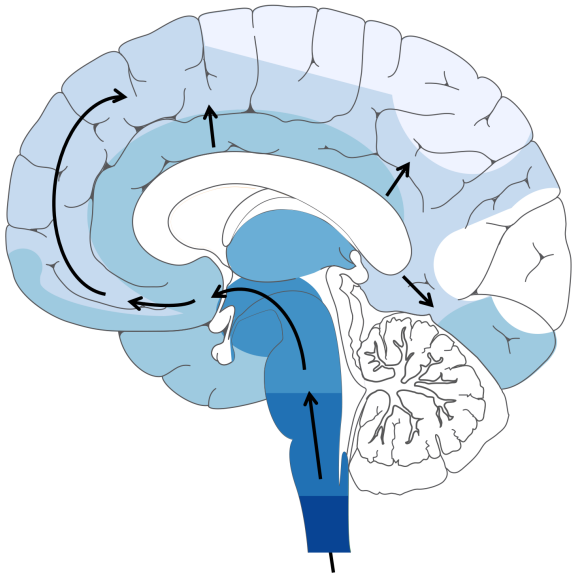

***Figure 3**: Proposed spread of Lewy pathology according to Braak et al.(52). The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.*

Whether PD first develops in the brain or in the peripheral autonomous nervous system is a matter of debate. Emerging data from postmortem and imaging studies have suggested that at least parts of the clinical and neuropathological diversity in PD can be explained by variable disease onset sites. This has led to a recent hypothesis that spread of Lewy pathology follow two different trajectories: "brain first" and "body first" (66-68). In "brain first", Lewy pathology first develops in one of the cerebral hemispheres with secondary spread to the peripheral autonomous nervous system. Accordingly, the clinical symptoms are predominantly unilateral at onset and there are few autonomic symptoms. In the "body first" subtype, Lewy pathology arises in the peripheral autonomous nervous system and then spreads to the brain. Consequently, autonomic symptoms are an early feature. Further, Lewy pathology is expected to spread to both brain hemispheres almost simultaneously through the ascending left and right vagus nerve, leading to early development of cognitive impairment(68). Whether the Braak hypothesis or the brain-first/body-first model best explains the progression of Lewy pathology remains elusive without a validated biomarker that enables longitudinal monitoring of pathology from the pre-symptomatic to late stages of disease.

1.3.2 Alzheimer's disease pathology

In addition to Lewy pathology, varying degrees of concomitant AD-pathology are often present. The two major hallmark protein pathologies of AD are extracellular deposits containing amyloid-β

peptides, referred to as amyloid-β plaques and intracellular aggregates of hyperphosphorylated tau, known as neurofibrillary tangles (NFT)(69). The burden of AD co-pathology in PD patients varies between studies, in part because of different staging systems and cut-off criteria used, but in general levels of amyloid-β and tau pathology sufficient to meet a secondary diagnosis of AD is more common in PDD than in non-demented PD(63, 64, 70, 71). While AD co-pathology is present in 20-30 % of PD patients upon autopsy when cognitive status is not accounted for, as many as 40-90 % of demented patients may have AD co-pathology(72). Although AD pathology is associated with aging and frequently observed in the brains of elderly individuals, several lines of evidence suggest it contributes to the clinical phenotype and rate of progression in PD. AD co-pathology has in particular been associated with reduced overall survival, cognitive decline and dementia(62, 73). Interestingly, AD co-pathology is also associated with a greater burden of Lewy pathology, suggesting a potential synergistic relationship between Lewy and AD-co pathology(62). However, the parallel increase in co-occurring neuropathologies makes it challenging to determine the individual contribution of each pathology to the clinical course of PD.

Accumulation of amyloid-β plaques and tau NFT also follow a unique pattern of spread. In contrast to Lewy pathology, amyloid-β plaques usually first appears in the neocortex. Subsequently amyloid-β deposits extend to the allocortical region and involve key structures such as the hippocampus and amygdala, before spreading to the subcortical region, the brainstem and finally the cerebellum(74) (Figure 4A). Tau NFTs are usually first detected in the transentorhinal cortex, then spread into the entorhinal region and further into the amygdala and hippocampus before extending to most of the neocortex(75). This sequence differs from that observed for amyloid-β plaques (Figure 4B).

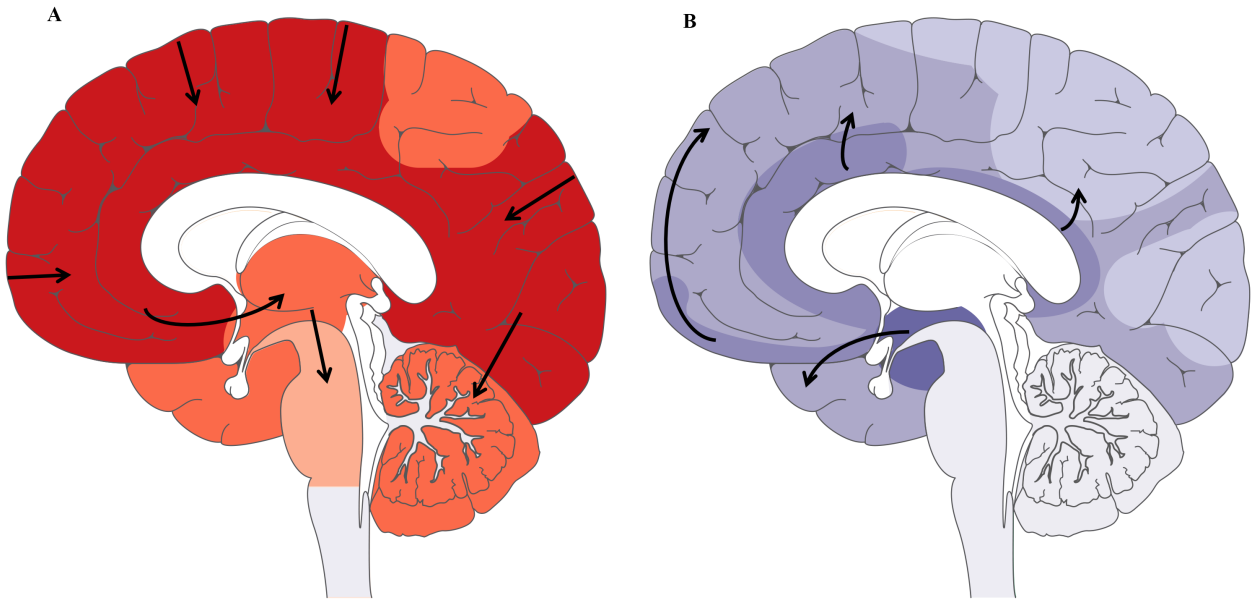

***Figure 4**: The proposed progression of A) amyloid-β plaques according to Thal et al.(74) and B) tau neurofibrillary tangles according to Braak et al.(75). The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.*

1.4 Non-genetic and genetic risk factors

The association between PD and age has been well documented in epidemiological studies(16, 17), and aging is recognized as the primary risk factor for the development of PD. Aging is associated with dysregulation of several cellular and molecular processes, including genomic instability, epigenetic alterations, altered immune response and dysfunction of mitochondria and protein degradation pathways(76), many of which also have been linked to the pathogenesis of PD, as discussed below. Tissues composed of primarily post-mitotic cells, such as the brain, are believed to be especially vulnerable to these changes. However, most elderly people do not develop PD meaning that aging alone is not sufficient to cause PD. In addition to aging, both genetic and non-genetic risk factors are believed to modify the risk of disease(77).

1.4.1 Non-genetic risk factors

A broad range of non-genetic risk factors such as environmental exposure, lifestyle factors, drug use and comorbidities have been linked to PD(78). However, the search for non-genetic risk factors has proven difficult as an exposure could potentially occur decades before disease onset. Moreover,

non-genetic risk factors such as environmental exposure are constantly changing. Most studies to date have been retrospective case-control studies, making them prone to various forms of bias, including reverse causation (i.e., the outcome influences the exposure) or recall bias (i.e., participants may remember or report information inaccurately). The discovery that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can cause parkinsonism by dopamine neuron degeneration provided a link between PD and environmental exposure(79). Later, exposure to several pesticides such as rotenone, maneb and paraquat have shown a positive association with PD risk(80, 81). In a comprehensive review, the following factors associated with PD risk were supported by prospective studies: Consumption of dairy products, diabetes, hormone replacement therapy, depression, mood disorder, bipolar disorder and the use of aspirin were positively associated with PD(78). Contrary, physical activity, smoking, caffein consumption, fat intake, the use of drugs such as ibuprofen, calcium channel blockers, statins and thiazolidinediones and high serum urate levels were negatively associated with PD(78). According to the Braak hypothesis, PD pathogenesis may be initiated in the olfactory bulb and enteric nerves of the digestive system. While biologically plausible that environmental triggers may access the nervous system through the nose or the gut and potentially initiate the disease, this remains to be proven. Moreover, genetic and non-genetic risk factors are believed to interact, with one possible mechanism being through epigenetic modification(82). Epigenetic modifications refer to changes in gene expression and include mechanism such as DNA methylation and histone modification.

1.4.2 The genetic landscape of PD

Although it has been long known that 10-15 % of cases have affected relatives, PD was until the 1980s regarded as a disease with a negligible genetic basis(83). We now know that genetic factors are likely to contribute to virtually all PD cases across a continuum from causal, rare variants to common variants with low effect sizes that only marginally increase the disease susceptibility (Figure 5). By our current understanding of the genetic architecture of PD, a differentiation between monogenic PD (also known as familial or Mendelian PD) and idiopathic PD (also known as sporadic PD) is often made. High penetrance single-gene variants are usually associated with monogenic PD, while low-penetrance risk alleles predispose to idiopathic PD. While this distinction may be practical in a clinical setting, this is likely an oversimplification as there is a considerable overlap between genes associated with monogenic and idiopathic PD and the pathways that they exert their effect on, as will be discussed below.

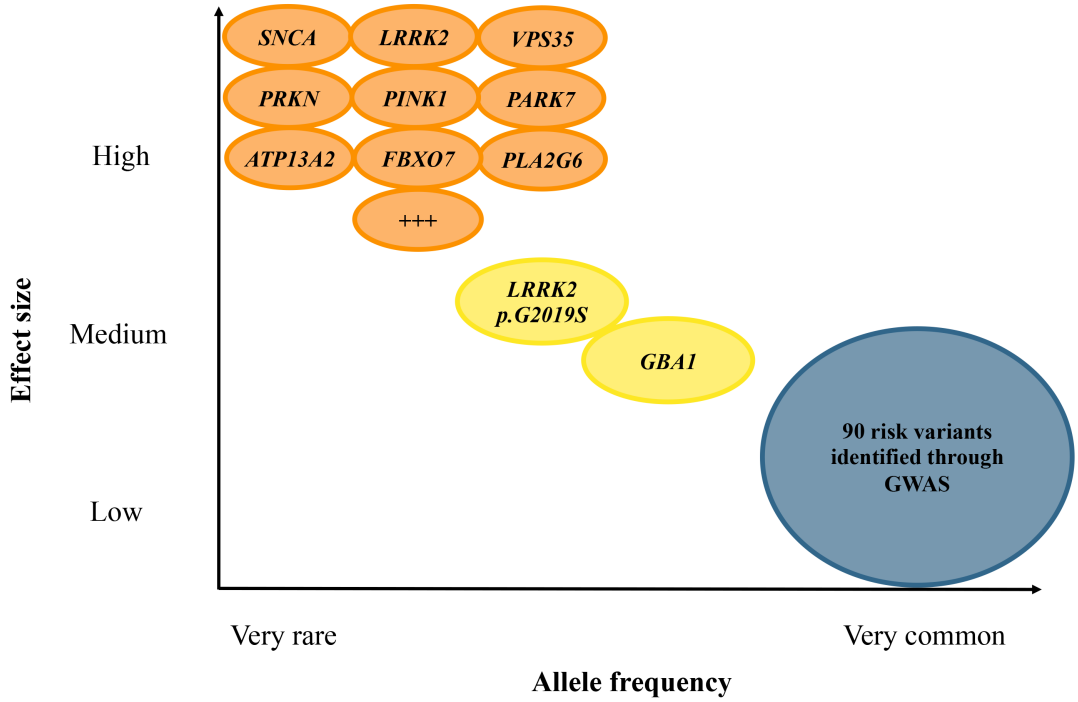

***Figure 5**: The genetic landscape of PD across a continuum of allele frequencies and effect sizes. +++ indicates additional known variants not included in the plot. Adapted from: The Genetics of Parkinson's disease and Implications for clinical practice by Day JO and Mullin S(84), 2021, licensed under CC BY 4.0.*

1.4.3 Monogenic Parkinson's disease

More than 20 genes have been reported as causal for monogenic PD, although only a few have unequivocally been associated with a phenotype resembling idiopathic PD(85). Many genes still lack replication, and their relevance in PD are debated. Genes reported as causative for PD are listed in Table 1. Broadly, monogenic PD often display reduced penetrance and variability in expressivity such as age at onset, clinical presentation, and progression, even among carriers of identical mutations within the same families. These observations suggest that additional genetic and non-genetic factors may contribute to the disease(85). In support of this hypothesis, one study reported that more than 30 % of patients with monogenic PD had one or more additional variants of unknown significance in other PD genes, apparently modifying age at onset(86).

The first definitive monogenic cause of PD was discovered in 1997 when variants in the *SNCA* gene, the gene encoding α-synuclein, were found to cause autosomal dominant PD(87). Later, duplications and triplications of the entire *SNCA* locus have been discovered(88-90). Since then,

variants in several genes have been shown to cause autosomal dominant or autosomal recessive PD. Variants in *SNCA*, *LRRK2*, *VPS35* cause autosomal dominant disease(87, 91-94). Broadly, autosomal dominant PD more frequently display a phenotype resembling idiopathic PD, with good levodopa response, albeit with an earlier age at onset (age at onset ~ 50 years)(95). However, in patients with *SNCA* duplications, and in particular triplications, the age at onset is even lower and the phenotype may be more similar to DLB(90, 96). Pathogenic variants in *PRKN*, *PINK1* and *PARK7* cause autosomal recessive PD(97-99). Clinically, variants in these genes predominantly result in early onset PD (age at onset <40 years) with typical PD symptoms, good levodopa response and slow disease progression. However, dystonia is more prevalent and cognitive decline less frequent compared to idiopathic PD(100). Variants in *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1* and *VPS13C* also cause autosomal recessive PD with an early or even juvenile onset(101-105). Unlike the former group, patients display a more complex phenotype with additional neurological signs and symptoms such as dementia, spasticity or abnormal ocular movements, have a more rapid disease progression and poor or absent levodopa response. (Reviewed by (106)). Additionally, several other genes have been implicated in monogenic PD such as *UCHL1*, *HTRA2*, *GIGYF2*, *EIF4G1*, *DNAJC13*, *TMEM230*, *LRP10*, *CHCHD2* and ATP10B(84, 85, 107).These genes either lack replication in independent families or studies have shown conflicting results.

***Table 1**: Confirmed and unconfirmed genes associated with monogenic PD.*

	Inheritance	**Clinical features**	**Genes**
Confirmed PD genes	Autosomal dominant	Classical PD symptoms	*SNCA*, *LRRK2*, *VPS35*
	Autosomal recessive	Classical PD symptoms	*PRKN*, *PINK1*, *PARK7*
		Atypical PD symptoms	*ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1, VPS13C*
Unconfirmed PD genes	Autosomal dominant	-	*UCHL1*, *HTRA2*, *GIGYF2*, *EIF4G1*, *DNAJC13*, *TMEM230*, *LRP10, CHCHD2*
	Autosomal recessive	-	*ATP10B*

In most populations only 5-10 % of PD cases are known to have monogenic forms with mendelian inheritance(106). However, some variants display population specific frequencies. For example, the most common *LRRK2*-variant (p.G2019S) has a high prevalence among North African Arabs and Ashkenazi Jews (36 % and 28 % of patients with hereditary PD respectively), while being uncommon in east Asians(108). While monogenic PD only account for a small proportion of the total PD cases in most populations, these variants provide insight into the pathways associated with PD. It is also apparent from patients harboring monogenic mutations that PD is phenotypically diverse, and that there is a significant overlap with atypical forms of parkinsonism.

1.4.4 GBA1

Falling between a monogenic variant with reduced penetrance and a strong genetic risk factor is the *GBA1* gene. *GBA1* encodes the lysosomal enzyme glucocerebrosidase (GCase). Homozygous and compound heterozygous variants in *GBA1* cause the lysosomal storage disorder Gaucher disease (GD). GD is divided into clinical subtypes according to the involvement of the central nervous system: Type I (mild, non-neuronopathic), type II and type III (severe, neuronopathic). Clinical observations identified an increased frequency of parkinsonism among heterozygous relatives of GD patients(109, 110). Later, a large multicenter study of PD patients and controls of different genetic origins confirmed an overall fivefold increased risk of PD in heterozygous and homozygous *GBA1* variant carriers(111). Subsequent genetic investigations successfully reproduced these findings, providing evidence that variants in the *GBA1* gene serve as the numerically most important genetic risk factor for PD. The frequency of *GBA1* variants in PD patients is population-specific and varies between 3 and 20 %, with the highest carrier frequency found among Ashkenazi Jews(111). Approximately 300 variants in *GBA1* have been associated with GD, many of which have also been observed in PD patients(112). However, the pathogenicity of each individual *GBA1* variant varies. Rare variants that cause Gaucher disease in the homozygous state (hereafter referred to as Gaucher-causing *GBA1* variants) can be stratified as severe variants (causing GD type II or III, e.g., p.L444P) and mild variants (causing GD type I, e.g., p.N370S). Severe Gaucher-causing *GBA1* variants are associated with a higher risk of PD (odds ratio (OR) >10) than mild variants (OR >2)(113). Interestingly, low-frequency *GBA1* variants that do not cause GD in the homozygous state such as p.E326K(p.E365K) and p.T369M (hereafter referred to as non-Gaucher causing *GBA1* variants) have also been identified as risk factors for PD(114, 115). These variants confer the lowest risk for PD with OR <2(114, 115).

Gaucher-causing *GBA1* variants are low penetrant and large population studies suggest that only about 9.1 % of carriers will develop PD(116). Further, the penetrance is age-dependent(117, 118) and may be modified by additional genetic risk variants(119). Therefore Gaucher-causing *GBA1* variants are regarded as a risk factor for developing PD, rather than a mendelian cause of disease.

On an individual level, PD patients with Gaucher-causing *GBA1* variants are clinically indistinguishable from PD patients not carrying *GBA1* variants. On a group level, carriers of Gaucher-causing *GBA1* variant have an earlier age at onset, more rapid disease progression, increased risk of dementia and increased mortality(120-123). Further, the severity of the variant correlates with the risk of developing cognitive impairment(124). Upon autopsy PD patients carrying Gaucher-causing *GBA1* variants show similar neuropathological features as non-carriers, with nigrostriatal degeneration and widespread Lewy pathology(125), with some reports suggesting a more severe Lewy pathology(126).

1.4.5 Idiopathic Parkinson's disease

For the majority of individuals with PD no causative single-gene variant can be identified. Rather, the disease is likely resulting from a complex interplay between genetic and non-genetic factors. These cases are known as idiopathic or sporadic, although PD neither develops from a completely unknown cause or spontaneously as these terms may suggest. The genetic underpinnings of idiopathic PD have been advanced by landmark efforts including the human genome project(127), the international HapMap project(128) and the 1000 Genomes project(129). Collectively, these projects have provided databases on the human genome sequence, genetic structure and variation that have been essential for understanding the impact of the human genome on health and disease.

Although the human genome sequence is remarkably similar, every unrelated individual differs by millions of base pairs (bp)(129) that potentially can increase or decrease the susceptibility for disease. Single nucleotide variants (SNV) are the most abundant type of genetic variability, in which individuals differ in a single genomic position. Other forms of variation come from insertions, deletions and larger structural changes such as copy number variation (CNV). Genetic variants are classified by the frequency of the least common allele (minor allele frequency (MAF)) in a population. By convention, variants are considered common (MAF >1 %), low-frequency (MAF 0.1-1 %) and rare (MAF <0.1 %), although different cutoffs in the literature exist. Common SNVs are usually referred to as single nucleotide polymorphisms (SNPs). Further, the human genome exhibits a haplotype block structure where recombination occurs at relatively few

recombination hotspots, while the regions between these hotspots tend to have low recombination rates(128). Consequently, SNPs in physical proximity tend to be inherited together more often than expected by chance, i.e., they are in linkage disequilibrium (LD), and genotyping only a few common genetic variants is strongly predictive of variants within the same haplotype block.

In parallel, advances in technology have made it feasible to conduct large scale genetic studies. Development of cost-effective SNP-arrays which genotype 300,000-1,000,000 selected variants with genome wide coverage, and imputation of variants that have not been assayed directly, have been instrumental to conduct genetic studies in large population samples. By tagging nearby variants through patterns of linkage disequilibrium (LD), these SNP-arrays should give a representation of the entire genome. In this context, genome wide association studies (GWAS) have been a successful approach in linking genetic variants to disease(130). GWAS systematically and in a hypothesis-free manner assess million of common genetic variants for association with a phenotype by comparing differences in allele frequencies. For PD, the commonly investigated phenotype has been case vs. control status (i.e., PD susceptibility). However, it could potentially also encompass continuous variables like age at onset or time-to-event such as time to dementia(85). More recently, a transition towards whole exome sequencing (WES) and whole genome sequencing (WGS) techniques, which provide a denser coverage and high genotype precision have emerged. These advances hold promise to uncover rare variants and other types of genetic variation including CNVs that are not readily detected with SNP-arrays(131). However, due to the substantial computational and financial resources required, the sample sizes in WES and WGS studies are still small compared to GWAS.

Over the past two decades, significant progress has been made in identifying genetic risk variants associated with idiopathic PD, largely through the use of GWAS. The two initial PD GWAS were published in 2005 and 2006 respectively. However, due to limited samples size these early investigations had limited power to reliably detect risk loci(132, 133). In 2009 the two first genomewide significant PD loci were identified in European cases, specifically in *SNCA* and *MAPT*(134). Concurrently in Japanese PD cases, significant loci were identified in *PARK16*, *BST1* and *LRRK2*(135). Since then, a substantial number of PD GWAS with progressively larger sample size have been published, and a considerable proportion of risk loci have been replicated, suggesting that they represent true associations. Meta-analyses, combining data from several GWAS have become an important approach to enhance discovery of PD-associated variants. The most recent PD GWAS based on meta-analysis was published in 2019 and involved a large cohort

of 37,700 PD cases, 18,000 proxy cases (first degree relatives of PD patients) and 1.4 million controls(136). This study identified 90 genetic variants across 75 loci associated with PD susceptibility, providing further evidence for the polygenic architecture of PD. Heritability estimates for PD range from 27-34 % bases on twin-studies(137, 138) to 22-27 % based on GWAS (SNP-based heritability)(136, 139). However, identified GWAS loci only explain ~1/3 (16-36 %) of the estimated heritability, suggesting a large proportion of genetic variants are yet to be discovered(136). For a summary of some important GWAS publications in PD, please see Figure 6.

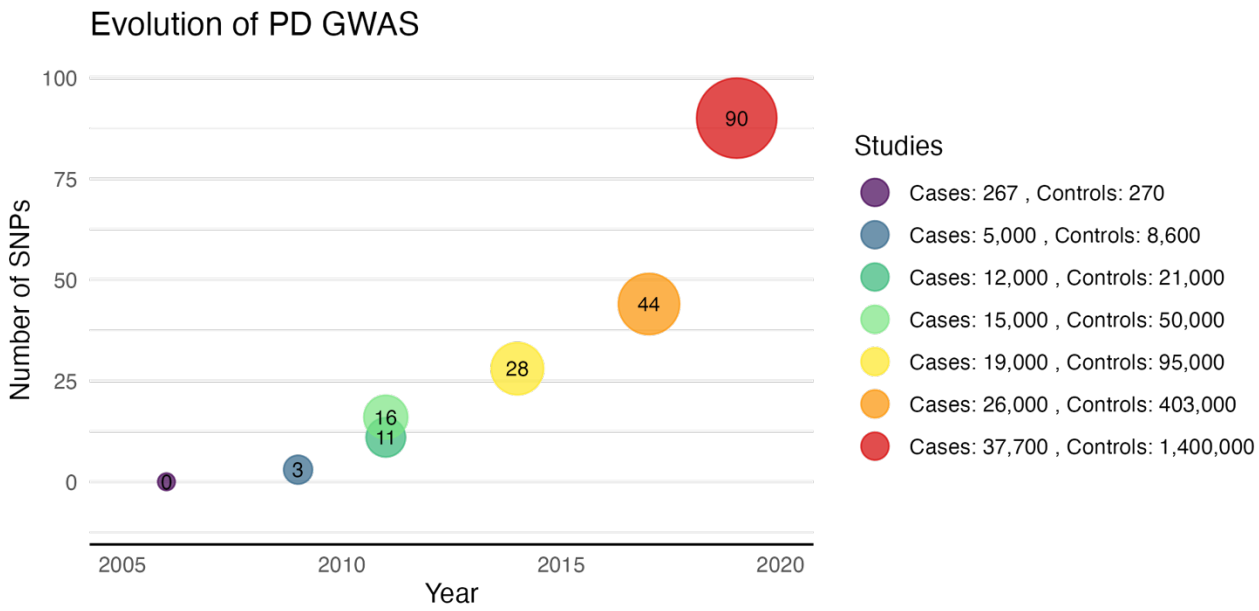

***Figure 6**: Evolution of PD GWAS from 2006 to 2019. Each bubble represents a GWAS(133, 134, 136, 140-142) and the number of genome wide significant SNPs associated with PD are displayed in the center of each bubble. Note that the 2019 GWAS in addition to 37,700 PD cases also included 18,000 proxy cases(136).*

Genetic factors do not only influence the risk of developing PD, but likely also contribute to the progression and heterogeneity in manifestation of motor and non-motor symptoms. Evidence from studies on monogenic PD highlights that different mutations lead to diverse phenotypes characterized by variations in age at onset, disease progression and susceptibility to dementia. Genetic factors that influence the expression or severity of the disease are called genetic modifiers. The search for genetic modifiers has gained significance interest, as these factors could potentially serve as targets for therapeutic interventions. Through the identification of genetic modifiers that influence key disease milestones, such as the time at which certain motor or non-motor symptoms appear, novel avenues for drug development could be uncovered. While much attention has been

directed towards known PD risk loci, a more unbiased investigative approach has begun to gain traction in recent large-scale studies. Nevertheless, such approaches have been limited by the scarcity of comprehensive patient cohorts with extensive phenotyping, adequate sample size and sufficient longitudinal follow-up. Nevertheless, using a GWAS approach, Bluwendraat et al. showed that *SNCA* and *TMEM175*, both established PD susceptibility loci, were associated with an earlier age at onset(143). Moreover, the heritability for age at onset was estimated to 11 %, much lower than the heritability estimates for PD, perhaps related to the subjective nature of the outcome. Subsequent studies have confirmed the role of TMEM175 in age at onset, and a novel *BST1* locus has been nominated(144, 145). Similarly, in GWAS on longitudinally followed cohorts *GBA1* and two loci not known to alter PD susceptibility, *APOE* and *RIMS2*, have been associated with cognitive decline(145-148). As discussed above, Gaucher-causing variants in *GBA1* associate with a higher rate of cognitive decline and dementia. The *APOE* gene, located on chromosome 19, has three common alleles (E2, E3 and E4). While the *APOE* E4 allele is a major genetic risk factor for AD(149) and DLB(150), it does not appear to alter PD susceptibility. However, several early studies have reported significant associations between the E4 allele and cognitive decline in PD, although results have been inconsistent(151). Variants in the *MAPT* locus have robustly been associated with PD risk in GWAS(134). *MAPT* encodes the tau protein which is the main component of neurofibrillary tangles (NFTs) and has consistently been related to several other neurodegenerative disorders, including frontotemporal dementia (FTD), PSP, CBD and AD(152). Therefore, *MAPT* has become an attractive candidate gene for cognitive decline in PD. Some studies have indicated a potential role for *MAPT* in influencing cognitive decline in PD, however findings have been inconsistent(151), and the more recent GWAS on cognitive progression have not confirmed the association(145-148). Collectively these studies suggest that the there is only a partial overlap between the genetic architecture of PD susceptibility and progression, indicating distinctive genetic factors are at play for these two aspects of the disease.

1.4.6 Lessons learned from GWAS

Following the successful identification of genetic risk loci through GWAS, several valuable insights have been gained. GWAS variants individually only exert a modest effect on disease risk, typically exhibiting an odds ratio less than 1.5. To detect these variants with small effect sizes, large population samples are required to ensure sufficient statistical power. In addition to discovering novel loci, several GWAS loci are in close proximity of known monogenic PD genes indicating shared biological pathways in both forms. Examples of such pleomorphic loci are *SNCA*, *LRRK2*, *GBA1* and *VPS13C*(85). Further, PD also shares genetic influence with other neurodegenerative

diseases such as DLB (*SNCA*, *GBA1*, *TMEM175*)(153) and AD (*HLA* locus and *MAPT*)(154, 155), suggesting shared genetic etiology of potential clinical importance. Such insight can potentially pave the way for identifying convergent therapeutic targets and advance our understanding of the interplay among these neurodegenerative diseases.

Despite the progress highlighted above, the translation of discovered genetic variants into improved clinical care has been limited. One of the bottlenecks lies in functionally validating the disease-associated variants. Only a few PD loci have been functionally validated, among them *SNCA*, *LRRK2*, *GBA1* that are also implicated in monogenic PD. GWAS variants are most often not causative, but rather inherited together, i.e. in high linkage disequilibrium (LD) with one or more causal variants(156). Further, most GWAS variants are located in non-protein coding regions of the genome without any obvious effect on normal protein function. Rather, non-coding variants are more likely to be located in regulatory regions(157), thereby contributing to disease risk though regulation of transcription or expression of one or more nearby or distant genes(158). Adding to the complexity, the functional role of the disease associated variant may be context dependent, as the regulatory function is expected to be tissue and cell type specific. Thus, identification of causal variants and the target genes remain challenging and requires follow-up studies using various approaches including fine-mapping, and integrating several sources of functional data from disease relevant tissue and cell-types. A detailed discussion on the topic can be found in (156, 159).

1.5 Pathways associated with Parkinson's disease

Apart from discovering causative genes, another challenge is to understand the mechanisms through which these factors contribute to disease. It is now recognized that genes may operate collectively within biological pathways rather than in isolation. In a comprehensive analysis involving ~26,000 PD patients and ~403,000 controls, a total of 2,199 curated gene sets representing biological pathways were assessed for association with PD risk. Among these, 46 partly overlapping gene sets were linked to PD susceptibility in both the testing and replication phase of the study(160). Both genes leading to monogenic PD and genes nominated through GWAS appear to converge on common pathways. In particular pathways involved in α-synuclein misfolding and aggregation, lysosomal dysfunction, endosomal trafficking, mitochondrial dysfunction and immune response have been highlighted(160-162). Consequently, as we currently only know a proportion of PD

associated genes, collective rather than individual assessment of variants may be a promising approach to understand the biological underpinnings of PD.

1.5.1 Alpha synuclein aggregation and misfolding

The discovery of mutations and multiplications in the *SNCA* gene as a cause of monogenic PD, identification of α-synuclein as a major component of Lewy pathology, as well as common variation in the *SNCA* locus increasing the risk for idiopathic PD collectively point to a pivotal role of α-synuclein in the pathogenesis of PD. The physiological role of endogenous α-synuclein remains poorly understood, although it appears to play a role in synaptic vesicle function, vesicular trafficking and neurotransmitter release(163, 164). α-synuclein acquires pathogenic properties through a polymerization process where soluble monomers form oligomers that ultimately may aggregate into large insoluble fibrils that may be incorporated in Lewy pathology(165). However, both oligomeric and fibrillar α-synuclein conformations have been reported to exhibit toxic properties, yet which is most relevant in the induction and progression of PD remains unresolved(165). More recently, different strains of α-synuclein have been recognized. These strains are distinct forms of α-synuclein exhibiting different conformational arrangements and properties, including differences in toxicity(165). While the precise mechanisms underlying the spread of α-synuclein in the nervous system remain incompletely understood, emerging evidence indicates that α-synuclein can have prion-like properties. At the center of this hypothesis is the notion that α-synuclein can self-aggregate(166), transmit from one cell to another(167), and act as a seed to induce aggregation of α-synuclein in the recipient cell(168). Human evidence supporting such prion-like properties of α-synuclein comes from reports of Lewy pathology acquired in grafted fetal mesencephalic neurons(169, 170). The potential prion-like spread of α-synuclein fits into the current hypothesis of Lewy pathology progression, perhaps explaining the fairly consistent anatomical progression of interconnected brain regions. Collectively, these properties suggest potential self-propagating features of α-synuclein that often are referred to as prion-like properties, although there is no evidence to support direct transmission of PD between individuals, unlike prion diseases.

1.5.2 Lysosomal pathway

Lysosomes are cellular organelles that have a pivotal role in maintain cellular homeostasis. Specifically, they function as the terminal degradative station for multiple cellular trafficking routes, including the autophagy and endosomal trafficking pathways(171). Importantly, lysosomes

are involved in α-synuclein degradation, in particular through the autophagy-lysosomal pathway(172, 173). An intricate, bi-directional relationship between α-synuclein and lysosomes have been suggested: Inhibition of lysosomal function increases intracellular accumulation of α-synuclein(173). Moreover, aggregated α-synuclein has been shown to inhibit the autophagy-lysosomal pathway, either by impeding lysosomal uptake(172), or by causing a more generalized lysosomal dysfunction(174) thus impairing its own degradation. Consequently, lysosomal dysfunction may impede α-synuclein clearance, promoting its aggregation and propagation.

Genes causing rare forms of monogenic PD such as *VPS13C*, *ATP13A2* and *PLA2G6* have been suggested to be involved in lysosomal functions(175). Additionally, although involved in several biological pathways, one key role *LRRK2* is potentially maintaining lysosomal homeostasis(176). Genetic studies on idiopathic PD have further highlighted a broad contribution of genes implicated in lysosomal storage disorders(177). Among the lysosomal genes, *GBA1* variants are confirmed as a major risk factors for both PD(111) and DLB(178), as previously discussed in section 1.4.4. In addition to *GBA1*, several other genes involved in lysosomal functions including lysosomal enzymes, lysosomal membrane proteins and proteins involved in lysosomal trafficking and autophagy have been nominated by GWAS as risk factors for PD. These include loci in the proximity of *TMEM175*, *SCARB2*, *GAK*, *GALC*, *VPS13C*, *CTSB*(136, 140-142, 179), that have been replicated, and loci in the proximity of *ATP6V0A1*, *GRN*, *GUSB*, and *NEU1* that been nominated(136, 142). A functional association between lysosomal impairment and α-synuclein aggregation has been suggested for several of these(174, 180, 181). Collectively, these studies point to a major contribution of the lysosomal pathway in the pathogenesis of PD where both rare and common variants influence disease susceptibility. For a more comprehensive review of lysosomal genes associated with PD, please refer to(175, 182).

1.5.3 Endosomal trafficking pathway

The endosomal trafficking pathway is a network of membrane-enclosed structures involved in collection, sorting and dissemination of protein and lipid cargo between the plasma membrane and the intracellular compartment(183). Internalization of membrane-bound protein and lipid cargo through endocytosis is the first step in the endosomal trafficking pathway. Following endocytosis, the cargo converges in the early endosome, the initial sorting station of the pathway. Once in the early endosome, cargo can either be recycled back to the plasma membrane, transported to the trans-Golgi network or be retained in the early endosome which matures into a late endosome. The

late endosome ultimately fuses with the lysosome for degradation of proteins and lipids, linking the endosomal trafficking and lysosomal pathways(184). A significant proportion of genetic variation in PD has been linked to the endosomal trafficking pathway. This includes both monogenic genes such as *VPS35*(94), *DNAJC6*(105), and *SYNJ1*(185, 186) and genes nominated by GWAS such as *GAK*, *VAMP4*, *NOD2*, *RAB29,* and *SH3GL2*(136, 141, 142). These genes have been linked to various steps in the endosomal trafficking pathway, supporting the notion that the endosomal trafficking pathway is of particular importance in PD. Also, *LRRK2* is linked to vesicle trafficking, in particular as a regulator of endocytosis(187). Bandres-Ciga et al. conducted a recent study on ~29,000 PD cases and 22,000 controls, focusing on 252 genes associated with the endosomal trafficking pathway(161). Their findings emphasize the involvement of this pathway in PD susceptibility and identified additional genes that go beyond the associations previously identified through GWAS.

1.5.4 Mitochondrial pathway

Mitochondria are essential in energy metabolism and regulation of cell death via apoptosis. Decline in mitochondrial function has been demonstrated to occur in humans during aging, and neurons, which have high metabolic requirements, are particularly susceptible to these age-related impairments(76). Mitochondrial dysfunction represents another well-established mechanism in the pathogenesis of PD. The first evidence of mitochondrial dysfunction in PD came from cases exposed to the neurotoxin MPTP which target nigrostriatal dopaminergic neurons through blocking mitochondrial respiration(188). A deficiency of the complex I of the mitochondrial respiratory chain has further been found in post-mortem brains from patients with idiopathic PD, implicating mitochondrial dysfunction as a more general phenomenon in PD(189). PD genetics have further strengthened the link between PD and mitochondria. Mutations in autosomal recessive PD genes including *PRKN*, *PINK1*, *PARK7*, and *FBXO7* directly impact mitochondrial function, in particular mitophagy, by which damaged mitochondria are selectively removed via autophagy and ultimately degraded in the lysosome(190-193). Additionally, *SNCA*, *LRRK2* and *VPS35* have been reported to indirectly regulate mitochondrial function, suggestion crosstalk between disease pathways (reviewed in (194)). The discovery of putative mitochondrial genes through GWAS has been limited, although some exceptions exist. Common variation in the *MCCC1* gene, which encodes a subunit of the mitochondrial enzyme MCC, has been consistently replicated(136, 141, 142), while *COQ7* and *ALAS1* have been nominated(142). More recently, Billingsley et al. nominated

additionally 14 genes associated with mitochondrial dysfunction using Mendelian randomization(162).

1.5.5 Immune system

While not considered an autoimmune disease, compelling evidence also implicate innate and adaptive immune mechanisms in PD. Whether these processes are a cause or effect of neurodegeneration remains unresolved. Activated microglia in the substantia nigra in post-mortem brains of PD-patients were described several decades ago(195), and increased levels of pro-inflammatory cytokines have been reported in the cerebrospinal fluid (CSF) and brain-tissue(196). More recently, the involvement of the adaptive immune system has been suggested, and in particular CD4+ and CD8+ T cells have been shown to accumulate in the substantia nigra of PD patients(197). Immune mechanisms have also been highlighted by PD GWAS where non-coding variants in the human leukocyte antigen (*HLA*) and bone marrow stromal cell antigen 1 (*BST1*) locus have been consistently associated with PD-risk(135, 136, 141, 198). Intriguingly, while both common and rare variants in *LRRK2* are strongly linked to PD, *LRRK2* has in GWAS been identified as a susceptibility factor for the autoimmune disorder Crohn's Disease(199). Moreover, there is a considerable genetic overlap between PD and seven autoimmune diseases, in particular Crohn's disease(200). More recently, a significant cell-type heritability enrichment for microglia has been reported in PD(155). These findings suggest that immune dysregulation may serve as a common pathway underlying more PD associated genes than previously recognized. For a more comprehensive discussion of immune dysfunction in PD, a recent review has been published(201).

As discussed above, genetic studies have revealed several pathways associated with PD. Moreover, many of these pathways are interconnected, with the lysosome appearing to be a central nexus as the terminal degrative station for several intracellular trafficking routes(171). Consequently, dysfunction in one pathway can result in secondary effects in another pathway, as highlighted by the reciprocal relationship between mitochondria and lysosomes. Dysfunctional mitochondria impair lysosomal function and vice versa, suggesting a mutually destructive relationship(202, 203). Importantly, impairment of the lysosomal, endosomal and mitochondrial pathways as well as immune mediated mechanisms are not only limited to PD, but appear to converge in several neurodegenerative diseases, pointing to shared pathological mechanisms(204).

2 Thesis aims

There is a current lack of disease-modifying therapies for PD. The projected doubling in the prevalence of PD within the next generation underscores the pressing need for effective interventions. Numerous genetic risk factors for PD have been identified, and they converge on shared pathways. However, how these genetic risk factors either individually or through common pathways modify the disease, and how they relate to the key neuropathological substrate of PD remains poorly understood. Addressing these knowledge gaps forms the foundation for the studies presented in this thesis. The overall aim of my PhD work is to increase our understanding of the interplay between genetic and neuropathological heterogeneity and how they contribute to cognitive deterioration in PD.

Paper 1: In this paper we explored the influence of common variation in the Apolipoprotein E (*APOE*) and microtubule associated protein tau (*MAPT*) loci, and time to development of dementia in PD, in neuropathologically characterized samples.

Paper 2: In this paper we investigated how common genetic variants associated with PD and AD influence Lewy and AD co-pathology respectively in patients with PD and DLB. With the hypothesis that differential genetic mechanisms may influence Lewy pathology depending on the level of AD co-pathology, samples were stratified by the presence or absence of AD co-pathology.

Paper 3: In this paper we examined if the lysosomal PD polygenic risk score (PRS) highlighted in Paper 2 was associated with an earlier onset of dementia in PD patients with a reduced vulnerability to AD co-pathology.

3 Summary of results

3.1 Paper 1

APOE and MAPT Are Associated With Dementia in Neuropathologically Confirmed PD

Genetic risk factors are potential predictors for cognitive decline and dementia. Both the *APOE* and *MAPT* loci have previously been linked to cognitive decline in PD, but the results have been conflicting. In this paper we investigated whether SNPs tagging the *APOE* (rs429358 and rs7412) and *MAPT* (rs1800547) loci were associated with the onset of dementia. We conducted survival analysis on 152 samples with PD from the Netherlands Brain Bank (NBB), taking advantage of the neuropathological confirmed diagnoses. We showed that both the *APOE* E4 allele and the *MAPT* H1 haplotype were associated with an accelerated onset of dementia. Further, we demonstrated the influence of *APOE* E4 on the level of amyloid-β pathology, indicating *APOE* influence dementia development through deposition of amyloid-β plaques. Identifying PD patients at high risk for early dementia has prognostic implication and can potentially improve the selection for clinical trials in a precision medicine context, and aid in identification of potential therapeutic targets.

3.2 Paper 2

Lysosomal polygenic risk is associated with the severity of neuropathology in Lewy body disease

In paper 2 we investigated how common genetic variants associated with AD and PD risk influence the key neuropathologies in patients with PD and DLB. The study was divided into a discovery phase in samples from the NBB (n=217) where associations were nominated for replication in the Mayo Clinic Jacksonville Brain Bank (n = 394). We constructed AD-PRS and assessed the relationship with measures of amyloid-β and tau pathology. Further, we constructed a PD-PRS and PRS reflecting 6 different pathways and 2 cell types previously implicated in PD, and investigated their relationship with Lewy pathology in samples with and without significant AD co-pathology. We found that a higher polygenic burden for AD (AD-PRS) was associated with the level of amyloid-β and tau pathology in both cohorts. In a sensitivity analysis where the *APOE* region was removed from the AD-PRS, associations with measures of amyloid-β and tau pathology were still

significant in the Mayo Clinic cohort, suggesting that variants beyond the well-established *APOE* locus contribute to the level of AD pathology. Moreover, our data showed that the PRS reflecting the lysosomal pathway (lysosomal PD-PRS) was associated with Lewy pathology in the subset of samples without significant AD co-pathology in both the discovery and replication cohort. The association between lysosomal PD-PRS and Lewy pathology was more consistent than the genome-wide PD-PRS, suggesting that only a subset of SNPs associated with PD risk contribute to the level of Lewy pathology. Further, we showed that the lysosomal PD-PRS associated with an earlier onset of dementia in the subset of individuals without significant AD co-pathology from the NBB.

Our results extend the current knowledge about the contribution of both AD and PD risk variants on the heterogenous neuropathologies in PD and DLB. In particular, our data highlight variants within the lysosomal pathway as relevant to the level of Lewy pathology and development of dementia in the absence of significant AD co-pathology. Our findings provide evidence that PRS may serve as potential markers to enhance the selection of participants for clinical trials.

3.3 Paper 3

Lysosomal polygenic burden is associated with cognitive progression in Parkinson's disease patients with low risk of Alzheimer co-pathology

In Paper 3 we followed up our results from Paper 2 and investigated whether the lysosomal PD-PRS was associated with faster progression to cognitive impairment in longitudinally followed patients with a low vulnerability to AD co-pathology. We included patients from two longitudinal PD cohorts, the Parkinson's Progression Markers Initiative (PPMI) (n = 374) and the National Institute of Neurological Disorders and Stroke (NINDS) Parkinson's Disease Biomarker Program (PDBP) (n = 777). In the absence of gold-standard assessment of AD co-pathology, we stratified patients based on CSF measures of amyloid-β and tau, as well as AD-PRS. The optimal AD-PRS threshold for discriminating between patients with and without significant AD co-pathology was determined in neuropathologically verified LBD samples from the NBB (n = 217).

Our results show that the lysosomal PD-PRS was associated with an earlier onset of cognitive impairment in patients with a low vulnerability to AD co-pathology, both based on CSF measures of amyloid-β and AD-PRS in the PPMI samples. The association between lysosomal PD-PRS and cognitive impairment in samples with a low vulnerability to AD co-pathology was replicated in the

independent PDBP samples. Further, our results suggest that the association between the lysosomal PD-PRS and cognitive impairment extends beyond the well-established *GBA1* locus.

Our results underscore the role of lysosomal polygenic burden in the cognitive progression of PD patients with a low vulnerability of AD co-pathology, replicating our results from Paper 2. Moreover, our data provide compelling evidence for the potential use of AD-PRS as a means to discriminate between patients with and without vulnerability to AD co-pathology.

4 Methodological considerations

4.1 Subjects

In paper 1, 2 and 3 we included neuropathologically verified donors with LBD from the Netherlands Brain Bank (NBB) (www.brainbank.nl). Donors enrolled from 1989 to 2017 (n = 3,853) were considered for study inclusion, and cases with available Lewy and AD pathology staging, clinical information and genotype data were included.

For Paper 1, our focus was on cases with neuropathologically confirmed PD with and without dementia, resulting in a sample size of 152. Cases were diagnosed based on the combination of the UK Parkinson's Disease Society Brain Bank criteria(205), moderate to severe loss of neurons in the substantia nigra and Lewy pathology at minimum within the brainstem. Cases with DLB were excluded from this study based on the presence of dementia within the first year of disease onset(42). Dementia was diagnosed during life by a neurologist or geriatrician, or retrospectively based on neuropsychological test results indicating impairment in at least two core cognitive domains(206), or a Mini-Mental State Examination (MMSE) score below 20.

In paper 2 we included 222 subjects from the NBB with PD or DLB. DLB cases had a clinical diagnosis of probable DLB(42) in combination with the presence of limbic-transitional or diffuse-neocortical Lewy pathology. After excluding extreme age outliers (n = 1) and cases with an atypical distribution of Lewy pathology (n = 4) 217 cases remained for the final analysis. Additionally, neuropathologically healthy controls (n = 82) and AD cases (n = 64) were included to assess the discriminative ability of AD- and PD-PRS. Furthermore, we included autopsy-confirmed cases (n = 402) with an antemortem diagnosis of PD(205) or DLB(42) from the Mayo Clinic Jacksonville Brain Bank for Neurodegenerative Disorders for replication of results. 394 cases were included in the final analysis after removing extreme age outliers (n = 8).

In paper 3 the same LBD cases from NBB used in Paper 2 were included (n = 217). These cases were used to identify the optimal cut-point for AD-PRS to distinguish between cases with and without AD co-pathology. For the main analysis in Paper 3 we included patients from two longitudinal cohorts: the Parkinson's Progression Markers Initiative (PPMI) and the National Institute of Neurological Diseases and Stroke (NINDS) Parkinson's Disease Biomarker Program

(PDBP)(207, 208). PPMI (www.ppmi-info.org) is a multicenter prospective longitudinal study including PD patients over 30 years of age within 2 years of diagnosis and not requiring symptomatic therapy at baseline. The study started in 2011, and recruitment is still ongoing. Patients enrolled in the initial phase of the study with a clinical diagnosis of PD and positive DaT-SPECT imaging (n = 423) were considered for inclusion. Clinical variables from baseline and annual study visits for the first five years were used. PDBP (https://pdbp.ninds.nih.gov) is a consortium of sites collecting data to well characterized longitudinal cohorts. Patients are included at various stages and duration of disease, irrespective of dopaminergic therapy. PDBP is designed to mimic a broad PD population, thereby representing prototypical candidates for future clinical trials(208). Inclusion criteria are a diagnosis of PD according to the UK PD Society Brain Bank criteria(205). Additional inclusion criteria, such as positive DaT-SPECT imaging, age requirements or response to dopaminergic therapy, apply at the various sites contributing to the consortium(208). A total of 374 individuals from PPMI and 777 from PDBP fulfilling diagnostic criteria for PD and with available genotypes, demographic variables and cognitive assessment were included.

4.1.1 Advantages and limitations of post-mortem samples

Identification of relevant genetic associations rely on precise phenotypes. Due to overlap of symptoms with other neurodegenerative diseases, the diagnostic error rates of PD and DLB are high, in particular in the early stages of disease, as previously discussed. While advances in clinical diagnostic criteria have been made, autopsy remains the gold standard for confirming the disease. Consequently, brain bank cohorts of neuropathologically confirmed cases offer a more accurate phenotype essential to research into neurodegenerative diseases. However, the access to brain bank samples is limited, likely reflecting the substantial financial and time investments required to recruit, collect, characterize and store human brain tissue(209). Nevertheless, brain-bank cohorts are inherently cross-sectional in nature and clinical data often collected retrospectively, limiting longitudinal assessment. Moreover, systematic clinical examination may not be consistently conducted, particularly in the last years of life when clinical deterioration often hinders assessment. Further, samples are biased towards the advanced disease stages, with most cases displaying end-stage pathology. As Lewy and AD co-pathology are expected to evolve over the entire disease course, even in the prodromal phases before clinical symptoms are evident, brain bank cohorts are not representative of the early stages of disease.

4.1.2 Combining PD and DLB

In Paper 2, we chose to focus on the genetics of neuropathology in individuals with PD and DLB collectively. The clinical differentiation between PD and DLB has become less clear as dementia at onset no longer is an exclusion criterion for PD(23). From a clinical point of view, it is meaningful to maintain a distinction between PD and DLB, in terms of prognostic implications and treatment strategies. However, PD and DLB also have overlapping pathological hallmarks and genetic risk factors, pointing to a shared underlying pathological process. While neuropathological differences between PD and DLB have been described on a group level(210), these distinctions alone are insufficient for definitive differentiations in the absence of clinical information(211). A significant genetic correlation between PD and DLB has also been reported(212) and variants in the proximity of *SNCA*, *GBA1* and *TMEM175* have in GWAS been nominated as risk loci in both diseases(153). Combining PD and DLB has certain advantages, such as increase in the statistical power and the potential to uncover associations that are common to both entities. However, if the underlying disease mechanisms in PD and DLB are fundamentally different, the combination of the two conditions would introduce statistical noise and lead to overgeneralization of results beyond their relevant context.

4.2 Cognitive assessment

In Paper 3, cognition was assessed using the Montreal Cognitive Assessment (MoCA)(213). The MoCA is a brief screening tool of global cognition covering multiple cognitive domains, including assessment of short-term memory recall, visuospatial abilities, executive functions, phonemic fluency, verbal abstraction, attention, concentration, working memory, language and orientation. One additional point is added for people with less than 12 years of education, with a maximum total score of 30. While comprehensive neuropsychological evaluation is more reliable for a diagnosis of PDD(206), these assessments are resource-intensive and often challenging to obtain for the number of patients required for genetic studies. Consequently, researchers will often have to settle for more crude measures of cognition like the MoCA score due to practical limitations. The majority of PPMI subjects had more detailed cognitive evaluation available, yet cognitive assessment of PDBP samples relied on the MoCA score. Consequently, to harmonize between the two cohorts, the MoCA score was chosen in Paper 3. The MoCA is well-recognized for detecting cognitive symptoms in individuals with PD(214, 215). Moreover, the MoCA score has also been demonstrated to differentiate between various cognitive stages in individuals with PD and healthy

controls(215). While various cut-off scores have been proposed, we chose <21/30 to classify cognitive impairment in Paper 3, which has previously been determined as the optimal cut-off for PDD(215).

4.3 Staging of neuropathology

In Paper 1 (NBB), 2 (NBB and the Mayo Clinic) and 3 (NBB) neuropathological characterized samples were included. All brain autopsies were performed by experienced neuropathologists. A. J. M. Rozemuller and W. D. J. v. d. Berg conducted the brain autopsies at NBB and D. W. Dickson at the Mayo Clinic.

4.3.1 Lewy pathology

Lewy bodies and Lewy neurites were immunostained by antibodies against α-synuclein (Mouse monoclonal anti α-synuclein, clone KM51, 1:500 Monosan Xtra, the Netherlands and Rabbit polyclonal anti α-synuclein, 1:3000 Mayo Clinic antibody, FL). Various staging schemes have been devised to systematically assess and evaluate the extent of Lewy pathology. These staging systems offer valuable frameworks to better understand the progression and impact of Lewy pathology, enhance diagnostic accuracy and facilitate research. Kosaka et al. were the first to introduce a standardized staging of Lewy pathology. They classified cases with LBD into three pathological subtypes according to the distribution of Lewy pathology: Brainstem LBD (BLBD), transitional LBD (TLBD) and diffuse LBD (DLBD)(57). Building on the concept of a caudal to rostral spread of Lewy pathology delineated by Kosaka, Braak et al. described a more detailed six stage progression of Lewy pathology in PD(52, 53). In addition to refining the staging system of the brainstem and cortex, Braak also suggested that Lewy pathology originated outside the central nervous system. Similarly, the DLB consortium later adapted the staging by Kosaka in the pathological criteria for DLB as described by McKeith and colleagues(56). In addition, several other staging systems have been developed but are beyond the scope of this thesis to discuss.

The donors from NBB were assigned a Braak Lewy pathology stage (3-6 for LBD donors) using the BrainNet Europe (BNE) Consortium protocol(216). In the BNE protocol the presence or absence of lesions rather than lesion counts are assessed, improving the inter-rater agreement(216). In the Mayo Clinic donors, Lewy pathology was staged as BLBD, TLBD and DLBD according to Kosaka(57). Comparison between the two staging schemes have previously been published(60) and

is provided in Table 2. While an approximate conversion from Braak Lewy pathology stage to Kosaka stage has been proposed, such adaptations are prone to inaccuracies, as Braak Lewy pathology stage 3 could represent both the BLBD and TLBD, and Braak Lewy pathology stage 5 could represent both TLBD and DLBD if assessment instructions are strictly followed(216). Importantly, our study design involved an initial discovery analysis in the NBB cohort followed by an independent replication attempt in the Mayo Clinic cohort, and we did not consider it justified to subsequently revise the original discovery analysis based on a potentially suboptimal conversion from Braak Lewy pathology stage to Kosaka stage. The difference in staging schemes represent an important limitation to the study. However, one may also argue that methodological differences are most concerning in cases where findings are not reproduced, whereas an association that still replicates across somewhat different cohorts is an indication of a robust signal.

Table 2*: Staging of Lewy pathology according to the two major classifications schemes by Braak et al.(52) and Kosaka et al.(57). Used with permission of John Wiley & Sons – Books, from Evidence in Favor of Braak Staging of Parkinson's Disease, Dickson DW et al.(60), Vol. 25, Suppl. 1, 2010; permission conveyed through Copyright Clearance Center Inc.*

Anatomical region	**Braak LP stage**	**Kosaka LBD types**
Anterior olfactory nucleus	**1**	(Not assessed)
Dorsal motor nucleus of vagus		**Brainstem**
Locus coeruleus	**2**	
Substantia nigra	**3**	
Basal nucleus of Meynert		**Transitional**
Amygdala	**4**	
Parahippocampal and cingulate limbic cortices		
Multimodal association cortices of temporal, frontal, and parietal lobes	**5**	**Diffuse**
Primary motor and visual cortices	**6**	

4.3.2 Alzheimer's disease pathology

Current criteria for the neuropathological assessment of AD from the National Institute on Aging-Alzheimer's Association Guidelines (NIA-AA) incorporates two staging systems for Amyloid-β pathology and one score for tau pathology(217).

Amyloid-β pathology is measured by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuritic plaque score and Thal amyloid-β(Aβ)-phase. The CERAD score is a semi-quantitative measure of the density of neuritic plaques in three areas of the cortex (frontal, temporal and parietal) graded as none, sparse, moderate or frequent(218) (Table 3). The Thal Aβ-phase reflects the anatomical distribution of amyloid-β plaques (considering both diffuse and neuritic plaques)(74) (Table 4). In the NBB samples (Paper 1, 2 and 3) Thal Aβ-phases were analyzed in the medial temporal lobe (MTL) (Thal Aβ-MTL)(219). As the cerebellum was often not sampled for these cases to distinguish between Thal Aβ-phase 4 or 5, a 4-tier version of the Thal Aβ-phase was used. For the Mayo Clinic samples (Paper 2), the 5-tier version of Thal Aβ-phase was used. Although differences in protocols used to determine Thal Aβ-phase may limit the comparability between the two cohorts, a strong correlation between Thal Aβ-MTL and Thal Aβ-phase has been reported(220), which in our view justifies the comparison in the context of polygenic risk score associations. In both brain banks tau NFTs were scored according to Braak, ranging from 0 to VI(75) (Table 5).

***Table 3**: CERAD score for the density of neuritic plaques in the neocortex(218).*

CERAD score	Description
0	None
A/1	Sparse
B/2	Moderate
C/3	Frequent

***Table 4**: Thal-Aβ phase and corresponding hallmark brain regions(74).*

Thal Aβ-phase	Hallmark region
Thal Aβ-phase 0	No amyloid-β pathology
Thal Aβ-phase 1	Amyloid-β pathology exclusively in the neocortex
Thal Aβ-phase 2	Amyloid-β pathology spread to the allocortex
Thal Aβ-phase 3	Amyloid-β pathology spread to the subcortical nuclei (diencephalon/striatum)
Thal Aβ-phase 4	Amyloid-β pathology spread to the brainstem
Thal Aβ-phase 5	Amyloid-β pathology spread to the pontine nuclei and cerebellum

***Table 5**: Braak NFT stages and corresponding hallmark brain regions(75).*

Braak NFT stage	Hallmark region
0	No NFT pathology
I	NFT pathology in the transentorhinal region
II	NFT pathology in the entorhinal region
III	NFT pathology in the neocortex of the fusiform and lingual gyri
IV	NFT pathology in the neocortical association areas
V	NFT pathology in the frontal, parietal and occipital (peristriate) regions
IV	NFT pathology in the primary and secondary neocortical regions and striate area in the occipital lobe

According to the NIA-AA criteria, the "ABC score" is a composite of the three abovementioned AD pathology scores, where the combination of Thal Aβ-phase (A), Braak NFT stage (B) and CERAD neuritic plaque score (C) designate donors as having no, low, intermediate or high AD neuropathological change(217). Donors with cognitive impairment and an intermediate or high "ABC score" fulfill criteria for AD, while cases with a not or low "ABC score" do not, regardless of cognitive function(217). The NBB donors had all three neuropathology scores available, while the Mayo Clinic donors had been scored according to Thal Aβ-phase and Braak NFT stage. To harmonize the classification of AD pathology between the two cohorts, we used an adaptation of the NIA-AA criteria considering the combination of Thal Aβ-phase and Braak NFT stage (hereafter referred to as composite AD-score). Following the NIA-AA criteria, Thal Aβ-phases were categorized as 0, 1-2, 3 and 4-5 and Braak NFT stages were categorized as 0, I-II, III-IV and V-VI (Table 6).

***Table 6**: The composite AD-score, an adaptation of the "ABC score", encompassing Thal-Aβ phase and Braak NFT stage to NBB and Mayo Clinic donors.*

	Braak NFT 0	Braak NFT I-II	Braak NFT III-IV	Braak NFT V-VI
Thal Aβ-phase 0	No	No	No	No
Thal Aβ-phase 1-2	No	Low	Low	Low
Thal Aβ-phase 3	No	Low	Intermediate	Intermediate
Thal Aβ-phase 4-5	No	Low	Intermediate	High

A major obstacle in discerning the genetic contribution to Lewy and AD co-pathology in LBD is the neuropathological heterogeneity. The level of AD co-pathology is positively correlated with the severity of Lewy pathology, which challenges the interpretation of the underlying genetic relationship with the extent of each pathology(221, 222). Thus far, most clinico-pathological studies have compared patients regardless of AD co-pathology. However, two recent studies have suggested a distinct genetic architecture dependent on the level of concomitant AD-pathology in DLB(223, 224). These studies show that non-Gaucher causing *GBA1* variants are associated with a "pure" form of DLB i.e., without any significant AD co-pathology, while *APOE* E4 is a risk factor for DLB with AD co-pathology. Thus, the genetic contribution to Lewy and AD co-pathology may be more clearly assessed by stratifying patients based on the level of AD co-pathology. Therefore, we divided the LBD donors into two groups in Paper 2, where LBD without AD co-pathology had a "no" or "low" composite AD-score and LBD with AD co-pathology had an "intermediate" or "high" composite AD-score. However, this categorization is a crude division, and one could hope that more sensitive and biologically accurate models incorporating both Lewy and AD co-pathology could be developed in the future when larger cohorts of neuropathological characterized samples become available.

4.4 Genotyping and quality control

For the NBB (Paper 1, 2 and 3) and Mayo Clinic samples (Paper 2) human brain tissue was obtained in the course of autopsy, and specimens were genotyped. Genotyping of NBB samples was carried out on the Infinium® NeuroChip Consortium Array (Illumina, San Diego, CA US)(225). The NeuroChip is a SNP-array with an extensive genome-wide backbone of ~ 300,000 variants enriched for variants associated with neurodegenerative diseases (~ 180,000). Genotype calling from raw intensity files for NBB samples was conducted in Illumina GenomeStudio by JA Tunold and L Pihlstrøm(226). The Mayo Clinic brain bank samples were genotyped on the Infinium® OmniExpress-24 (version 1.3) array (Illumina, San Diego, CA) including ~ 714,000 variants(227).

For the PPMI and PDBP cases used in Paper 3, WGS data was available. DNA was extracted from whole blood and sequenced on the Illumina HiSeq X Ten Sequencer. Genotypes were obtained from the Accelerating Medicines Partnership-Parkinson's Disease program (AMP-PD; www.amp-pd.org).

Despite the decrease in the cost of genome sequencing facilitated by next-generation technologies, the expense of WGS remains a significant barrier for many studies lacking a substantial financial backbone. Thus, SNP-arrays remain the mainstay in many genetic studies, providing a cost-effective approach to assess common genetic variants across a wide range of samples. Variants not directly genotyped can be statistically inferred by comparing each sample to a reference panel, known as genotype imputation. While different genotyping arrays were used for the NBB and Mayo Clinic cohorts, genotype imputation ensured the inclusion of most common SNPs for both cohorts although not necessarily directly assayed on the genotyping array.

No genotyping method is perfectly accurate, and genotyping errors can introduce both random errors and bias. Further, sample mix-up and population stratification, referring to the systematic differences in allele frequencies between subpopulations, are potential sources of bias(228). Hence, implementing robust quality control (QC) measures becomes crucial to ensure reliability of genetic data and prevent spurious associations in downstream analyses. Quality control is performed on variants and samples (individuals), and follows similar sequential steps across studies as summarized below. Several publications explaining the QC steps in more detail have been published, including(228). The thresholds used may vary slightly across the different studies due to variations in sample size(228). For details, please refer to the individual papers.

For Paper 1 and Paper 2, sample and variant QC was carried out by L Pihlstrøm in PLINK version 1.9(229). Individuals with genotyping call rate <0.95, excess heterozygosity (>±4 standard deviations (SD) from mean), conflicting sex assignment, cryptic relatedness (pi-hat >0.125) or ancestry outliers assessed by genetic principal component plots were excluded. Variants were excluded if genotyping call rate was <0.95, MAF <0.05 or if the genotype distribution departed from the Hardy-Weinberg equilibrium ($p < 10^{-6}$). Sex-chromosomes and multi-allelic variants were removed. Variants were imputed on the Michigan Imputation Server(230), using reference data from the Haplotype Reference Consortium(231), and SNPs with an imputation $r^2 < 0.3$ were filtered out.

The WGS data from PPMI and PDBP samples used in Paper 3 underwent similar QC steps and were performed by H Leonard and H Iwaki prior to our acquisition of the data. The steps are described in detail in(232).

4.5 Polygenic risk scores

PRS for paper 2 and 3 were calculated by MXX Tan.

Since each genetic risk variant identified through GWAS can only explain a small proportion of the disease susceptibility, the predictive value of any individual variant is limited. Polygenic risk scores (PRS) estimate the cumulative effect of many variants by summing the trait-associated alleles weighted by their effect size and can thus be seen as an individual-level estimate of genetic liability(233). Correspondingly, a higher PRS indicates an increased number of risk alleles, reflecting a greater genetic risk for the outcome.

4.5.1 Genomewide polygenic risk scores

PRS analysis require input from two data sets: Base data, which is usually summary statistics containing allele weights and p-values from GWAS, and targe data containing genotypes and phenotypes from independent samples. As base data for AD-PRS in Paper 2 and 3 we used summary statistics from Jansen et al. AD GWAS(234). As *APOE* is a major AD-susceptibility locus, we also calculated the AD-PRS excluding the *APOE* region (GRCh37 chr:bp 19:45116911-46318605) in Paper 2. At the time of calculating AD-PRS, two recent AD GWAS had been published: Jansen et al. (71,880 cases and 383,378 controls, 29 risk loci) and Kunkle et al. (35,274 cases and 59,163 controls, 25 risk loci)(234, 235). We chose the former based on the larger sample size and more risk loci identified. However, it should be noted that a large proportion of the AD cases in the Jansen et al. GWAS were AD proxy cases, i.e., cases with a parental history of AD, as well as proxy-controls, i.e., unscreened controls. The use of proxy cases in AD GWAS has been problematized(236). In essence, the clinical diagnostic accuracy of AD is generally low, and by including a large number of unscreened proxy-cases the proportion of actual AD cases will be further diluted. The use of proxy cases is believed to be the explanation for heritability estimates for AD decreasing as the sample size (and number of proxy cases) are increasing(236). Consequently, GWAS using proxy cases or other patients with minimal phenotyping may capture genetic effects not related to AD specifically.

For PD-PRS in Paper 2 and 3, the largest PD-GWAS meta-analysis to date was used as base data(136). This meta-analysis also contains proxy-cases. However, the use of poxy-cases in PD-GWAS seems to be less problematic, as heritability estimates have been fairly consistent, even after

inclusion of proxy cases(236). This may in part be because the diagnostic accuracy of PD is higher than for AD.

After standard QC of the target data as described above, we used the software PRSice2 to calculate the individual PRS(237). To ensure the accuracy of PRS and that the variants included are independent of each other and thus additive, LD between GWAS variants had to be accounted for. PRSice2 uses a method called clumping and thresholding (C+T). Clumping selects the SNP with the lowest p-value association with the trait in each LD block, and variants that are only weakly correlated with each other, measured by r^2, are retained(233). In the thresholding step, SNPs with a p-value larger than a chosen threshold are removed. The 1000 Genomes European samples (n = 503) were used to calculate LD structure and the standard clumping algorithm that identifies SNPs within a 250 kb window in LD with an r^2 greater than 0.1 was used. PRS were calculated over a range of p-value thresholds ($5x10^{-8}$-0.05). Selecting the optimal p-value threshold is an important tuning parameter for PRS to balance the signal to noise ratio. A less stringent threshold yields a greater number of variants, including non-related genetic risk variants, to the PRS, thus increasing the noise. A more significant p-value threshold favors the inclusion of variants more likely associated with the disease, increasing the signal, but at the cost of potentially excluding disease associated variants just above the threshold. No single p-value threshold maximizes the PRS accuracy in all circumstances, and it is therefore customary to evaluate a range of thresholds when developing PRS. To evaluate the various p-value thresholds we examined the ability of AD- and PD-PRS to discriminate between AD and PD cases respectively and neurologically healthy controls. This assessment was conducted through area under the receiver-operating characteristic (ROC) curve (AUC) analysis. For a more detailed description of ROC-AUC analysis, please see section 4.6.3. The best performance was defined as the maximum AUC, and for both AD-PRS and PD-PRS a p-value thresholds of $p < 5\times10^{-8}$ outperformed the PRS with p-value threshold of $p < 1\times10^{-5}$ and $p < 0.05$ (Figure 7). The PRS with p-value threshold of $p < 5\times10^{-8}$ were thus chosen for the subsequent analyses.

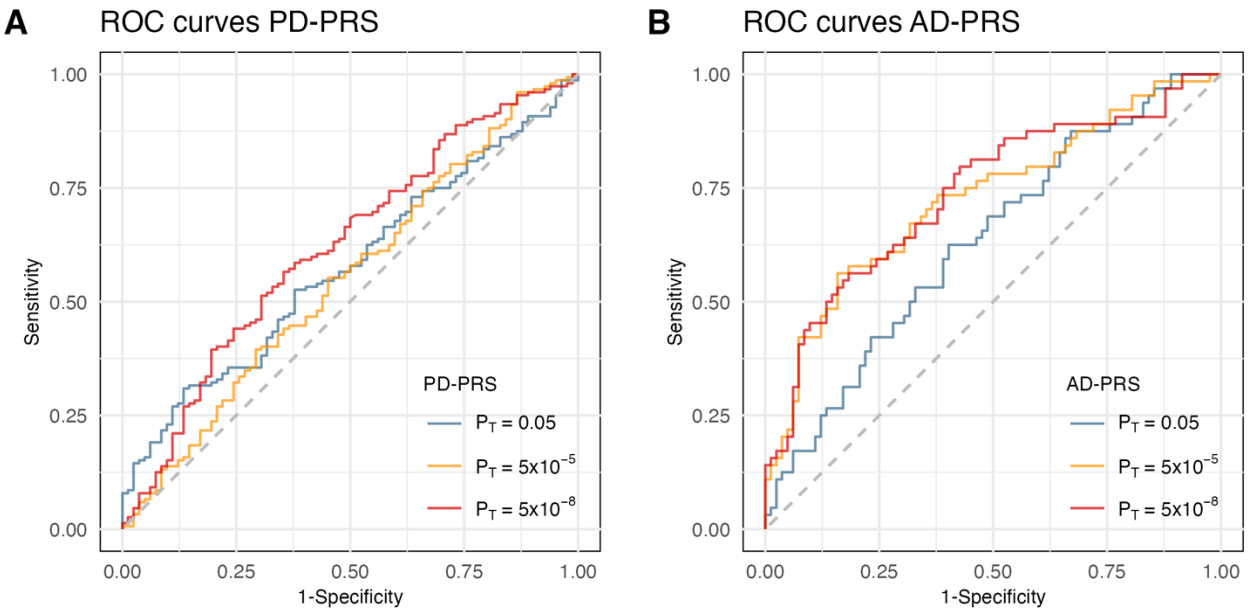

***Figure** 7: Area under the receiver-operating characteristic (ROC) curve (AUC) for case-control discrimination. A) PD-PRS. P_T 0.05: AUC = 0.58, P_T 5×10^{-5}: AUC = 0.55 and P_T 5×10^{-8}: AUC = 0.61. B) AD-PRS. P_T 0.05: AUC = 0.63, P_T 5×10^{-5}: AUC = 0.72 and P_T 5×10^{-8}: AUC = 0.73. P_T = P-value threshold.*

PRS were calculated independently for NBB and Mayo clinic samples. Only minor differences in the SNPs included from the PRSice algorithm were seen for some PRS, likely caused by different genotyping arrays used between the two cohorts. However, imputation ensured most variants were available. See Table 7 for comparison of the number of SNPs included in AD- and PD-PRS for cases from NBB and the Mayo Clinic.

***Table** 7: Number of SNPs in the genome-wide AD-PRS with and without the APOE region and the genome-wide PD-PRS for NBB and Mayo Clinic samples from Paper 2.*

PRS	Number of SNPs NBB	Number of SNPs Mayo Clinic	SNPs in common
AD-PRS	81	82	78
AD-PRS excluding *APOE*	34	34	34
PD-PRS	181	186	180

4.5.2 Stratified polygenic risk scores

Another approach to compute PRS is to select variants based on knowledge of biological pathways, molecular processes or cell-types, referred to here as stratified PRS (Figure 8). Stratified PRS may help nominate biological pathways of etiological relevance to the disease. Further, this approach may be more appropriate in discovering genetic influence on disease endophenotypes, as not all variants associated with disease risk may contribute to a certain endophenotype as discussed in section 1.4.5. For a review of pathway-based analyses in the context of PD, please refer to(238). As discussed in section 1.5 one previous report linked 46 partly overlapping gene-sets reflecting biological pathways to PD(160). To ensure sufficient statistical power, we narrowed our focus to a few extensively investigated pathways and cell types, considering the relatively smaller sample size compared to the former study. In Paper 2 we chose to investigate six pathways (adaptive and innate immune system, α-synuclein, endosomal trafficking, lysosomal and mitochondrial pathways) and two cell types (microglia and monocytes) that have been previously reported to associate with PD susceptibility(155, 160-162) (Table 8). Pathway gene-sets were obtained from the Molecular Signatures Database (MSigDB)(239), while curated lists of genes involved in the mitochondrial pathway and the endosomal trafficking pathway were selected from previous publications(161, 162). For the stratified PRS reflecting pathways, SNPs were mapped to genes using physical gene boundaries, while the stratified PRS reflecting cell-types were based on publicly available data on open chromatin regions mapped by Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq)(240, 241). In Paper 3 we only calculated the lysosomal PD-PRS for replication of results from Paper 2.

Following the association between the lysosomal PD-PRS and Lewy pathology (Paper 2) and time to dementia (Paper 2 and Paper 3), we also calculated the lysosomal PD-PRS excluding the *GBA1* region. This step was taken due to our suspicion that variants in the *GBA1* region were driving the association. We excluded SNPs in the whole locus 1 region from the most recent PD GWAS including *GBA1* and variants in *PMVK*, *KRTCAP2* (GRCh37 1:154898185-155214653, GRCh38 1:154925709-155244670).

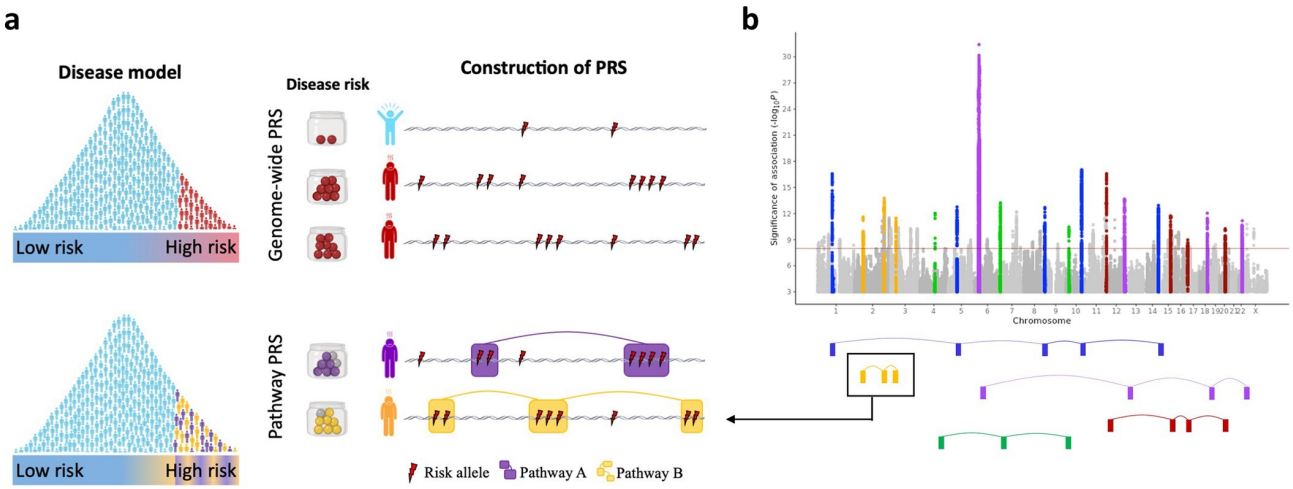

Figure 8: *Colored boxes represent genes, while the lines connect genes belonging to the same pathway. A) The upper model represents the genome-wide PRS where all SNPs below a certain threshold are aggregated. The lower model represents the stratified PRS where risk-alleles annotated to biological pathways or cell-types are aggregated. B) Manhattan plot representing summary statistics from which genome-wide and stratified PRS are calculated. Each GWAS signal corresponds to an alternative functional route to disease. Reused from: The pathway polygenic risk score approach by ©Choi SW et al 2023(242), under the terms of the CC BY 4.0 license.*

***Table 8**: Number of SNPs in the stratified PD-PRS for NBB and Mayo Clinic samples from Paper 2. Although different*

PRS	Number of SNPs NBB	Number of SNPs Mayo Clinic	SNPs in common
Adaptive immunity	17	17	17
Alpha synuclein	9	7	7
Endosomal trafficking	10	9	9
Innate immunity	10	10	10
Lysosomal	12	12	11
Lysosomal excluding *GBA1*	11	11	11
Microglia	45	45	45
Mitochondria	2	2	2
Monocytes	44	44	44

4.5.3 Limitations of PRS

The use of polygenic risk scores come with several limitations needed to be considered in order to ensure their appropriate interpretation. GWAS have proven the polygenic architecture of PD, yet these variants only account for a small proportion of PD heritability, meaning the genetic architecture of PD remains incompletely understood. It is crucial to recognize that PRS are derived from genetic variation captured by GWAS only, and therefore do not account for the complete genetic architecture of PD, which may include rare and structural variants, gene-gene or gene-environment-interactions yet to be discovered(243). Furthermore, as highlighted in section 1.4.5, the heritable component of PD is estimated to 22-27 %, indicating that additional and largely unknown factors contribute to the overall heritability of the disease, thus limiting the predictability based on genetic risk factors alone. Moreover, another concern is the lack of diversity in study populations(244, 245). The majority of GWAS have focused on populations of European genetic ancestry, leading to an underrepresentation of a large proportion of the global population. As a consequence of distinct genetic backgrounds, the applicability of PRS across different populations becomes problematic, thus limiting their transferability(246, 247).

In the stratified PRS, genes are annotated to specific biological pathways or cell types, and variants within the physical boundaries of these genes or within open chromatin regions respectively, are aggregated. For the majority of PD GWAS loci, the implicated genes remain uncertain, as highlighted in section 1.4.6. Consequently, determining which pathway they belong to is even more complex. One clear limitation of the stratified PRS approach is the assumption that SNPs primarily affect the nearest gene, although it is now recognized that they can also regulate genes located more than 1 Mb away(158). Further, pathways are not well-defined, and may represent partially overlapping biological mechanisms. Most of our gene-sets were obtained from the MSigDB, which is one of the most comprehensive repositories of curated gene sets. However, we acknowledge that our mapping of SNPs to pathways is limited by the current incomplete knowledge about PD GWAS variants, target genes and their contributions to biological pathways. Finally, when calculating PRS, target data should ideally be independent of the samples in the base data in order to prevent inflation of the association with the outcome. In Paper 3, both PPMI and PBDP samples were included in the PD GWAS used to derive the PRS weights. However, the extent of inflation aligns with the degree of overlap between target and base data(248), which was limited in Paper 3. Moreover, we investigated a completely different outcome (i.e., cognitive impairment in cases only) than in PD GWAS (i.e., disease status among cases and controls). Recalculating the base data

excluding PPMI and PDBP samples would have been preferable, but this would require access to individual level genotypes for the entire study, which were unavailable to us.

4.6 Statistical analyses

JA Tunold conducted statistical analyses for Paper 1-3 using the statistical software package R v.4.0.2, v.4.2.1 and v4.3.1(249).

Medical research starts with a question that can be translated into a testable hypothesis. Two competing hypotheses are formulated: A null hypothesis (H_0) and an alternative hypothesis (H_a). The null hypothesis states that there is no difference or relationship between the variables under investigation, and often represents the default position researchers aim to challenge. The alternative hypothesis proposes that the null hypothesis is untrue, and usually represents the researcher's claim. Through statistical hypothesis testing, we seek to evaluate the evidence provided by the data to either accept or reject the null hypothesis in favor of the alternative hypothesis. Prior to conducting statistical tests, a significance level denoted alpha (α) is selected to decide whether or not the null hypothesis is rejected. For Paper 1-3 the significance level was set to $\alpha = 0.05$. The p-value is the probability of obtaining a result equal to or more extreme than the observed result, assuming the null hypothesis is true(250). Consequently, there are two possible types of errors that can occur, leading to incorrect conclusions about the null hypothesis. Type I errors refer to situations when a true null hypothesis is mistakenly rejected (false positive), while type II errors occur when a false null hypothesis is not rejected (false negative)(251).

4.6.1 Survival analysis

In paper 1, 2 and 3 we assessed the relationship between survival time and genetic variants (Paper 1) or PRS (Paper 2 and 3), using Cox proportional-hazards regression models. Cox proportional-hazards regression models are multivariate models for survival analysis, allowing adjustment for the impact of confounders that may influence the outcome(252). Confounding refers to a situation where the relationship between the independent variable and the outcome is influenced by the presence of a third variable (confounder). In genetic association studies, population stratification is a potential confounder due to systematic differences in allele frequencies between subpopulations. As discussed in section 4.4, calculating genetic principal components capture the underlying genetic variation in a population and are commonly included as covariates to correct for population

stratification. Additionally, we included sex, age at onset and level of education (only Paper 3) as covariates to account for potential differences in the risk of survival outcome. The effect measure of Cox proportional-hazards regression is the hazard ratio (HR) which is the probability of an event at a given time. If HR = 1, there is no effect of the independent variable on survival, while HR >1 indicates an increased hazard and HR<1 a reduced hazard. A fundamental assumption of Cox proportional-hazards models is that the relative hazard remains constant over time, known as the proportional hazards assumption(252). The proportional hazards assumption was assessed by a goodness-of-fit test assessing the correlation between the Schoenfeld residuals and survival time, using the *cox.zph* function in the R package "survival". In addition, a graphical diagnostic was conducted by plotting the Schoenfeld residuals with the *ggcoxcph* function. There was no evidence of violation of the proportional hazards assumption in the Cox models.

4.6.2 Proportional odds ordinal logistic regression analysis

In Paper 2 we assessed the relationship between measures of neuropathology and PRS. The neuropathological scores represent ordinal outcomes that can be ranked in a meaningful order, but the differences between each category is not quantifiable as they lack measurable units. Therefore, specific statistical tests designated for ordinal data are required to analyze and interpret the results accurately. We used proportional odds (PO) ordinal logistic regression models (hereafter referred to as ordinal logistic regression models) to account for the ordered nature of outcomes(253). Age at death, sex and genetic principal components were added as covariates. The effect measure of ordinal logistic regression models is the odds ratio (OR). Since the PRS were converted to z-scores, the effect sizes were interpreted as the OR per 1 SD increase in PRS. A key assumption of ordinal logistic regression models is the proportional odds assumption(253). This assumption states that the relationship between the independent variables and the outcome variable is consistent across all levels of the outcome variable. Several methods to assess the proportional odds assumption, including plotting, statistical tests and comparison to other non-proportional odds models exist(254). Some of these methods may be over conservative, rejecting the proportional odds assumption even if it may be more meaningful to use such a model. To assess the proportional odds assumption, we fitted partial proportional odds model (PPO) where the proportional odds assumption was relaxed for the explanatory variable (i.e., PRS). When comparing PO to PPO models the likelihood ratio test p-values were non-significant, indicating that the PPO models were not superior to the PO models, and that the PO assumptions were reasonable.

4.6.3 Area under the receiver operating characteristic curve

In Paper 2 and Paper 3 we developed classification models to discriminate between high risk and low risk individuals. In Paper 2 we assessed how well AD- and PD-PRS discriminated between cases and controls, how well an AD-risk score discriminated between cases with and without AD co-pathology, and how well a Lewy pathology risk score discriminated between cases with and without neocortical Lewy pathology. In Paper 3 we assessed the ability of AD-PRS to discriminate between cases with and without AD co-pathology and to determine the optimal cut-point to distinguish these subgroups. The area under the receiver operating characteristic (ROC) curve (AUC) statistics were used to assess the discriminative ability of the classification models. Common metrics to assess the performance of a classifier are sensitivity, referring to the ability to correctly identify patients with a condition (e.g., disease or AD co-pathology), and specificity which is the ability to correctly identify patients without the condition. The ROC is a graphical representations of the diagnostic performance, generated by plotting 1-specificity on the x-axis against sensitivity on the y-axis across a range of cut-points(255). The AUC quantifies the global performance of the prediction model and can take any value between 0.5 and 1. An AUC of 0.5 indicates performance no better than chance, while an AUC of 1 refers to perfect discrimination (i.e., 100 % sensitivity and 100 % specificity). What is regarded as a good discriminative ability may depend on the context. While an AUC >0.80-0.90 may be required for individual level discrimination in clinical practice, a lower AUC may still be meaningful for discrimination on a group level.

The ROC can also be used to determine the optimal cut-point for a classifier. Taking advantage of having a neuropathological assessment of the presence or absence of AD co-pathology in the NBB samples, we sought to determine the optimal cut-point for AD-PRS to discriminate between samples with and without AD co-pathology in Paper 3. A good practice in developing a predictive model is to train the model on one set of data and then test the model performance in independent data. 70 % of NBB samples were allocated for training the model, and the remaining 30 % were held out as an independent test set, with equal proportions of AD-positive samples in both groups. When developing a prediction model a critical concern to address is overfitting, which refers to the model's tendency to fit the training data so well that it fails to generalize to new data, leading to unreliable predictions. We used k-fold (k = 10) repeated (r = 3) cross-validation to reduce the risk of overfitting. In short, the method included dividing the data into k (k = 10) equally sized folds, that are trained and evaluated k (k = 10) times, each time using a different fold as the test data and the remaining folds as training data. This process was repeated three times. The final model

achieved an AUC of 0.72 and was validated on the held-out test data where it achieved an AUC of 0.71. To determine the optimal cut-point we used the maximum value of the Youden index (J)(256), defined by (sensitivity+specificity-1). Using this method, the optimal cut-point of AD-PRS was 0.29 SD.

4.6.4 Chances of statistical errors

To account for type I errors in Paper 2 and 3 we applied a two-stage design, where identified associations passing $\alpha = 0.05$ in the discovery cohorts (NBB and PPMI respectively) were selected for replication in the independent samples from the Mayo Clinic cohort and PDBP respectively. Only associations replicating at $\alpha = 0.05$ with a consistent direction of effect in both the discovery and replication stages were considered positive findings. In Paper 2, the subset of NBB samples with AD co-pathology was considerably smaller than the subset without AD co-pathology, and few cases had lower Braak Lewy pathology stages (Braak LP <5) in the former. The restricted samples size and uneven distribution of Lewy pathology limited the utility of ordinal logistic regression in this subset. As a result, Braak LP stages 3-5 were collapsed and associations with Lewy pathology were assessed with logistic regression analysis. Limited statistical power to detect associations in this subset increases the risk of type II error. Selecting the optimal p-value threshold for calculating PRS is a tradeoff between type I and type II error. A higher p-value threshold allows for inclusion of more variants and potentially capturing a broader spectrum of genetic influence, at the cost of increasing the risk of type I error. Contrary, a lower p-value threshold includes fewer SNPs, thus reducing the risk of type I error, but increasing the risk of type II error. As discussed in section 4.5.1 we selected SNPs passing the genome-wide significance level for calculating PRS in Paper 2 and Paper 3. Consequently, the risk of type I error was reduced, at the cost of potentially not detecting true associations.

4.7 Ethical considerations

The studies included in thesis (Papers 1-3) were approved by the Regional Committees for Medical Research Ethics South East Norway (REK30552) and the data protection representative at Oslo University hospital. Written informed consent was obtained from the participants included in Papers 1-3. For the brain bank samples from NBB and the Mayo Clinic, written informed consent was obtained from the donors or their next of kin, while written informed consent from the PPMI and PDBP cohorts were obtained from all participants directly. The NBB follow the ethical principles

for brain banking outlined in the BrainNet Europe Code of Conduct(257), which have further been approved by the VU University Medical Center, Amsterdam, the Netherlands. Similarly, the procedures of the Mayo Clinic Jacksonville Brain Bank of Neurodegenerative diseases were approved by the Mayo Clinic institutional review board. The PPMI and PDBP study included patients from various sites in the United States, Europe, Israel and Australia, with approval from local institutional review boards or ethics committees prior to study initiation.

With the advancement of faster and more cost-effective genetic analyses, next-generation sequencing methods like WES or WGS are often preferred over more focused sequencing approaches in research studies. The genome contains information related to disease susceptibility, potential monogenic conditions, and carrier status for recessive diseases that may be unknown to the individual participant, and unrelated to the main scope of investigation. An ongoing ethical concern is whether and when to provide feedback to research participants about such incidental findings. None of the studies included in this thesis involved Norwegian patients. However, handling potential incidental findings is highly relevant in the context of the Prospective Study of Parkinsonism in Oslo (PROSPOS), to which I have enrolled patients throughout the course of my PhD work. At inclusion, the appropriate management of incidental findings is discussed with participants and addressed in the informed consent documentation. Guidelines for management and disclosure of incidental findings have been provided by The Norwegian National Research Ethics Committee(258). Nevertheless, the determination of which incidental findings should be reported is a matter of discussion and is anticipated to evolve over time, although guidelines have been published(259). In this context, actionability, i.e., if there is an effective preventive treatment available, plays a pivotal role. While effective preventive measures are available for conditions like hereditary breast and ovarian cancer or familial hypercholesterolemia, the same does not hold true for neurodegenerative diseases. This will hopefully change in the future.

5 Discussion

As for most complex diseases, the primary focus of genetic research in idiopathic PD thus far has been identification of genetic variants that increase the individual risk of disease. We now know that PD is highly polygenic with a range of variants influencing disease risk. It is becoming increasingly apparent that genetic variation also can influence disease progression, where one potential mechanism is though the aggregation of disease relevant protein pathologies. However, the remarkable clinical and pathological heterogeneity likely reflects a complex interplay between genetic variants and protein pathologies. The common theme for Papers 1-3 is linking genetics, neuropathology and cognitive outcomes in PD. Identification of genetic risk factors contributing to protein pathology and clinical outcomes may advance our understanding of the molecular mechanisms leading to disease development and progression.

PD and other neurodegenerative diseases such as DLB and AD have in common that protein aggregates represent pathological hallmark lesions. By convention, pathological classification of these neurodegenerative diseases is based on the predominant protein pathology, which in the case of PD are the α-synuclein immunoreactive inclusions collectively called Lewy pathology. Although the temporal sequence of protein pathology in PD is yet to be determined, neuropathological studies have described a progressive pattern of Lewy pathology that spreads to interconnected regions within the nervous system, implying that Lewy pathology becomes more severe as the disease progresses(52). These observations are supported by experimental studies suggesting that pathogenic forms of α-synuclein can seed misfolding of endogenous α-synuclein(168) and potentially spread through cell-to-cell transmission(167).

In addition to Lewy pathology, variable degrees of amyloid-β plaques and tau NFTs, changes primarily associated with AD, are common(72). Acknowledging that a substantial proportion of patients with PD and DLB have concomitant proteinopathies may provide important insight into disease pathogenesis, and potentially be an important aspect in distinguishing between disease subtypes. In general, concomitant AD-pathology is associated with a poorer prognosis and in particular dementia(64, 71, 73). However, studies differ in whether Lewy pathology(72) or the combination of AD and Lewy pathology(62) is more important for development of dementia. A crucial question to expand our understanding of PD pathogenesis is thus how α-synuclein, amyloid-

β and tau pathology develop and spread over time, and how these processes shape clinical symptoms in the individual patient.

5.1 Linking genetics to Lewy pathology

While accumulating evidence point to α-synuclein aggregation and Lewy body formation as central events in the pathogenesis of PD, the genetic influence on these processes remains largely unknown. One way to link genetic variants to neuropathological endophenotypes is to make the endophenotypes the outcome of GWAS. Yet, the sample size required to perform GWAS is limited by the challenges associated with the collection of biological specimens such as brain tissue. Thus far, no GWAS focusing on neuropathological outcomes in patients with PD or DLB has been published, according to the GWAS catalog (https://www.ebi.ac.uk/gwas/). To date, only a few genetic association studies of Lewy pathology in patients with idiopathic PD or DLB have been published, and early studies have primarily focused on candidate variants. In a study on patients with autopsy-confirmed LBD, no associations between 28 PD susceptibility variants and Lewy body counts or LBD subtype were detected(260). SNPs tagging the *MAPT* H1 haplotype have consistently been associated with PD risk in GWAS. While *MAPT* encodes the tau protein found in neurofibrillary tangles, two small studies on neuropathologically confirmed PD and DLB cases, reported that *MAPT* H1/H1 carriers had a higher burden of neocortical Lewy pathology or total α-synuclein score respectively, compared to non-carriers(261, 262). However, these results are not consistent, and have been opposed by others(62, 260). Perhaps surprisingly, a post-mortem study on individuals with LBD demonstrated that the *APOE* E4 was associated with increased Lewy pathology in samples with low AD co-pathology(263), suggesting that *APOE* E4 impacts the severity of Lewy pathology independently of amyloid-β and tau-pathology. These results were supported by an earlier study where the *APOE* E4 allele associated with patients with DLB and PDD with no or low levels of AD co-pathology(264). However, a more recent study on neuropathologically characterized DLB samples found no association between the *APOE* E4 allele and “pure DLB”, i.e., DLB without AD co-pathology(223). Contrary, in this study, the SNP tagging the non-Gaucher causing *GBA1* variant p.E365K (p.E326K) was significantly associated with “pure DLB”.

Several factors may be responsible for the inconsistent associations between genetic variants and neuropathological endophenotypes. These include weak effects of most variants, limited availability

of neuropathological characterized samples, and different criteria used to define pathology. Another strategy to discover associations between susceptibility variants and neuropathological endophenotypes is by assessing the polygenic contribution of genetic variants on measures of neuropathology. However, in a study on autopsy-confirmed LBD samples from the Mayo Clinic, Heckman and colleagues did not find any associations between PD-PRS consisting of 28 variants derived from PD GWAS and measures of Lewy pathology(260). Cerebrospinal fluid (CSF) measures of α-synuclein have been investigated as a potential biomarkers and in-vivo proxy of Lewy pathology. Total CSF α-synuclein is modestly, but significantly decreased in patients with PD compared to age-matched controls(265). However, CSF α-synuclein has low sensitivity and specificity for PD, and no clinical utility yet. Several previous studies on PD patients in various disease stages have found no association between PD-PRS and cross-sectional or longitudinal measures of total CSF α-synuclein(266-268). While the lack of association may result from low statistical power, it also remains unclear whether total CSF α-synuclein levels accurately reflect the pathological accumulation of α-synuclein in the brain.

In an attempt to overcome previous limitations, we calculated PD-PRS derived from a more recent GWAS meta-analysis, including a larger number of significant association signals in Paper 2. Moreover, it is increasingly recognized that many PD susceptibility variants may exert their influence in the context of interconnected pathways or within specific cell types. Thus, PRS may be further enhanced by generating stratified PRS. This approach can potentially discover connections between the involved pathways and their influence on endophenotypes, such as Lewy pathology. We therefore sought to explore the cumulative contribution of genetic variants annotated to selected pathways and cell-types previously enriched for PD susceptibility. The use of stratified PRS is still in its infancy within the PD research field, and to our knowledge a novel approach to dissect LBD cases for their pathological patterns. As discussed in section 4.3.2 the positive correlation between Lewy and AD co-pathology precludes the ability to fully examine the genetic contribution to each protein pathology. One approach to overcome this obstacle is to stratify patients by the level of AD co-pathology, a strategy that previously has identified potential differences in the underlying genetic architecture within the LBD continuum(223, 224). The aim of such stratification is to leverage the signal-to-noise ratio and increasing the chances for successful identification of influential genetic markers while moderating the need for a large sample size. In Paper 2, we therefore sought to explore how genetics influence the level of Lewy pathology in patients with and without significant concomitant AD-pathology. We found a significant association between the

genome-wide PD-PRS and Lewy pathology in the NBB samples without significant AD co-pathology. However, this association was not replicated in the Mayo clinic samples, indicating that not all PD susceptibility variants act through mechanisms that promote Lewy pathology. These findings are generally consistent with prior research that has found no significant association between a genome-wide PD-PRS and measures of Lewy pathology or CSF levels of α-synuclein(260, 266-268).

However, in Paper 2, we showed that the lysosomal PD-PRS was associated with Lewy pathology, an effect that was specific to the subgroup of samples without AD co-pathology. This was the only signal nominated in the NBB cohort that was replicated in the independent samples from the Mayo Clinic cohort (Figure 9). As discussed in section 1.5.2, mounting evidence imply lysosomal mechanisms in PD liability and pathogenesis, best documented for *GBA1*. PD patients carrying Gaucher-causing *GBA1* variants have in most brain bank studies been found to have widespread cortical Lewy pathology(125). In most(126, 269-271), but not all(123) reports, PD and DLB patients carrying Gaucher-causing *GBA1* variants have been found to have more widespread Lewy pathology compared to non-carriers. In accordance with our results, autopsy studies have also suggested that PD and DLB individuals carrying Gaucher-causing *GBA1* variants have a "purer" Lewy body disease with advanced Lewy pathology and less prevalent AD co-pathology(221, 223, 270, 272). Corroborating these results, in a study where CSF biomarkers were used as an in vivo proxy of AD co-pathology, the authors found that the non-Gaucher causing *GBA1* variant p.E365K (p.E326K) was more strongly associated with "pure" DLB than DLB with AD co-pathology(224). Consequently, we strongly suspect variants in the *GBA1* locus to drive the association signal. *GBA1* encodes the lysosomal enzyme GCase which has been found to be reduced in post-mortem brains of PD patients with and without Gaucher-causing *GBA1* variants(273). Moreover, experimental studies have suggested that loss of GCase function causes accumulation of α-synuclein, and that α-synuclein inhibits GCase activity(174), functionally linking lysosomal impairment to α-synuclein aggregation. Although *GBA1* currently is the best documented lysosomal gene in PD and DLB, a broader contribution of lysosomal genes to the pathogenesis has been reported(177). Aligning with the former study, the association between the lysosomal PD-PRS and Lewy pathology remained significant in the Mayo Clinic samples in Paper 2, even after excluding the *GBA1* component. Our data do not provide evidence about which additional variant(s) associate with Lewy pathology. However, in addition to *GBA1*, the lysosomal PD-PRS included SNPs annotated to *CTSB*, *GALC*, *SCARB2*, *LAMP3*, *ABCB9* and *IDUA*. *CTSB*, *GALC*, *SCARB2* and LAMP3 have previously been

associated with PD risk through GWAS(136, 140-142). *IDUA* is among the candidate genes in locus 19 on chromosome 4 in the lates PD-GWAS(136), while *ABCB9* has been associated with hypomethylation in a whole-blood epigenome wide association study (EWAS) in PD(274). The genetic influence of lysosomal function comes in addition to the previously reported age-related failure of the lysosomal pathway(275), perhaps further lowering the threshold for developing Lewy pathology.

The underlying cause for the observed variability in genetic associations depending on the level of AD co-pathology remains uncertain. One explanation may be that AD-pathology render the brain more susceptible to additional neuropathological change, creating a vulnerable environment where genetic risk factors for PD are less important. In conclusion, it may be hypothesized that alteration in the lysosomal pathway resulting from the interplay between genetic factors and the aging process may be a common mechanism underlying PD pathogenesis through increased Lewy pathology in a subset of patients where AD co-pathology is absent.

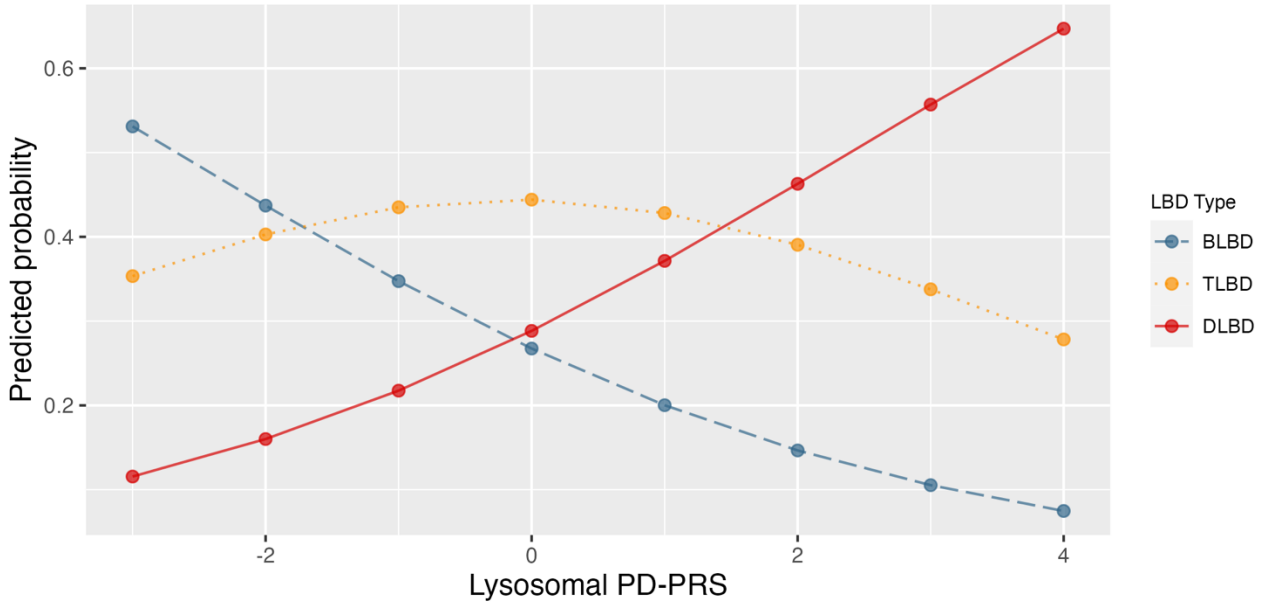

***Figure 9**: Predicted probability of Lewy pathology vs. lysosomal PD-PRS for Mayo Clinic samples without AD co-pathology. BLBD (Brainstem Lewy Body Disease), TLBD (Transitional Lewy Body Disease), DLBD (Diffuse Lewy Body Disease).*

While aggregation and accumulation of α-synuclein are considered a hallmark feature of PD, the biological significance of Lewy pathology is not fully understood. It may be assumed that α-synuclein aggregates are the driving force in PD pathogenesis, directly neurotoxic or at least

intimately linked to neuronal death and dysfunction. However, it is possible that α-synuclein aggregates serve a protective mechanism to reduce the exposure of harmful proteins to neurons, or merely an epiphenomenon without pathogenic or protective properties(46). These theories are fueled by studies indicating that Lewy pathology seems neither necessary nor sufficient to develop clinical PD(222, 276-279).

While most individuals with PD have widespread Lewy pathology at autopsy, a significant proportion of patients harboring mutations in *LRRK2* and *PRKN* do not develop discernible Lewy pathology(276-278). Additionally, in idiopathic PD, some reports suggest that cases fulfilling clinical diagnostic criteria for PD lack Lewy pathology upon autopsy(222). While it is possible that Lewy pathology may not always develop in PD, these observations do not exclude a role for α-synuclein in its pathogenesis. Lewy pathology represents late-stage α-synuclein aggregation. Earlier stages of α-synuclein aggregation such as α-synuclein oligomers are however not detected using routine immunohistochemistry protocols. Indeed, studies using newer techniques such as proximity ligation assay (PLA) have detected widespread α-synuclein oligomers, including in brain regions not affected by Lewy pathology(280). These α-synuclein oligomers, which precede the formation of Lewy pathology, are also suggested to be more toxic than later stages of α-synuclein conformations(281), although still a matter of debate(165). It is therefore possible that cases lacking Lewy pathology at autopsy have earlier stages of α-synuclein aggregation, not visible on routine histopathological assessment(282). Conversely, Lewy pathology is not an exclusive feature of PD and DLB but is also found regularly in other neurodegenerative diseases such as AD(283) as well as in elderly neurologically unimpaired subjects referred to as incidental Lewy body disease (iLBD)(205). While iLBD cases with relatively little Lewy pathology may represent a prodromal state of disease, cases lacking clinical parkinsonism or dementia with extensive Lewy pathology (Braak LP stage 5-6) have also been reported(279), challenging this view. Differences in the total burden of Lewy pathology, which are not always measured in post-mortem studies, between iLBD and PD cases may potentially explain why cases with a similar distribution of Lewy pathology do not display identical phenotypes(284). Given the widespread presence of Lewy pathology, it is highly likely that it plays a significant role in the pathogenesis of PD. However, association does not imply causation, and additional factors may be required for disease initiation and progression. Consequently, a deeper understanding of the underlying biological processes involved in α-synuclein aggregation, accumulation and dissemination is needed.

5.2 Linking genetics to AD pathology

It is evident that PD neuropathologically is characterized by the presence of multiple protein aggregates, with amyloid-β and tau NFT aggregates frequently observed alongside Lewy pathology. AD co-pathology is so frequently encountered that is has been proposed that cognitively normal PD patients with incipient AD pathology may be ideal for clinical trials on targeted AD-therapy(285). Following the recent approval of the anti-amyloid-β monoclonal antibody aducanumab by the Food and Drug Administration (FDA), this scenario has become more likely. Although the approval is controversial, due to concerns regarding the clinical efficacy of the drug(286), it highlights the importance of gaining insights into underlying pathological process leading to AD pathology, not only within the context of AD itself, but also in related neurodegenerative disorders like PD and DLB. However, studies need to clarify the relationship between amyloid deposits and clinical symptoms as well as how to most efficiently detect patients with incipient pathology. In general, AD co-pathology is associated with an unfavorable prognosis, in particular reduced overall-survival, faster cognitive decline and development of dementia(62, 73). While it is likely that AD co-pathology may contribute to the clinical heterogeneity of PD, the relative contribution of each pathology to the clinical course of PD is unknown. Studies differ in whether tau(221) or amyloid-β(287) is the primary driver of this dysfunction, rather than the combination of the two. Consequently, understanding the genetic architecture of AD co-pathology may aid in diagnostic accuracy and prognosis, provide insight into potentially shared underlying molecular mechanisms between PD and AD, and identify genes or pathways that could be targets for disease-modifying treatments.

Several studies have previously explored the genetic contribution to AD co-pathology in patients with idiopathic PD or DLB and been supported by studies from the AD research field. The *APOE* E4 allele has in post-mortem studies been associated with more severe AD co-pathology in individuals with PD or DLB, although best documented for amyloid-β pathology(62, 263, 270, 288, 289). The association between *APOE* E4 and amyloid-β pathology is further supported by biomarker studies on patients with PD or DLB. In these studies the *APOE* E4 allele has been associated with lower levels of the 42 amino acid isoform of amyloid-β ($A\beta_{1-42}$) in the CSF and greater cortical binding of amyloid-β tracers on PET imaging(267, 290-293). Importantly, a mendelian randomization analysis has suggested a causal association between the *APOE* locus, CSF $A\beta_{1-42}$ and PD(267).

A broader contribution of AD genetic risk factors to AD co-pathology has been suggested by a study on post-mortem samples with PD or DLB(294). In this study, a clinical genetic risk score based on age at onset, the number of *APOE* E4 alleles and the genotype for two additional AD risk-variants (*BIN1* and *SORL1*) predicted intermediate or high AD co-pathology(294). In univariate analysis, only the number of *APOE* E4 alleles was significantly associated with AD co-pathology. However, the combined model, selected through backward stepwise regression, including all four variables, outperformed the univariate association judged by the Akaike Information Criterion (AIC).

As anticipated, a robust association was observed between the AD-PRS and AD co-pathology, including both measures of amyloid-β and tau NFT in Paper 2. Considering the substantial evidence supporting *APOE*-mediated co-pathology in LBD, we expected that *APOE* was the driver of the association signal. However, in the larger of the two datasets, the association remained significant even after removing the *APOE* region from the AD-PRS. Our study expands on the current knowledge by demonstrating association between a genome-wide AD-PRS and the two hallmark pathologies of AD in LBD samples. Moreover, our study demonstrates a polygenic contribution to AD co-pathology beyond the potent influence of *APOE*. Our results are in line with post-mortem and biomarker studies suggesting genetic variants beyond *APOE* E4 influence amyloid-β and tau pathology in LBD(268, 294).

Studies in patients with LBD are corroborated by neuropathology GWAS performed in patients with a clinico-pathological diagnosis of AD(295, 296). In the first of these studies, the association between *APOE* and measures of both amyloid-β and tau-pathology was confirmed(295). Three additional loci were also nominated for association with amyloid-β pathology. Moreover, previously identified AD susceptibility variants, showed nominal associations with amyloid-β (including *BIN1*) and tau pathology (including *BIN1* and *SORL1*)(295). In the second study, *BIN1* was associated with both amyloid-β and tau pathology, yet only the association with tau passed the genome-wide significance threshold(296). Moreover, a preprint of a neuropathology GWAS has recently been published(297). In this study, patients with various neurodegenerative diseases were stratified based on the presence or absence of AD and Lewy pathology. The association between both *APOE* E4 and *BIN1* and AD-pathology were replicated. However, the majority of samples in this study had pure AD pathology (n = 2004) compared to patients with pure Lewy pathology (n = 97), although a considerable amount of samples had combined AD and Lewy pathology (n = 787)(297). This likely reflects a high proportion of samples with clinical AD. While our results are

consistent with studies on patients with AD, it should be emphasized that the patient population in Paper 2 is different (i.e., LBD). Therefore, these studies complement each other, both contributing to our understanding of the genetic influence on AD pathology.

While the physiological role of APOE is to mediate lipid transport, animal and cellular studies have suggested that APOE among many functions mediates amyloid-β aggregation, amyloid-β clearance and tau aggregation(298). *BIN1* and *SORL1* encode proteins involved in endosomal trafficking(299). Basic research studies have mechanistically linked *BIN1* to processes related to both amyloid-β and tau pathology(300). *SORL1* has more consistently been linked to processes related to amyloid-β, where overexpression of *SORL1* reduces amyloid-β levels, and loss of *SORL1* increases amyloid-β levels(301).

Collectively, post-mortem, CSF and PET imaging studies support the *APOE* E4 allele as a major driver of AD co-pathology in LBD. However, the relationship may be complex, considering studies suggesting *APOE* E4 independently promotes Lewy pathology(223, 264). Beyond *APOE*, we and others have provided evidence that support additional genetic influence on AD co-pathology, with one study highlighting *BIN1* and *SORL1* as potential risk loci in LBD, supported by studies in AD. Functional studies have also provided evidence for these genes in processes related to amyloid-β and tau pathology.

5.3 Linking genetics and protein pathology to dementia

The risk of dementia in PD is considerably increased compared to age- and sex-matched controls with a cumulative prevalence of 80 %(30). However, the onset of cognitive decline and rate of progression to dementia shows considerable variability. Age is the most important risk factor for dementia, but dementia can also occur at a younger age as is evident in many forms of monogenic PD. It is therefore likely that the individual genetic background also may influence the onset of dementia. As the number of people diagnosed with PD is predicted to increase in the coming years as a result of an aging population, there is an urgent need to better understand the molecular basis of dementia. Neuropathologically, PDD is strongly correlated with limbic and neocortical Lewy pathology(72). Nevertheless, amyloid-β and tau pathology is also common in patients with PDD, and independently contribute to cognitive decline(72). It may therefore be expected that genetic risk

factors contributing to more advanced Lewy and AD co-pathology also increase the susceptibility to cognitive impairment and dementia.

A large number of studies have previously investigated the genetic contribution to dementia in patients with idiopathic PD or DLB. While most previous studies have been candidate gene studies, GWAS have more recently confirmed some of these associations. Cross-sectional studies have reported that the inheritance of the *APOE* E4 allele is associated with an increased risk for dementia in PD(302, 303), faster cognitive decline(304) and lower performance on neuropsychological testing(305). Although not all studies have found this association, and in-between-study heterogeneity and publication bias may confound the results, meta-analyses have weighted in favor of an effect of *APOE* E4 on dementia risk(306, 307). Moreover, longitudinal studies have found faster cognitive decline measured by global cognitive function tests such as MoCA and MMSE(308-310), and more comprehensive neuropsychological assessment among *APOE* E4 carriers(311, 312). On the contrary, the *APOE* E4 allele did not show any significant association with cognitive decline or dementia during the 10-year follow-up period in the CamPaIGN study, which is a UK-based incident cohort of PD patients(313, 314). Moreover, the *APOE* E4 allele was not found to be associated with a shorter time to dementia in another longitudinal study(315). However, in hypothesis free studies such as GWAS, the association between *APOE* and cognitive decline has more recently been confirmed(147, 148). Subsequent, after the publication of our paper, a study combining six population-based longitudinal PD cohorts found that the *APOE* E4 allele had the strongest effect on cognitive decline and progression to dementia(124).

MAPT has also been studied as a risk factor for cognitive decline and dementia. In the previously mentioned CamPaIGN study, the *MAPT* H1 haplotype was linked to a faster rate of cognitive decline and earlier onset of dementia in individuals with PD(313, 316). In two separate studies including longitudinal follow-up of PD patients, associations between the *MAPT* H1 haplotype and lower scores on the memory subscale of the Mattis Dementia Rating Scale (DRS-2) and MMSE were observed(311, 317). However, no significant associations were found between the *MAPT* H1 haplotype and overall rate of cognitive decline. In the former study, the authors hypothesized that the association signal observed in the CamPaIGN study may be specifically related to development of dementia early in the disease course(311) because the CamPaIGN patients were enrolled at the time of diagnosis, and evaluated for progression to dementia after 3 and 5 years. Notably, the *MAPT* H1 haplotype was the only genetic factor associated with dementia in the 10-year follow-up of the CamPaIGN cohort(314). The association between the *MAPT* H1 haplotype and dementia has been

independently replicated, and a novel H1 sub-haplotype (H1p) also implicated(318), although contradictory results have been reported by several, including larger cross-sectional and longitudinal studies(124, 147, 148, 309, 319).

Results concerning non-Gaucher causing *GBA1* variants have been mixed, likely explained by the complexity of the *GBA1* gene, and the extensive range of variants linked to PD. In the first longitudinal study to examine non-Gaucher causing *GBA1* variants, an association with dementia was only found after adjusting for *MAPT* haplotype(320). Conversely, in a much larger multi-center study no association between non-Gaucher causing *GBA1* variants and global cognitive impairment or cognitive decline was detected(321). In another study, combining PD patients from three longitudinal cohorts, a modest yet significant association between non-Gaucher causing *GBA1* variants and dementia was reported(322). More recently, the association between non-Gaucher causing *GBA1* variants and cognitive decline has been confirmed in GWAS(145, 146, 148).

In Paper 1 we investigated the association between risk variants in the two candidate genes *MAPT* and *APOE* and time to dementia by retrospective survival analysis. We took advantage of studying neuropathologically well characterized samples where the risk of misdiagnosis was small and clinical data from the patient's entire lifespan were available. We showed that both the *APOE* E4 allele and the *MAPT* H1 haplotype were associated with a faster progression to dementia. Further, we showed that the *APOE* E4 had a dose dependent effect with carriers of two E4 alleles having a more than three-fold increase in risk of dementia compared to non-carriers. Consistent with our findings, a dose-dependent effect of the E4 allele on dementia risk has later been replicated, albeit with higher hazard ratios (HR) compared to our results(124). Further, our data from Paper 1 do not support the proposed explanation for previously inconsistent findings regarding *MAPT* H1, where *MAPT* has been proposed to contribute to dementia early in the disease course(311). The participants in our study progressed to dementia on average 9 years after onset of motor symptoms, which does not align with this proposed explanation.

The chromosome 17q21 region, where *MAPT* is located, exhibits a highly complex architecture with multiple genes and a high number of variants with complete LD. An inversion polymorphism within the region has led to two distinct haplotypes(323). The H1 haplotype is prevalent across all populations, while the H2 haplotype is almost exclusively found in populations of European genetic ancestry(323). This complex architecture poses a significant challenge in localizing the genetic signal, and may be one reason for previous inconsistent results regarding the role of *MAPT* H1 in

PDD. Although *MAPT* appears to be the most obvious candidate gene within this region, given its relation with protein tau and association with other neurodegenerative diseases(152), in theory, the association signal could be related to any of the genes in high LD within the region.

The precise mechanism through which *APOE* and *MAPT* may promote dementia remains uncertain, although neuropathological investigations suggest protein aggregation is central in this association, as discussed in section 5.2. Our results from Paper 1 demonstrated that individuals with dementia had more advanced amyloid-β, tau and Lewy pathology than non-demented individuals. Moreover, *APOE* E4 was associated with more severe amyloid-β pathology, suggesting *APOE* E4 exerts its risk on dementia through increasing amyloid-β neuropathology. In contrast, we found no associations between the *MAPT* H1 haplotype and neuropathology scores in Paper 1. Although *MAPT* encodes tau which is a common protein pathology associated with dementia(221), a post-mortem study on various neurodegenerative diseases including patients with dementia and movement disorders reported the *MAPT* H1 haplotype associated with reduced NFT pathology in certain brain regions(324). Yet other studies have found a higher burden of Lewy pathology in *MAPT* H1 haplotype carriers, as discussed above(261, 262). Tau and α-synuclein have been found to co-localize in Lewy bodies, and both proteins may synergistically promote the fibrillization of each other(325, 326). Collectively, these studies provide supporting evidence of an intricate relationship between α-synuclein and tau, but also mechanistically linking *MAPT* to LBD.

The polygenic contribution to dementia has also previously been investigated. In a longitudinal study, Paul et al. demonstrated an association between a PD-PRS consisting of 23 SNPs and cognitive decline, defined as a four-point decrease in MMSE score from baseline(327). Contrary to this, no association between a PD-PRS consisting of the 90 lead SNPs from the latest PD GWAS and conversion to PDD was detected in a more recent, large scale longitudinal study with PD patients from 15 cohorts(148). In Paper 2, we showed that the lysosomal PD-PRS was significantly associated with an earlier onset of dementia in samples with no or low AD co-pathology, but not in samples with intermediate or high AD co-pathology. In paper 3, we investigated whether these results could be extended to cognitive impairment in PD, early in the disease process. We showed that the lysosomal PD-PRS was associated with an earlier onset of cognitive impairment in samples with a low AD risk based on CSF measures and AD-PRS in two longitudinal cohorts. To our knowledge, the use of stratified PRS is a novel approach to investigate the polygenic contribution to dementia in PD. Our results are supported by longitudinal studies and GWAS that have shown an association between non-Gaucher-causing *GBA1* variants and cognitive outcomes in PD(145, 146,

148, 322). Moreover, cross-sectional studies have observed a higher frequency of dementia among patients carrying Gaucher-causing *GBA1* variants(126), and longitudinal studies have consistently shown that these variants confer a higher risk of cognitive progression(124, 320-322). Further, these studies have demonstrated that the risk of cognitive impairment increases with the severity of the variant, as patients carrying severe Gaucher-causing variants have a greater risk than patients carrying mild Gaucher-causing variants(124, 322). Despite our initial expectation that *GBA1* was the main driver of the association signal, the significant relationship between the lysosomal PD-PRS and earlier cognitive impairment persisted even after removing *GBA1* from the PRS in both PPMI samples with a negative (i.e., high) CSF $A\beta_{1-42}$ and PDBP samples with a low AD-PRS – both indicative of a low vulnerability to AD co-pathology. Notably, the effect-sizes increased rather than decreased, although the corresponding p-values showed a slight decrease in strength. Thus, our results provide novel evidence for a role of lysosomal variants beyond *GBA1* on cognitive progression in PD. As discussed above, *GBA1* and lysosomal variants have been linked to increased α-synuclein pathology. As advanced Lewy pathology has been reported to be the main substrate of dementia in PD(72), it may be hypothesized that *GBA1* and other lysosomal variants alter pathways involved in α-synuclein clearance leading to a faster development of cortical Lewy pathology, and thus faster cognitive decline.

5.4 Potential utility for PRS in risk stratification

A fundamental principle of precision medicine involves intervention strategies towards individuals with the greatest risk of disease by considering a combination of individual-level risk factors. In this context, PRS has emerged as an attractive tool to stratify patients based on the genetic risk for disease or endophenotypes. In Paper 2, we showed that the Lewy pathology risk score, derived from NBB samples, discriminated between LBD samples with and without neocortical Lewy pathology in the Mayo clinic samples with an AUC of 0.76 (95 % CI 0.71-0.81). Further, the AD co-pathology risk score including AD-PRS, sex and age at onset discriminated between LBD samples with and without AD co-pathology with an AUC of 0.70 (95 % CI 0.65-0.75). Although not sufficient for individual level prediction yet, these results show a potential for PRS as enrichment markers of Lewy and AD co-pathology. In Paper 3, we extended on these results, and sought to identify the optimal cut-point for AD-PRS to stratify patients for the vulnerability to AD co-pathology. The cut-point was determined in NBB samples with neuropathological assessment of AD co-pathology. By applying the pre-determined cut-point to patients from the PPMI and PDBP

cohorts, we were able to replicate our findings from Paper 2, as the lysosomal PD-PRS was associated with an earlier onset of dementia in samples with a low vulnerability to AD co-pathology based on AD-PRS. While we lacked a gold-standard assessment of AD co-pathology for the PPMI and PDBP samples, our results suggest that the AD-PRS could serve a similar purpose in meaningful stratification into subgroups, although with obvious limitations compared to neuropathological assessment. CSF measures of Aβ and tau are well established biomarkers of AD, and have been validated in samples with post-mortem confirmation of AD pathology(328) and amyloid PET imaging(329, 330). However, their utility in PD is less clear. Among the AD CSF biomarkers, the strongest association with cognitive decline in PD is found for low CSF $A\beta_{1-42}$ levels(293, 331, 332). Few studies have validated AD CSF biomarkers in autopsy-confirmed samples or against PET imaging in PD. However, one study showed that CSF $A\beta_{1-42}$ correlated with global cerebral amyloid-β score and a weaker, yet significant correlation was also reported for CSF measures of total tau (t-tau) and neuropathological tau score(333). However, the optimal cut-points for CSF AD biomarkers in PD remains uncertain, although there is some evidence to suggest that they diverge from those established in AD patients(333, 334). In Paper 3, we also used CSF AD biomarkers to stratify between cases with and without AD co-pathology. In lack of established cut-offs for PD patients, we used cut-offs determined in patients from the Alzheimer's Disease Neuroimaging Initiative (ADNI)(329, 335). In Paper 3, the lysosomal PD-PRS was significantly associated with an earlier development of cognitive impairment in PPMI individuals with a low risk of AD co-pathology based on CSF $A\beta_{1-42}$, but not CSF t-tau or tau phosphorylated at threonine 181 (p-tau). Further, CSF $A\beta_{1-42}$ was significantly lower in PPMI individuals with a high vulnerability to AD co-pathology based on the AD-PRS, potentially reflecting a higher level of AD co-pathology in these samples. $A\beta_{1-42}$ continues to decrease over the course of disease(336), which may potentially parallel the increase in AD co-pathology(333, 337). In contrast, PRS offers a benefit compared to fluid biomarkers, as it allows for risk evaluation at an early stage of disease, preceding the usual trajectories for fluid biomarkers throughout the disease course. Although the AD-PRS cut-point suggested in Paper 3 should be interpreted with precaution and validated against PET or neuropathological assessment of AD co-pathology in a large sample, our results highlight the potential prognostic use of AD-PRS to identify patients with an elevated vulnerability to AD co-pathology on a group level.

5.5 Conclusion

The interplay between neuropathological and genetic factors are intricate and multifaceted. Alpha-synuclein, amyloid-β and tau are highly correlated as they increase in parallel(62), and their relationship is likely complex, highlighted by the interactions between them(338). Adding to the complexity are the number of genes implicated in PD, and our incomplete understanding of the pathways they alter. However, an intriguing overlap among genes linked to the predominant protein pathologies and dementia risk in PD may be discerned. The most consistently observed neuropathological features of PDD are advanced limbic and neocortical Lewy pathology and AD-related amyloid-β and tau pathologies(72). Reflecting this dual nature of neuropathology, the risk loci established for cognitive decline include both *GBA1*, which is linked to Lewy pathology, and *APOE* which is strongly associated with more severe AD co-pathology. Although this most likely represents an oversimplification, it underscores a relationship between genetic variants and pathways leading to different types of pathology where dementia is a common outcome. An increased understanding of genetic mechanisms underlying endophenotypes such as Lewy and AD co-pathology or clinical outcomes, such as dementia can be expected to improve clinical care. The most immediate application of such knowledge is individualized predictions of the disease course. Further, identification of causative genes and biological pathways important to disease pathogenesis can facilitate development of targeted therapeutics. Ultimately, characterization of distinct disease subtypes and individuals likely to respond to specific treatments based on their genetic makeup can lead to a more targeted approach in managing the disease.

6 Future directions

The accumulation of evidence is steadily uncovering the molecular etiology and pathogenic mechanisms underlying PD, offering prospects of disease-modifying therapies. While the core motor-features (bradykinesia, rigidity and rest tremor) remain the mainstay for a clinical diagnosis of PD, it is evident that the pathological process leading to disease starts years if not decades before development of motor symptoms, hindering an early diagnosis. Further, the unitarian view of PD has been questioned following the identification of genetic subtypes and involvement of only partially overlapping disease pathways, but also providing an opportunity for a biological diagnosis of PD within a precision medicine context(339). For AD, the amyloid, tau, neurodegeneration (A/T/N) classification scheme provides an updated biological definition of AD relying solely on biomarkers(340). This framework provides an opportunity for selection of patients within the AD continuum for enrichment of clinical trials.

Similar biological definitions of PD have recently been proposed, based on the presence of neuronal α-synuclein and dopaminergic dysfunction(341) or genetics, α-synuclein pathology and neurodegeneration(342). Both these biological staging systems emphasize the importance of identifying pathologic α-synuclein. However, there is a pressing need for a reliable approach to detect α-synuclein pathology in living patients. Although there are currently no biomarkers that can reliably distinguish PD patients from individuals with other parkinsonian disorders and healthy controls, α-synuclein seed amplification assays (SAA) have shown promising results(343). These assays can detect small amounts of misfolded α-synuclein in various tissue, although best documented for CSF, and reliably distinguish between patients with PD/DLB, controls and other neurodegenerative diseases such as PSP and CBD(reviewed in (344)). Notably, preliminary results also suggest that SSAs may have the potential to distinguish between PD and MSA(345).

While α-synuclein SAAs may improve the diagnostic accuracy of PD, the current dichotomous outcome of the test does not allow for tracking the course of disease. Neuroimaging has the potential to investigate the progression of protein pathology in vivo. PET radiotracers for amyloid pathology have been available for more than a decade, and a tau PET radiotracer has just recently been approved by the FDA(346, 347). Contrary there is currently no equivalent method for visualizing α-synuclein aggregates in living patients. The development of a suitable α-synuclein

PET tracer has faced several challenges including a relatively low concentration of α-synuclein compared to amyloid-β and tau aggregates in the brain(348). Additionally, the co-existence and structural similarity between aggregated forms of α-synuclein, amyloid-β and tau, makes it difficult to selectively target α-synuclein(348). An α-synuclein PET tracer offers several advantages, including non-invasive early detection of pathology, monitoring disease progression and assessment of the effectiveness of potential therapeutic interventions.

Despite the ongoing progress in uncovering the genetic underpinnings of idiopathic PD, we can only explain a proportion of disease heritability by variants captured through current research methods. Increasing GWAS sample size, shifting focus towards rare and structural variants enabled by next generations sequencing technique as well as investigating gene-gene and gene-environment interactions are expected to uncover some of this missing heritability(349). However, a major concern of genetic research in general is the lack of diversity in study populations(244, 245). While the sample size and number of PD GWAS conducted have increased, they are not representative for the global population, as most studies to date have been performed in populations of European genetic ancestry. Expanding GWAS to include underrepresented populations is expected to increase the yield of new risk loci as was recently proven in a PD GWAS meta-analysis in East Asian populations(350). Although the few studies conducted in PD patients of non-European genetic ancestry have revealed overlapping genetic risk loci, they have also highlighted genetic heterogeneity. For example, *LRRK2* appears to be a common risk factor for PD in European and East Asian populations. However, the *LRRK2* p.G2019S risk variant which is common in individuals of European ancestry is rare among East Asians, where the p.A419V, p.G2385R and p.R1628P variants are more common(351, 352). Population-specific differences in LD pattern, allele frequencies, gene-gene and gene-environment interactions are all factors likely to explain genetic heterogeneity across diverse populations(353). Further, the lack of diversity in genetic research may have broader implications for the underrepresented populations. As discussed in section 4.5.3 the accuracy of PRS may be compromised when applied to a population of different genetic ancestry than the population in which the PRS was developed, not only leading to inaccurate risk predictions, but also potentially increasing health care inequities(247). Consequently, an understanding of the genetic architecture of PD in ancestrally diverse populations is an unmet research need. Efforts to address the fundamental gap in the genetics of understudied PD populations are a central focus of global initiatives, such as the Global Parkinson's disease

Genetics Program (GP2)(354). Such endeavors hold potential to greatly benefit the global PD population.

While GWAS have successfully identified disease susceptibility variants through large cross-sectional studies, a comprehensive understanding of genetic influence on the disease course and the discovery of disease relevant biomarkers necessitates longitudinal cohorts with greater depth of phenotyping. The AMP-PD and PPMI cohorts used in Paper 2 serve as prime examples of such longitudinal studies designed to discover and replicate PD biomarkers. Efforts to combine data from multiple longitudinal studies have discovered promising progression markers as discussed in section 1.4.5. However, the sample sizes remain relatively small compared to case-control GWAS. To ensure generalizability, additional cohorts are imperative for replication, confirmation and validation of these findings(355). An ongoing effort, the PROSPOS study, is collecting a comprehensive range of clinical and biological data from Norwegian patients with PD and atypical parkinsonism. The study harmonizes data collection with collaborators from the International Parkinson's Disease Genomics Consortium (IPDGC) and GP2, with the first 96 PROSPOS samples already submitted to GP2. Throughout my PhD, I have enrolled patients in the PROSPOS study, with a specific focus on including patients for cognitive assessment and amyloid-β PET imaging using the radiotracer ^{18}F-Flutemetamol. Since enrollment started as recent as February 2020, with further delay arising from the COVID-19 pandemic, limited follow-up time prevented the inclusion of PROSPOS data in my PhD thesis. Nevertheless, the extensive longitudinal data expected to be generated in this study holds promise in providing a deeper insight into the long-term progression of PD.

Current treatment options for PD primarily aim at alleviating symptoms, and do not modify disease progression. In parallel with our increased understanding of PD pathophysiology, a diverse range of therapeutic candidates are being explored. These include treatments targeting α-synuclein, organelles such as mitochondria or lysosomes, or proteins such as LRRK2 or GCase(356). Nevertheless, none of the examined treatments have yet successfully evolved into clinically verified disease modifying therapies. Clinical trial failures may arise due to a number of reasons, one of which may be the inability to account for heterogeneity in PD(356). As discussed in section 1.4.5 and demonstrated in Paper 1, 2 and 3 variations in progression of PD may in part be attributed to differences in the underlying genetic architecture. Thus, having an uneven distribution of fast progressors in either the treatment or placebo group could yield misleading conclusions about the drug's efficacy. Consequently, it has been suggested that clinical trials, in addition to age- and

gender matching, should account for genetic imbalances(357). Further, it is unlikely that one drug will benefit the broader PD group, as the underlying pathophysiology may differ between patients. To address this heterogeneity, patient stratification may aid in identifying individuals most likely to benefit from a targeted therapy. Stratification based on genetic predisposition such as type of *LRRK2* or *GBA1* variant, provides one approach to cluster more homogenous patients. Despite the myriad of genetic variants associated with PD, the discovery that many of these converge in highly coherent disease pathways offers promise for the development of therapies with broad applicability. For the larger idiopathic PD population, stratified PRS may be one method to identify patients with a similar underlying disease process, thereby improving the signal-to-noise ratio in clinical trials focusing on targets like lysosomes or mitochondria. However, given the likely perturbation of multiple pathways in individual PD patients, a combination of therapies, rather than one single therapy, may be a more likely scenario for future disease modifying treatment(358). Nevertheless, each medication will likely need to be proven efficacious on their own before a multi-drug regime can be implemented. Moreover, an AD PRS above a predetermined threshold might serve as a means to select patients with an increased vulnerability to AD co-pathology likely to benefit from treatments targeting amyloid-β, as suggested in Paper 3. However, considering the current limited predictive ability of PRS, it is plausible that a combination of PRS with other biomarkers will be a more promising approach. This is the goal of precision medicine, where treatment strategies are customized to the individual´s disease subtype.

7 References

1. Raudino F. The Parkinson disease before James Parkinson. Neurol Sci. 2012;33(4):945-8.
2. Parkinson J. An Essay on the Shaking Palsy. London: Whittingham and Rowland for Sherwood, Neely and Jones; 1817.
3. Charcot JM. Lectures on the diseases of the nervous system: delivered at la Salpêtrière: HC Lea; 1879.
4. Blocq P, Marinesco G. Sur un cas de tremblement parkinsonien hémiplégique symptomatique d'une tumeur du pédoncule cérébral. Comptes Rendus Séances Société Biol. 1893;5:105–11.
5. Lewy FH. Paralysis agitans. I. Pathologische anatomie. In: Lewandrowsky M, editor. Handbuch der neurologie. 3. Berlin: Springer-Verlag; 1912. p. 920-33.
6. Trétiakoff C. Contribution à l'étude de l'Anatomie pathologique du Locus Niger de Soemmering avec quelques déduction relatives à la pathogénie des troubles du tonus musculaire et de la maladie de Parkinson [Thesis]. Paris: Université de Paris; 1919.
7. Carlsson A, Lindqvist M, Magnusson T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature. 1957;180(4596):1200.
8. Carlsson A, Lindqvist M, Magnusson T, Waldeck B. On the presence of 3-hydroxytyramine in brain. Science. 1958;127(3296):471.
9. Ehringer H, Hornykiewicz O. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. Klin Wochenschr. 1960;38:1236-9.
10. Hornykiewicz O. [The tropical localization and content of noradrenalin and dopamine (3-hydroxytyramine) in the substantia nigra of normal persons and patients with Parkinson's disease]. Wien Klin Wochenschr. 1963;75:309-12.
11. Poirier LJ, Sourkes TL. [Influence of Locus Niger on the Concentration of Catecholamines in the Striatum]. J Physiol (Paris). 1964;56:426-7.
12. Birkmayer W, Hornykiewicz O. [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. Wien Klin Wochenschr. 1961;73:787-8.
13. Cotzias GC, Papavasiliou PS, Gellene R. Modification of Parkinsonism--chronic treatment with L-dopa. N Engl J Med. 1969;280(7):337-45.
14. Cotzias GC, Papavasiliou PS, Fehling C, Kaufman B, Mena I. Similarities between neurologic effects of L-dopa and of apomorphine. N Engl J Med. 1970;282(1):31-3.

15. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. Nat Rev Dis Primers. 2017;3(1):17013.
16. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014;29(13):1583-90.
17. Hirsch L, Jette N, Frolkis A, Steeves T, Pringsheim T. The Incidence of Parkinson's Disease: A Systematic Review and Meta-Analysis. Neuroepidemiology. 2016;46(4):292-300.
18. de Rijk MC, Launer LJ, Berger K, Breteler MM, Dartigues JF, Baldereschi M, et al. Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology. 2000;54(11 Suppl 5):S21-3.
19. Twelves D, Perkins KS, Counsell C. Systematic review of incidence studies of Parkinson's disease. Mov Disord. 2003;18(1):19-31.
20. Taylor KS, Cook JA, Counsell CE. Heterogeneity in male to female risk for Parkinson's disease. J Neurol Neurosurg Psychiatry. 2007;78(8):905-6.
21. G. B. D. Neurological Disorders Collaborator Group. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017;16(11):877-97.
22. Dorsey ER, Bloem BR. The Parkinson Pandemic-A Call to Action. JAMA Neurol. 2018;75(1):9-10.
23. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591-601.
24. Santos Garcia D, de Deus Fonticoba T, Suarez Castro E, Borrue C, Mata M, Solano Vila B, et al. Non-motor symptoms burden, mood, and gait problems are the most significant factors contributing to a poor quality of life in non-demented Parkinson's disease patients: Results from the COPPADIS Study Cohort. Parkinsonism Relat Disord. 2019;66:151-7.
25. Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. Nat Rev Neurosci. 2017;18(7):435-50.
26. Pont-Sunyer C, Hotter A, Gaig C, Seppi K, Compta Y, Katzenschlager R, et al. The onset of nonmotor symptoms in Parkinson's disease (the ONSET PD study). Mov Disord. 2015;30(2):229-37.
27. Pedersen KF, Larsen JP, Tysnes OB, Alves G. Natural course of mild cognitive impairment in Parkinson disease: A 5-year population-based study. Neurology. 2017;88(8):767-74.
28. Aarsland D, Batzu L, Halliday GM, Geurtsen GJ, Ballard C, Ray Chaudhuri K, et al. Parkinson disease-associated cognitive impairment. Nat Rev Dis Primers. 2021;7(1):47.

29. Backstrom D, Granasen G, Domellof ME, Linder J, Jakobson Mo S, Riklund K, et al. Early predictors of mortality in parkinsonism and Parkinson disease: A population-based study. Neurology. 2018;91(22):e2045-e56.
30. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord. 2008;23(6):837-44.
31. de Bie RMA, Clarke CE, Espay AJ, Fox SH, Lang AE. Initiation of pharmacological therapy in Parkinson's disease: when, why, and how. Lancet Neurol. 2020;19(5):452-61.
32. Armstrong MJ, Okun MS. Diagnosis and Treatment of Parkinson Disease: A Review. JAMA. 2020;323(6):548-60.
33. Olanow CW, Kieburtz K, Odin P, Espay AJ, Standaert DG, Fernandez HH, et al. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. Lancet Neurol. 2014;13(2):141-9.
34. Senek M, Nielsen EI, Nyholm D. Levodopa-entacapone-carbidopa intestinal gel in Parkinson's disease: A randomized crossover study. Mov Disord. 2017;32(2):283-6.
35. Katzenschlager R, Poewe W, Rascol O, Trenkwalder C, Deuschl G, Chaudhuri KR, et al. Apomorphine subcutaneous infusion in patients with Parkinson's disease with persistent motor fluctuations (TOLEDO): a multicentre, double-blind, randomised, placebo-controlled trial. Lancet Neurol. 2018;17(9):749-59.
36. Limousin P, Foltynie T. Long-term outcomes of deep brain stimulation in Parkinson disease. Nat Rev Neurol. 2019;15(4):234-42.
37. Moosa S, Martinez-Fernandez R, Elias WJ, Del Alamo M, Eisenberg HM, Fishman PS. The role of high-intensity focused ultrasound as a symptomatic treatment for Parkinson's disease. Mov Disord. 2019;34(9):1243-51.
38. Seppi K, Ray Chaudhuri K, Coelho M, Fox SH, Katzenschlager R, Perez Lloret S, et al. Update on treatments for nonmotor symptoms of Parkinson's disease-an evidence-based medicine review. Mov Disord. 2019;34(2):180-98.
39. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. Neurology. 2016;86(6):566-76.
40. Heim B, Krismer F, De Marzi R, Seppi K. Magnetic resonance imaging for the diagnosis of Parkinson's disease. J Neural Transm (Vienna). 2017;124(8):915-64.
41. Meyer PT, Frings L, Rucker G, Hellwig S. (18)F-FDG PET in Parkinsonism: Differential Diagnosis and Evaluation of Cognitive Impairment. J Nucl Med. 2017;58(12):1888-98.

42. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017;89(1):88-100.
43. Rizzo G, Arcuti S, Copetti M, Alessandria M, Savica R, Fontana A, et al. Accuracy of clinical diagnosis of dementia with Lewy bodies: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2018;89(4):358-66.
44. Greffard S, Verny M, Bonnet AM, Beinis JY, Gallinari C, Meaume S, et al. Motor score of the Unified Parkinson Disease Rating Scale as a good predictor of Lewy body-associated neuronal loss in the substantia nigra. Arch Neurol. 2006;63(4):584-8.
45. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain. 1991;114 (Pt 5):2283-301.
46. Espay AJ, Vizcarra JA, Marsili L, Lang AE, Simon DK, Merola A, et al. Revisiting protein aggregation as pathogenic in sporadic Parkinson and Alzheimer diseases. Neurology. 2019;92(7):329-37.
47. Cooper CA, Jain N, Gallagher MD, Weintraub D, Xie SX, Berlyand Y, et al. Common variant rs356182 near SNCA defines a Parkinson's disease endophenotype. Ann Clin Transl Neurol. 2017;4(1):15-25.
48. den HJW, Bethlem J. The distribution of Lewy bodies in the central and autonomic nervous systems in idiopathic paralysis agitans. J Neurol Neurosurg Psychiatry. 1960;23(4):283-90.
49. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839-40.
50. Wakabayashi K, Tanji K, Odagiri S, Miki Y, Mori F, Takahashi H. The Lewy body in Parkinson's disease and related neurodegenerative disorders. Mol Neurobiol. 2013;47(2):495-508.
51. Shahmoradian SH, Lewis AJ, Genoud C, Hench J, Moors TE, Navarro PP, et al. Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. Nat Neurosci. 2019;22(7):1099-109.
52. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197-211.
53. Braak H, Del Tredici K. Neuroanatomy and pathology of sporadic Parkinson's disease. Adv Anat Embryol Cell Biol. 2009;201:1-119.
54. Djaldetti R, Lev N, Melamed E. Lesions outside the CNS in Parkinson's disease. Mov Disord. 2009;24(6):793-800.

55. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett. 2006;396(1):67-72.
56. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology. 2005;65(12):1863-72.
57. Kosaka K, Yoshimura M, Ikeda K, Budka H. Diffuse type of Lewy body disease: progressive dementia with abundant cortical Lewy bodies and senile changes of varying degree--a new disease? Clin Neuropathol. 1984;3(5):185-92.
58. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol. 2007;33(6):599-614.
59. Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, et al. Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol. 2009;117(6):613-34.
60. Dickson DW, Uchikado H, Fujishiro H, Tsuboi Y. Evidence in favor of Braak staging of Parkinson's disease. Mov Disord. 2010;25 Suppl 1:S78-82.
61. Jellinger KA. A critical evaluation of current staging of alpha-synuclein pathology in Lewy body disorders. Biochim Biophys Acta. 2009;1792(7):730-40.
62. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain. 2011;134(Pt 5):1493-505.
63. Horvath J, Herrmann FR, Burkhard PR, Bouras C, Kovari E. Neuropathology of dementia in a large cohort of patients with Parkinson's disease. Parkinsonism Relat Disord. 2013;19(10):864-8; discussion
64. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol. 2012;72(4):587-98.
65. Martin WRW, Younce JR, Campbell MC, Racette BA, Norris SA, Ushe M, et al. Neocortical Lewy Body Pathology Parallels Parkinson's Dementia, but Not Always. Ann Neurol. 2023;93(1):184-95.
66. Borghammer P, Van Den Berge N. Brain-First versus Gut-First Parkinson's Disease: A Hypothesis. J Parkinsons Dis. 2019;9(s2):S281-S95.
67. Horsager J, Andersen KB, Knudsen K, Skjaerbaek C, Fedorova TD, Okkels N, et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. Brain. 2020;143(10):3077-88.

68. Borghammer P. The alpha-Synuclein Origin and Connectome Model (SOC Model) of Parkinson's Disease: Explaining Motor Asymmetry, Non-Motor Phenotypes, and Cognitive Decline. J Parkinsons Dis. 2021;11(2):455-74.
69. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1(1):a006189.
70. Hamilton RL. Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain Pathol. 2000;10(3):378-84.
71. Sabbagh MN, Adler CH, Lahti TJ, Connor DJ, Vedders L, Peterson LK, et al. Parkinson disease with dementia: comparing patients with and without Alzheimer pathology. Alzheimer Dis Assoc Disord. 2009;23(3):295-7.
72. Smith C, Malek N, Grosset K, Cullen B, Gentleman S, Grosset DG. Neuropathology of dementia in patients with Parkinson's disease: a systematic review of autopsy studies. J Neurol Neurosurg Psychiatry. 2019;90(11):1234-43.
73. Jellinger KA, Seppi K, Wenning GK, Poewe W. Impact of coexistent Alzheimer pathology on the natural history of Parkinson's disease. J Neural Transm (Vienna). 2002;109(3):329-39.
74. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58(12):1791-800.
75. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112(4):389-404.
76. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol. 2019;15(10):565-81.
77. Pang SY, Ho PW, Liu HF, Leung CT, Li L, Chang EES, et al. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. Transl Neurodegener. 2019;8:23.
78. Chen Y, Sun X, Lin Y, Zhang Z, Gao Y, Wu IXY. Non-Genetic Risk Factors for Parkinson's Disease: An Overview of 46 Systematic Reviews. J Parkinsons Dis. 2021;11(3):919-35.
79. Langston JW, Ballard PA, Jr. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. N Engl J Med. 1983;309(5):310.
80. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011;119(6):866-72.
81. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am J Epidemiol. 2009;169(8):919-26.

82. Angelopoulou E, Paudel YN, Papageorgiou SG, Piperi C. Environmental Impact on the Epigenetic Mechanisms Underlying Parkinson's Disease Pathogenesis: A Narrative Review. Brain Sci. 2022;12(2).
83. Duvoisin RC. Is Parkinson's disease acquired or inherited? Can J Neurol Sci. 1984;11(1 Suppl):151-5.
84. Day JO, Mullin S. The Genetics of Parkinson's Disease and Implications for Clinical Practice. Genes (Basel). 2021;12(7).
85. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. Lancet Neurol. 2020;19(2):170-8.
86. Lubbe SJ, Escott-Price V, Gibbs JR, Nalls MA, Bras J, Price TR, et al. Additional rare variant analysis in Parkinson's disease cases with and without known pathogenic mutations: evidence for oligogenic inheritance. Hum Mol Genet. 2016;25(24):5483-9.
87. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997;276(5321):2045-7.
88. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet. 2004;364(9440):1167-9.
89. Ibanez P, Bonnet AM, Debarges B, Lohmann E, Tison F, Pollak P, et al. Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet. 2004;364(9440):1169-71.
90. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003;302(5646):841.
91. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 2004;44(4):595-600.
92. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004;44(4):601-7.
93. Vilarino-Guell C, Wider C, Ross OA, Dachsel JC, Kachergus JM, Lincoln SJ, et al. VPS35 mutations in Parkinson disease. Am J Hum Genet. 2011;89(1):162-7.
94. Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, et al. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am J Hum Genet. 2011;89(1):168-75.

95. Trinh J, Zeldenrust FMJ, Huang J, Kasten M, Schaake S, Petkovic S, et al. Genotype-phenotype relations for the Parkinson's disease genes SNCA, LRRK2, VPS35: MDSGene systematic review. Mov Disord. 2018;33(12):1857-70.
96. Konno T, Ross OA, Puschmann A, Dickson DW, Wszolek ZK. Autosomal dominant Parkinson's disease caused by SNCA duplications. Parkinsonism Relat Disord. 2016;22 Suppl 1(Suppl 1):S1-6.
97. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998;392(6676):605-8.
98. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 2004;304(5674):1158-60.
99. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science. 2003;299(5604):256-9.
100. Kasten M, Hartmann C, Hampf J, Schaake S, Westenberger A, Vollstedt EJ, et al. Genotype-Phenotype Relations for the Parkinson's Disease Genes Parkin, PINK1, DJ1: MDSGene Systematic Review. Mov Disord. 2018;33(5):730-41.
101. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet. 2006;38(10):1184-91.
102. Paisan-Ruiz C, Bhatia KP, Li A, Hernandez D, Davis M, Wood NW, et al. Characterization of PLA2G6 as a locus for dystonia-parkinsonism. Ann Neurol. 2009;65(1):19-23.
103. Shojaee S, Sina F, Banihosseini SS, Kazemi MH, Kalhor R, Shahidi GA, et al. Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. Am J Hum Genet. 2008;82(6):1375-84.
104. Di Fonzo A, Dekker MC, Montagna P, Baruzzi A, Yonova EH, Correia Guedes L, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. Neurology. 2009;72(3):240-5.
105. Edvardson S, Cinnamon Y, Ta-Shma A, Shaag A, Yim YI, Zenvirt S, et al. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One. 2012;7(5):e36458.
106. Deng H, Wang P, Jankovic J. The genetics of Parkinson disease. Ageing Res Rev. 2018;42:72-85.

107. Martin S, Smolders S, Van den Haute C, Heeman B, van Veen S, Crosiers D, et al. Mutated ATP10B increases Parkinson's disease risk by compromising lysosomal glucosylceramide export. Acta Neuropathol. 2020;139(6):1001-24.
108. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008;7(7):583-90.
109. Machaczka M, Rucinska M, Skotnicki AB, Jurczak W. Parkinson's syndrome preceding clinical manifestation of Gaucher's disease. Am J Hematol. 1999;61(3):216-7.
110. Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. Parkinsonism among Gaucher disease carriers. J Med Genet. 2004;41(12):937-40.
111. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med. 2009;361(17):1651-61.
112. Parlar SC, Grenn FP, Kim JJ, Baluwendraat C, Gan-Or Z. Classification of GBA1 Variants in Parkinson's Disease: The GBA1-PD Browser. Mov Disord. 2023;38(3):489-95.
113. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. Neurology. 2015;84(9):880-7.
114. Zhang Y, Shu L, Sun Q, Zhou X, Pan H, Guo J, et al. Integrated Genetic Analysis of Racial Differences of Common GBA Variants in Parkinson's Disease: A Meta-Analysis. Front Mol Neurosci. 2018;11:43.
115. Duran R, Mencacci NE, Angeli AV, Shoai M, Deas E, Houlden H, et al. The glucocerobrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. Mov Disord. 2013;28(2):232-6.
116. Riboldi GM, Di Fonzo AB. GBA, Gaucher Disease, and Parkinson's Disease: From Genetic to Clinic to New Therapeutic Approaches. Cells. 2019;8(4):364.
117. Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. Neurology. 2012;78(6):417-20.
118. Straniero L, Asselta R, Bonvegna S, Rimoldi V, Melistaccio G, Solda G, et al. The SPID-GBA study: Sex distribution, Penetrance, Incidence, and Dementia in GBA-PD. Neurol Genet. 2020;6(6):e523.
119. Blauwendraat C, Reed X, Krohn L, Heilbron K, Bandres-Ciga S, Tan M, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. Brain. 2020;143(1):234-48.

120. Clark LN, Ross BM, Wang Y, Mejia-Santana H, Harris J, Louis ED, et al. Mutations in the glucocerebrosidase gene are associated with early-onset Parkinson disease. Neurology. 2007;69(12):1270-7.
121. Brockmann K, Srulijes K, Pflederer S, Hauser AK, Schulte C, Maetzler W, et al. GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study. Mov Disord. 2015;30(3):407-11.
122. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: The mutation matters. Ann Neurol. 2016;80(5):662-73.
123. Adler CH, Beach TG, Shill HA, Caviness JN, Driver-Dunckley E, Sabbagh MN, et al. GBA mutations in Parkinson disease: earlier death but similar neuropathological features. Eur J Neurol. 2017;24(11):1363-8.
124. Szwedo AA, Dalen I, Pedersen KF, Camacho M, Backstrom D, Forsgren L, et al. GBA and APOE Impact Cognitive Decline in Parkinson's Disease: A 10-Year Population-Based Study. Mov Disord. 2022;37(5):1016-27.
125. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: Review of the literature. Mov Disord. 2017;32(11):1504-23.
126. Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. Brain. 2009;132(Pt 7):1783-94.
127. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature. 2001;409(6822):860-921.
128. International HapMap Consortium. A haplotype map of the human genome. Nature. 2005;437(7063):1299-320.
129. Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
130. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. 10 Years of GWAS Discovery: Biology, Function, and Translation. Am J Hum Genet. 2017;101(1):5-22.
131. Oh JH, Jo S, Park KW, Lee EJ, Lee SH, Hwang YS, et al. Whole-genome sequencing reveals an association between small genomic deletions and an increased risk of developing Parkinson's disease. Exp Mol Med. 2023;55(3):555-64.
132. Maraganore DM, de Andrade M, Lesnick TG, Strain KJ, Farrer MJ, Rocca WA, et al. High-resolution whole-genome association study of Parkinson disease. Am J Hum Genet. 2005;77(5):685-93.

133. Fung HC, Scholz S, Matarin M, Simon-Sanchez J, Hernandez D, Britton A, et al. Genome-wide genotyping in Parkinson's disease and neurologically normal controls: first stage analysis and public release of data. Lancet Neurol. 2006;5(11):911-6.
134. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009;41(12):1308-12.
135. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet. 2009;41(12):1303-7.
136. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol. 2019;18(12):1091-102.
137. Wirdefeldt K, Gatz M, Reynolds CA, Prescott CA, Pedersen NL. Heritability of Parkinson disease in Swedish twins: a longitudinal study. Neurobiol Aging. 2011;32(10):1923 e1-8.
138. Goldman SM, Marek K, Ottman R, Meng C, Comyns K, Chan P, et al. Concordance for Parkinson's disease in twins: A 20-year update. Ann Neurol. 2019;85(4):600-5.
139. Keller MF, Saad M, Bras J, Bettella F, Nicolaou N, Simon-Sanchez J, et al. Using genome-wide complex trait analysis to quantify 'missing heritability' in Parkinson's disease. Hum Mol Genet. 2012;21(22):4996-5009.
140. International Parkinson Disease Genomics Consortium, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet. 2011;377(9766):641-9.
141. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet. 2014;46(9):989-93.
142. Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat Genet. 2017;49(10):1511-6.
143. Blauwendraat C, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrom L, et al. Parkinson's disease age at onset genome-wide association study: Defining heritability, genetic loci, and alpha-synuclein mechanisms. Mov Disord. 2019;34(6):866-75.

144. Grover S, Kumar Sreelatha AA, Pihlstrom L, Domenighetti C, Schulte C, Sugier PE, et al. Genome-wide Association and Meta-analysis of Age at Onset in Parkinson Disease: Evidence From the COURAGE-PD Consortium. Neurology. 2022;99(7):e698-e710.
145. Iwaki H, Blauwendraat C, Leonard HL, Liu G, Maple-Grodem J, Corvol JC, et al. Genetic risk of Parkinson disease and progression:: An analysis of 13 longitudinal cohorts. Neurol Genet. 2019;5(4):e348.
146. Iwaki H, Blauwendraat C, Leonard HL, Kim JJ, Liu G, Maple-Grodem J, et al. Genomewide association study of Parkinson's disease clinical biomarkers in 12 longitudinal patients' cohorts. Mov Disord. 2019;34(12):1839-50.
147. Tan MMX, Lawton MA, Jabbari E, Reynolds RH, Iwaki H, Blauwendraat C, et al. Genome-Wide Association Studies of Cognitive and Motor Progression in Parkinson's Disease. Mov Disord. 2021;36(2):424-33.
148. Liu G, Peng J, Liao Z, Locascio JJ, Corvol JC, Zhu F, et al. Genome-wide survival study identifies a novel synaptic locus and polygenic score for cognitive progression in Parkinson's disease. Nat Genet. 2021;53(6):787-93.
149. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA. 1997;278(16):1349-56.
150. Guerreiro R, Ross OA, Kun-Rodrigues C, Hernandez DG, Orme T, Eicher JD, et al. Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study. Lancet Neurol. 2018;17(1):64-74.
151. Fagan ES, Pihlstrom L. Genetic risk factors for cognitive decline in Parkinson's disease: a review of the literature. Eur J Neurol. 2017;24(4):561-e20.
152. Leveille E, Ross OA, Gan-Or Z. Tau and MAPT genetics in tauopathies and synucleinopathies. Parkinsonism Relat Disord. 2021;90:142-54.
153. Chia R, Sabir MS, Bandres-Ciga S, Saez-Atienzar S, Reynolds RH, Gustavsson E, et al. Genome sequencing analysis identifies new loci associated with Lewy body dementia and provides insights into its genetic architecture. Nat Genet. 2021;53(3):294-303.
154. Arneson D, Zhang Y, Yang X, Narayanan M. Shared mechanisms among neurodegenerative diseases: from genetic factors to gene networks. J Genet. 2018;97(3):795-806.
155. Andersen MS, Bandres-Ciga S, Reynolds RH, Hardy J, Ryten M, Krohn L, et al. Heritability Enrichment Implicates Microglia in Parkinson's Disease Pathogenesis. Ann Neurol. 2021;89(5):942-51.

156. Gallagher MD, Chen-Plotkin AS. The Post-GWAS Era: From Association to Function. Am J Hum Genet. 2018;102(5):717-30.
157. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. Science. 2012;337(6099):1190-5.
158. Farrow SL, Schierding W, Gokuladhas S, Golovina E, Fadason T, Cooper AA, et al. Establishing gene regulatory networks from Parkinson's disease risk loci. Brain. 2022;145(7):2422-35.
159. Schilder BM, Navarro E, Raj T. Multi-omic insights into Parkinson's Disease: From genetic associations to functional mechanisms. Neurobiol Dis. 2022;163:105580.
160. Bandres-Ciga S, Saez-Atienzar S, Kim JJ, Makarious MB, Faghri F, Diez-Fairen M, et al. Large-scale pathway specific polygenic risk and transcriptomic community network analysis identifies novel functional pathways in Parkinson disease. Acta Neuropathol. 2020;140(3):341-58.
161. Bandres-Ciga S, Saez-Atienzar S, Bonet-Ponce L, Billingsley K, Vitale D, Blauwendraat C, et al. The endocytic membrane trafficking pathway plays a major role in the risk of Parkinson's disease. Mov Disord. 2019;34(4):460-8.
162. Billingsley KJ, Barbosa IA, Bandres-Ciga S, Quinn JP, Bubb VJ, Deshpande C, et al. Mitochondria function associated genes contribute to Parkinson's Disease risk and later age at onset. NPJ Parkinsons Dis. 2019;5:8.
163. Sulzer D, Edwards RH. The physiological role of alpha-synuclein and its relationship to Parkinson's Disease. J Neurochem. 2019;150(5):475-86.
164. Deleersnijder A, Gerard M, Debyser Z, Baekelandt V. The remarkable conformational plasticity of alpha-synuclein: blessing or curse? Trends Mol Med. 2013;19(6):368-77.
165. Wong YC, Krainc D. alpha-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. Nat Med. 2017;23(2):1-13.
166. Giasson BI, Uryu K, Trojanowski JQ, Lee VM. Mutant and wild type human alpha-synucleins assemble into elongated filaments with distinct morphologies in vitro. J Biol Chem. 1999;274(12):7619-22.
167. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A. 2009;106(31):13010-5.
168. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science. 2012;338(6109):949-53.

169. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med. 2008;14(5):504-6.
170. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med. 2008;14(5):501-3.
171. Navarro-Romero A, Montpeyo M, Martinez-Vicente M. The Emerging Role of the Lysosome in Parkinson's Disease. Cells. 2020;9(11):2399.
172. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science. 2004;305(5688):1292-5.
173. Lee HJ, Khoshaghideh F, Patel S, Lee SJ. Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. J Neurosci. 2004;24(8):1888-96.
174. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, et al. Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell. 2011;146(1):37-52.
175. Smolders S, Van Broeckhoven C. Genetic perspective on the synergistic connection between vesicular transport, lysosomal and mitochondrial pathways associated with Parkinson's disease pathogenesis. Acta Neuropathol Commun. 2020;8(1):63.
176. Eguchi T, Kuwahara T, Sakurai M, Komori T, Fujimoto T, Ito G, et al. LRRK2 and its substrate Rab GTPases are sequentially targeted onto stressed lysosomes and maintain their homeostasis. Proc Natl Acad Sci U S A. 2018;115(39):E9115-E24.
177. Robak LA, Jansen IE, van Rooij J, Uitterlinden AG, Kraaij R, Jankovic J, et al. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain. 2017;140(12):3191-203.
178. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. JAMA Neurol. 2013;70(6):727-35.
179. Do CB, Tung JY, Dorfman E, Kiefer AK, Drabant EM, Francke U, et al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. PLoS Genet. 2011;7(6):e1002141.
180. Jinn S, Drolet RE, Cramer PE, Wong AH, Toolan DM, Gretzula CA, et al. TMEM175 deficiency impairs lysosomal and mitochondrial function and increases alpha-synuclein aggregation. Proc Natl Acad Sci U S A. 2017;114(9):2389-94.
181. Park JS, Blair NF, Sue CM. The role of ATP13A2 in Parkinson's disease: Clinical phenotypes and molecular mechanisms. Mov Disord. 2015;30(6):770-9.

182. Senkevich K, Gan-Or Z. Autophagy lysosomal pathway dysfunction in Parkinson's disease; evidence from human genetics. Parkinsonism Relat Disord. 2020;73:60-71.
183. Elkin SR, Lakoduk AM, Schmid SL. Endocytic pathways and endosomal trafficking: a primer. Wien Med Wochenschr. 2016;166(7-8):196-204.
184. Scott CC, Vacca F, Gruenberg J. Endosome maturation, transport and functions. Semin Cell Dev Biol. 2014;31:2-10.
185. Krebs CE, Karkheiran S, Powell JC, Cao M, Makarov V, Darvish H, et al. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. Hum Mutat. 2013;34(9):1200-7.
186. Quadri M, Fang M, Picillo M, Olgiati S, Breedveld GJ, Graafland J, et al. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism. Hum Mutat. 2013;34(9):1208-15.
187. Cunningham LA, Moore DJ. Endosomal sorting pathways in the pathogenesis of Parkinson's disease. Prog Brain Res. 2020;252:271-306.
188. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci. 1985;36(26):2503-8.
189. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem. 1990;54(3):823-7.
190. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. 2010;8(1):e1000298.
191. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A. 2010;107(1):378-83.
192. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet. 2011;20(1):40-50.
193. Burchell VS, Nelson DE, Sanchez-Martinez A, Delgado-Camprubi M, Ivatt RM, Pogson JH, et al. The Parkinson's disease-linked proteins Fbxo7 and Parkin interact to mediate mitophagy. Nat Neurosci. 2013;16(9):1257-65.
194. Borsche M, Pereira SL, Klein C, Grunewald A. Mitochondria and Parkinson's Disease: Clinical, Molecular, and Translational Aspects. J Parkinsons Dis. 2021;11(1):45-60.

195. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology. 1988;38(8):1285-91.
196. Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. J Neural Transm Suppl. 2000(58):143-51.
197. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest. 2009;119(1):182-92.
198. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nat Genet. 2010;42(9):781-5.
199. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet. 2008;40(8):955-62.
200. Witoelar A, Jansen IE, Wang Y, Desikan RS, Gibbs JR, Blauwendraat C, et al. Genome-wide Pleiotropy Between Parkinson Disease and Autoimmune Diseases. JAMA Neurol. 2017;74(7):780-92.
201. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. Nat Rev Immunol. 2022;22(11):657-73.
202. Osellame LD, Rahim AA, Hargreaves IP, Gegg ME, Richard-Londt A, Brandner S, et al. Mitochondria and quality control defects in a mouse model of Gaucher disease--links to Parkinson's disease. Cell Metab. 2013;17(6):941-53.
203. Demers-Lamarche J, Guillebaud G, Tlili M, Todkar K, Belanger N, Grondin M, et al. Loss of Mitochondrial Function Impairs Lysosomes. J Biol Chem. 2016;291(19):10263-76.
204. Gan L, Cookson MR, Petrucelli L, La Spada AR. Converging pathways in neurodegeneration, from genetics to mechanisms. Nat Neurosci. 2018;21(10):1300-9.
205. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry. 1988;51(6):745-52.
206. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord. 2007;22(12):1689-707; quiz 837.
207. Marek K, Chowdhury S, Siderowf A, Lasch S, Coffey CS, Caspell-Garcia C, et al. The Parkinson's progression markers initiative (PPMI) - establishing a PD biomarker cohort. Ann Clin Transl Neurol. 2018;5(12):1460-77.

208. Rosenthal LS, Drake D, Alcalay RN, Babcock D, Bowman FD, Chen-Plotkin A, et al. The NINDS Parkinson's disease biomarkers program. Mov Disord. 2016;31(6):915-23.
209. Shepherd CE, Alvendia H, Halliday GM. Brain Banking for Research into Neurodegenerative Disorders and Ageing. Neurosci Bull. 2019;35(2):283-8.
210. Tsuboi Y, Dickson DW. Dementia with Lewy bodies and Parkinson's disease with dementia: are they different? Parkinsonism Relat Disord. 2005;11 Suppl 1:S47-51.
211. Irwin DJ, Hurtig HI. The Contribution of Tau, Amyloid-Beta and Alpha-Synuclein Pathology to Dementia in Lewy Body Disorders. J Alzheimers Dis Parkinsonism. 2018;8(4).
212. Guerreiro R, Escott-Price V, Darwent L, Parkkinen L, Ansorge O, Hernandez DG, et al. Genome-wide analysis of genetic correlation in dementia with Lewy bodies, Parkinson's and Alzheimer's diseases. Neurobiol Aging. 2016;38:214 e7- e10.
213. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695-9.
214. Chou KL, Amick MM, Brandt J, Camicioli R, Frei K, Gitelman D, et al. A recommended scale for cognitive screening in clinical trials of Parkinson's disease. Mov Disord. 2010;25(15):2501-7.
215. Dalrymple-Alford JC, MacAskill MR, Nakas CT, Livingston L, Graham C, Crucian GP, et al. The MoCA: well-suited screen for cognitive impairment in Parkinson disease. Neurology. 2010;75(19):1717-25.
216. Alafuzoff I, Ince PG, Arzberger T, Al-Sarraj S, Bell J, Bodi I, et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol. 2009;117(6):635-52.
217. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012;123(1):1-11.
218. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology. 1991;41(4):479-86.
219. Thal DR, Rub U, Schultz C, Sassin I, Ghebremedhin E, Del Tredici K, et al. Sequence of Abeta-protein deposition in the human medial temporal lobe. J Neuropathol Exp Neurol. 2000;59(8):733-48.
220. Thal DR, Ronisz A, Tousseyn T, Rijal Upadhaya A, Balakrishnan K, Vandenberghe R, et al. Different aspects of Alzheimer's disease-related amyloid beta-peptide pathology and their

relationship to amyloid positron emission tomography imaging and dementia. Acta Neuropathol Commun. 2019;7(1):178.
221. Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. Lancet Neurol. 2017;16(1):55-65.
222. Geut H, Hepp DH, Foncke E, Berendse HW, Rozemuller JM, Huitinga I, et al. Neuropathological correlates of parkinsonian disorders in a large Dutch autopsy series. Acta Neuropathol Commun. 2020;8(1):39.
223. Kaivola K, Shah Z, Chia R, International LBDGC, Scholz SW. Genetic evaluation of dementia with Lewy bodies implicates distinct disease subgroups. Brain. 2022;145(5):1757-62.
224. van der Lee SJ, van Steenoven I, van de Beek M, Tesi N, Jansen IE, van Schoor NM, et al. Genetics Contributes to Concomitant Pathology and Clinical Presentation in Dementia with Lewy Bodies. J Alzheimers Dis. 2021;83(1):269-79.
225. Blauwendraat C, Faghri F, Pihlstrom L, Geiger JT, Elbaz A, Lesage S, et al. NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. Neurobiol Aging. 2017;57:247 e9- e13.
226. Guo Y, He J, Zhao S, Wu H, Zhong X, Sheng Q, et al. Illumina human exome genotyping array clustering and quality control. Nat Protoc. 2014;9(11):2643-62.
227. Infinium OmniExpress 24 v1.3 Product Files [Internet]. https://www.illumina.com/: Illumina; 2023 [cited 2023 Aug 28]. Available from: https://support.illumina.com/downloads/infinium-omniexpress-24-v1-3-product-files.html.
228. Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, et al. A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. Int J Methods Psychiatr Res. 2018;27(2):e1608.
229. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7.
230. Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;48(10):1284-7.
231. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet. 2016;48(10):1279-83.
232. Whole Genome Data [Internet]. https://amp-pd.org: The Accelerating Medicine Partnership in Parkinson's Disease (AMP-PD); n.d [cited 2023 Jul 7]. Available from: https://amp-pd.org/whole-genome-data.

233. Choi SW, Mak TS, O'Reilly PF. Tutorial: a guide to performing polygenic risk score analyses. Nat Protoc. 2020;15(9):2759-72.
234. Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nat Genet. 2019;51(3):404-13.
235. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat Genet. 2019;51(3):414-30.
236. Escott-Price V, Hardy J. Genome-wide association studies for Alzheimer's disease: bigger is not always better. Brain Commun. 2022;4(3):fcac125.
237. Choi SW, O'Reilly PF. PRSice-2: Polygenic Risk Score software for biobank-scale data. Gigascience. 2019;8(7).
238. Hall A, Bandres-Ciga S, Diez-Fairen M, Quinn JP, Billingsley KJ. Genetic Risk Profiling in Parkinson's Disease and Utilizing Genetics to Gain Insight into Disease-Related Biological Pathways. Int J Mol Sci. 2020;21(19).
239. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417-25.
240. Corces MR, Buenrostro JD, Wu B, Greenside PG, Chan SM, Koenig JL, et al. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nat Genet. 2016;48(10):1193-203.
241. Nott A, Holtman IR, Coufal NG, Schlachetzki JCM, Yu M, Hu R, et al. Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. Science. 2019;366(6469):1134-9.
242. Choi SW, Garcia-Gonzalez J, Ruan Y, Wu HM, Porras C, Johnson J, et al. PRSet: Pathway-based polygenic risk score analyses and software. PLoS Genet. 2023;19(2):e1010624.
243. Dehestani M, Liu H, Gasser T. Polygenic Risk Scores Contribute to Personalized Medicine of Parkinson's Disease. J Pers Med. 2021;11(10):1030.
244. Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature. 2016;538(7624):161-4.
245. Schumacher-Schuh AF, Bieger A, Okunoye O, Mok KY, Lim SY, Bardien S, et al. Underrepresented Populations in Parkinson's Genetics Research: Current Landscape and Future Directions. Mov Disord. 2022;37(8):1593-604.
246. Martin AR, Gignoux CR, Walters RK, Wojcik GL, Neale BM, Gravel S, et al. Human Demographic History Impacts Genetic Risk Prediction across Diverse Populations. Am J Hum Genet. 2017;100(4):635-49.

247. Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. Nat Genet. 2019;51(4):584-91.
248. Wray NR, Yang J, Hayes BJ, Price AL, Goddard ME, Visscher PM. Pitfalls of predicting complex traits from SNPs. Nat Rev Genet. 2013;14(7):507-15.
249. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2022.
250. Goodman S. A dirty dozen: twelve p-value misconceptions. Semin Hematol. 2008;45(3):135-40.
251. Neyman J, Pearson ES. The testing of statistical hypotheses in relation to probabilities a priori. Mathematical Proceedings of the Cambridge Philosophical Society. 1933;29(4):492-510.
252. Cox DR. Regression Models and Life-Tables. Journal of the Royal Statistical Society: Series B (Methodological). 1972;34(2):187-202.
253. Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. Int J Epidemiol. 1997;26(6):1323-33.
254. Harrell FE Jr. Regression Modeling Strategies [Internet]. https://hbiostat.org/: hbiostat; 2022 [cited 2022 Dec 2]. Available from: https://hbiostat.org/rmsc/ordinal.html#sec-ordinal-ordinality.
255. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med. 2000;45(1-2):23-41.
256. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32-5.
257. Kliouеva NM, Rademaker MC, Dexter DT, Al-Sarraj S, Seilhean D, Streichenberger N, et al. BrainNet Europe's Code of Conduct for brain banking. J Neural Transm (Vienna). 2015;122(7):937-40.
258. Retningslinjer for informasjon og tilbakemelding av resultater til forskningsdeltakeren [Internet]. Etikkom: De nasjonale forskningsetiske komiteene; 2016 [cited 2023 Aug 23]. Available from: https://www.etikkom.no/forskningsetiske-retningslinjer/Medisin-og-helse/retningslinjer-for-bruk-av-genetiske-undersokelser-av-mennesker-i-medisinsk-og-helsefaglig-forskning/3.-retningslinjer-for-informasjon-og-tilbakemelding-av-resultater-til-forskningsdeltakeren/.
259. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(8):1381-90.

260. Heckman MG, Kasanuki K, Diehl NN, Koga S, Soto A, Murray ME, et al. Parkinson's disease susceptibility variants and severity of Lewy body pathology. Parkinsonism Relat Disord. 2017;44:79-84.
261. Robakis D, Cortes E, Clark LN, Vonsattel JP, Virmani T, Alcalay RN, et al. The effect of MAPT haplotype on neocortical Lewy body pathology in Parkinson disease. J Neural Transm (Vienna). 2016;123(6):583-8.
262. Colom-Cadena M, Gelpi E, Marti MJ, Charif S, Dols-Icardo O, Blesa R, et al. MAPT H1 haplotype is associated with enhanced alpha-synuclein deposition in dementia with Lewy bodies. Neurobiol Aging. 2013;34(3):936-42.
263. Dickson DW, Heckman MG, Murray ME, Soto AI, Walton RL, Diehl NN, et al. APOE epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. Neurology. 2018;91(12):e1182-e95.
264. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, et al. APOE epsilon4 increases risk for dementia in pure synucleinopathies. JAMA Neurol. 2013;70(2):223-8.
265. Eusebi P, Giannandrea D, Biscetti L, Abraha I, Chiasserini D, Orso M, et al. Diagnostic utility of cerebrospinal fluid alpha-synuclein in Parkinson's disease: A systematic review and meta-analysis. Mov Disord. 2017;32(10):1389-400.
266. Ibanez L, Dube U, Saef B, Budde J, Black K, Medvedeva A, et al. Parkinson disease polygenic risk score is associated with Parkinson disease status and age at onset but not with alpha-synuclein cerebrospinal fluid levels. BMC Neurol. 2017;17(1):198.
267. Ibanez L, Bahena JA, Yang C, Dube U, Farias FHG, Budde JP, et al. Functional genomic analyses uncover APOE-mediated regulation of brain and cerebrospinal fluid beta-amyloid levels in Parkinson disease. Acta Neuropathol Commun. 2020;8(1):196.
268. Lee YG, Jeong SH, Park M, Kang SW, Baik K, Jeon S, et al. Effects of Alzheimer's genetic risk scores and CSF biomarkers in de novo Parkinson's Disease. NPJ Parkinsons Dis. 2022;8(1):57.
269. Goker-Alpan O, Giasson BI, Eblan MJ, Nguyen J, Hurtig HI, Lee VM, et al. Glucocerebrosidase mutations are an important risk factor for Lewy body disorders. Neurology. 2006;67(5):908-10.
270. Clark LN, Kartsaklis LA, Wolf Gilbert R, Dorado B, Ross BM, Kisselev S, et al. Association of glucocerebrosidase mutations with dementia with lewy bodies. Arch Neurol. 2009;66(5):578-83.
271. Nishioka K, Ross OA, Vilarino-Guell C, Cobb SA, Kachergus JM, Mann DM, et al. Glucocerebrosidase mutations in diffuse Lewy body disease. Parkinsonism Relat Disord. 2011;17(1):55-7.

272. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, et al. GBA mutations increase risk for Lewy body disease with and without Alzheimer disease pathology. Neurology. 2012;79(19):1944-50.
273. Gegg ME, Burke D, Heales SJ, Cooper JM, Hardy J, Wood NW, et al. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. Ann Neurol. 2012;72(3):455-63.
274. Chuang YH, Paul KC, Bronstein JM, Bordelon Y, Horvath S, Ritz B. Parkinson's disease is associated with DNA methylation levels in human blood and saliva. Genome Med. 2017;9(1):76.
275. Martinez-Vicente M, Sovak G, Cuervo AM. Protein degradation and aging. Exp Gerontol. 2005;40(8-9):622-33.
276. Madsen DA, Schmidt SI, Blaabjerg M, Meyer M. Interaction between Parkin and alpha-Synuclein in PARK2-Mediated Parkinson's Disease. Cells. 2021;10(2).
277. Poulopoulos M, Levy OA, Alcalay RN. The neuropathology of genetic Parkinson's disease. Mov Disord. 2012;27(7):831-42.
278. Kalia LV, Lang AE, Hazrati LN, Fujioka S, Wszolek ZK, Dickson DW, et al. Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. JAMA Neurol. 2015;72(1):100-5.
279. Parkkinen L, Pirttila T, Alafuzoff I. Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance. Acta Neuropathol. 2008;115(4):399-407.
280. Sekiya H, Tsuji A, Hashimoto Y, Takata M, Koga S, Nishida K, et al. Discrepancy between distribution of alpha-synuclein oligomers and Lewy-related pathology in Parkinson's disease. Acta Neuropathol Commun. 2022;10(1):133.
281. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 2011;108(10):4194-9.
282. Alam P, Bousset L, Melki R, Otzen DE. alpha-synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. J Neurochem. 2019;150(5):522-34.
283. Robinson JL, Richardson H, Xie SX, Suh E, Van Deerlin VM, Alfaro B, et al. The development and convergence of co-pathologies in Alzheimer’s disease. Brain. 2021;144(3):953-62.
284. Milber JM, Noorigian JV, Morley JF, Petrovitch H, White L, Ross GW, et al. Lewy pathology is not the first sign of degeneration in vulnerable neurons in Parkinson disease. Neurology. 2012;79(24):2307-14.
285. Tropea TF, Chen-Plotkin A. Are Parkinson's Disease Patients the Ideal Preclinical Population for Alzheimer's Disease Therapeutics? J Pers Med. 2021;11(9):834.

286. Alexander GC, Emerson S, Kesselheim AS. Evaluation of Aducanumab for Alzheimer Disease: Scientific Evidence and Regulatory Review Involving Efficacy, Safety, and Futility. JAMA. 2021;325(17):1717-8.
287. Kotzbauer PT, Cairns NJ, Campbell MC, Willis AW, Racette BA, Tabbal SD, et al. Pathologic accumulation of alpha-synuclein and Abeta in Parkinson disease patients with dementia. Arch Neurol. 2012;69(10):1326-31.
288. Lashley T, Holton JL, Gray E, Kirkham K, O'Sullivan SS, Hilbig A, et al. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol. 2008;115(4):417-25.
289. Robinson JL, Lee EB, Xie SX, Rennert L, Suh E, Bredenberg C, et al. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. Brain. 2018;141(7):2181-93.
290. Ferreira D, Przybelski SA, Lesnick TG, Lemstra AW, Londos E, Blanc F, et al. beta-Amyloid and tau biomarkers and clinical phenotype in dementia with Lewy bodies. Neurology. 2020;95(24):e3257-e68.
291. Nedelska Z, Schwarz CG, Lesnick TG, Boeve BF, Przybelski SA, Lowe VJ, et al. Association of Longitudinal beta-Amyloid Accumulation Determined by Positron Emission Tomography With Clinical and Cognitive Decline in Adults With Probable Lewy Body Dementia. JAMA Netw Open. 2019;2(12):e1916439.
292. Oh YS, Yoo SW, Lyoo CH, Yoo JY, Yoon H, Ha S, et al. The Association of beta-Amyloid with Cognition and Striatal Dopamine in Early, Non-Demented Parkinson's Disease. J Parkinsons Dis. 2021;11(2):605-13.
293. Siderowf A, Xie SX, Hurtig H, Weintraub D, Duda J, Chen-Plotkin A, et al. CSF amyloid beta 1-42 predicts cognitive decline in Parkinson disease. Neurology. 2010;75(12):1055-61.
294. Dai DL, Tropea TF, Robinson JL, Suh E, Hurtig H, Weintraub D, et al. ADNC-RS, a clinical-genetic risk score, predicts Alzheimer's pathology in autopsy-confirmed Parkinson's disease and Dementia with Lewy bodies. Acta Neuropathol. 2020;140(4):449-61.
295. Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, et al. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. PLoS Genet. 2014;10(9):e1004606.
296. Katsumata Y, Shade LM, Hohman TJ, Schneider JA, Bennett DA, Farfel JM, et al. Multiple gene variants linked to Alzheimer's-type clinical dementia via GWAS are also associated with non-Alzheimer's neuropathologic entities. Neurobiol Dis. 2022;174:105880.

297. Talyansky S, Guen YL, Kasireddy N, Belloy ME, Greicius MD. APOE - epsilon 4 and BIN1 increase risk of Alzheimer's disease pathology but not specifically of Lewy body pathology. medRxiv. 2023:2023.04.21.23288938.
298. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. Nat Rev Neurol. 2019;15(9):501-18.
299. Small SA, Simoes-Spassov S, Mayeux R, Petsko GA. Endosomal Traffic Jams Represent a Pathogenic Hub and Therapeutic Target in Alzheimer's Disease. Trends Neurosci. 2017;40(10):592-602.
300. Gao P, Ye L, Cheng H, Li H. The Mechanistic Role of Bridging Integrator 1 (BIN1) in Alzheimer's Disease. Cell Mol Neurobiol. 2021;41(7):1431-40.
301. Andersen OM, Rudolph IM, Willnow TE. Risk factor SORL1: from genetic association to functional validation in Alzheimer's disease. Acta Neuropathol. 2016;132(5):653-65.
302. Pankratz N, Byder L, Halter C, Rudolph A, Shults CW, Conneally PM, et al. Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. Mov Disord. 2006;21(1):45-9.
303. Monsell SE, Besser LM, Heller KB, Checkoway H, Litvan I, Kukull WA. Clinical and pathologic presentation in Parkinson's disease by apolipoprotein e4 allele status. Parkinsonism Relat Disord. 2014;20(5):503-7.
304. Huertas I, Jesus S, Garcia-Gomez FJ, Lojo JA, Bernal-Bernal I, Bonilla-Toribio M, et al. Genetic factors influencing frontostriatal dysfunction and the development of dementia in Parkinson's disease. PLoS One. 2017;12(4):e0175560.
305. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Hurtig HI, Van Deerlin VM, et al. APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease. JAMA Neurol. 2014;71(11):1405-12.
306. Williams-Gray CH, Goris A, Saiki M, Foltynie T, Compston DA, Sawcer SJ, et al. Apolipoprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's disease. J Neurol. 2009;256(3):493-8.
307. Sun R, Yang S, Zheng B, Liu J, Ma X. Apolipoprotein E Polymorphisms and Parkinson Disease With or Without Dementia: A Meta-Analysis Including 6453 Participants. J Geriatr Psychiatry Neurol. 2019;32(1):3-15.
308. Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. Lancet Neurol. 2017;16(1):66-75.

309. Paul KC, Rausch R, Creek MM, Sinsheimer JS, Bronstein JM, Bordelon Y, et al. APOE, MAPT, and COMT and Parkinson's Disease Susceptibility and Cognitive Symptom Progression. J Parkinsons Dis. 2016;6(2):349-59.
310. Gomperts SN, Locascio JJ, Rentz D, Santarlasci A, Marquie M, Johnson KA, et al. Amyloid is linked to cognitive decline in patients with Parkinson disease without dementia. Neurology. 2013;80(1):85-91.
311. Morley JF, Xie SX, Hurtig HI, Stern MB, Colcher A, Horn S, et al. Genetic influences on cognitive decline in Parkinson's disease. Mov Disord. 2012;27(4):512-8.
312. Tropea TF, Xie SX, Rick J, Chahine LM, Dahodwala N, Doshi J, et al. APOE, thought disorder, and SPARE-AD predict cognitive decline in established Parkinson's disease. Mov Disord. 2018;33(2):289-97.
313. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. Brain. 2009;132(Pt 11):2958-69.
314. Williams-Gray CH, Mason SL, Evans JR, Foltynie T, Brayne C, Robbins TW, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. J Neurol Neurosurg Psychiatry. 2013;84(11):1258-64.
315. Phongpreecha T, Cholerton B, Mata IF, Zabetian CP, Poston KL, Aghaeepour N, et al. Multivariate prediction of dementia in Parkinson's disease. NPJ Parkinsons Dis. 2020;6:20.
316. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis SJ, Brown J, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. Ann Neurol. 2007;62(2):145-53.
317. Alafuzoff I, Arzberger T, Al-Sarraj S, Bodi I, Bogdanovic N, Braak H, et al. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol. 2008;18(4):484-96.
318. Seto-Salvia N, Clarimon J, Pagonabarraga J, Pascual-Sedano B, Campolongo A, Combarros O, et al. Dementia risk in Parkinson disease: disentangling the role of MAPT haplotypes. Arch Neurol. 2011;68(3):359-64.
319. Mata IF, Johnson CO, Leverenz JB, Weintraub D, Trojanowski JQ, Van Deerlin VM, et al. Large-scale exploratory genetic analysis of cognitive impairment in Parkinson's disease. Neurobiol Aging. 2017;56:211 e1- e7.
320. Winder-Rhodes SE, Evans JR, Ban M, Mason SL, Williams-Gray CH, Foltynie T, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. Brain. 2013;136(Pt 2):392-9.

321. Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, et al. Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. Ann Neurol. 2016;80(5):674-85.
322. Lunde KA, Chung J, Dalen I, Pedersen KF, Linder J, Domellof ME, et al. Association of glucocerebrosidase polymorphisms and mutations with dementia in incident Parkinson's disease. Alzheimers Dement. 2018;14(10):1293-301.
323. Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, et al. A common inversion under selection in Europeans. Nat Genet. 2005;37(2):129-37.
324. Wider C, Ross OA, Nishioka K, Heckman MG, Vilarino-Guell C, Jasinska-Myga B, et al. An evaluation of the impact of MAPT, SNCA and APOE on the burden of Alzheimer's and Lewy body pathology. J Neurol Neurosurg Psychiatry. 2012;83(4):424-9.
325. Ishizawa T, Mattila P, Davies P, Wang D, Dickson DW. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. J Neuropathol Exp Neurol. 2003;62(4):389-97.
326. Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT, et al. Initiation and synergistic fibrillization of tau and alpha-synuclein. Science. 2003;300(5619):636-40.
327. Paul KC, Schulz J, Bronstein JM, Lill CM, Ritz BR. Association of Polygenic Risk Score With Cognitive Decline and Motor Progression in Parkinson Disease. JAMA Neurol. 2018;75(3):360-6.
328. Iaccarino L, Burnham SC, Dell'Agnello G, Dowsett SA, Epelbaum S. Diagnostic Biomarkers of Amyloid and Tau Pathology in Alzheimer's Disease: An Overview of Tests for Clinical Practice in the United States and Europe. J Prev Alzheimers Dis. 2023;10(3):426-42.
329. Blennow K, Shaw LM, Stomrud E, Mattsson N, Toledo JB, Buck K, et al. Predicting clinical decline and conversion to Alzheimer's disease or dementia using novel Elecsys Abeta(1-42), pTau and tTau CSF immunoassays. Sci Rep. 2019;9(1):19024.
330. Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-beta PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. Alzheimers Dement. 2018;14(11):1470-81.
331. Parnetti L, Farotti L, Eusebi P, Chiasserini D, De Carlo C, Giannandrea D, et al. Differential role of CSF alpha-synuclein species, tau, and Abeta42 in Parkinson's Disease. Front Aging Neurosci. 2014;6:53.
332. Alves G, Lange J, Blennow K, Zetterberg H, Andreasson U, Forland MG, et al. CSF Abeta42 predicts early-onset dementia in Parkinson disease. Neurology. 2014;82(20):1784-90.

333. Irwin DJ, Xie SX, Coughlin D, Nevler N, Akhtar RS, McMillan CT, et al. CSF tau and beta-amyloid predict cerebral synucleinopathy in autopsied Lewy body disorders. Neurology. 2018;90(12):e1038-e46.
334. Weinshel S, Irwin DJ, Zhang P, Weintraub D, Shaw LM, Siderowf A, et al. Appropriateness of Applying Cerebrospinal Fluid Biomarker Cutoffs from Alzheimer's Disease to Parkinson's Disease. J Parkinsons Dis. 2022;12(4):1155-67.
335. Shaw LM, Waligorska T, Fields L, Korecka M, Figurski M, Trojanowski JQ, et al. Derivation of cutoffs for the Elecsys((R)) amyloid beta (1-42) assay in Alzheimer's disease. Alzheimers Dement (Amst). 2018;10:698-705.
336. Baek MS, Lee MJ, Kim HK, Lyoo CH. Temporal trajectory of biofluid markers in Parkinson's disease. Sci Rep. 2021;11(1):14820.
337. Buongiorno M, Antonelli F, Compta Y, Fernandez Y, Pavia J, Lomena F, et al. Cross-Sectional and Longitudinal Cognitive Correlates of FDDNP PET and CSF Amyloid-beta and Tau in Parkinson's Disease1. J Alzheimers Dis. 2017;55(3):1261-72.
338. Spires-Jones TL, Attems J, Thal DR. Interactions of pathological proteins in neurodegenerative diseases. Acta Neuropathol. 2017;134(2):187-205.
339. Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurol. 2021;20(5):385-97.
340. Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87(5):539-47.
341. Chahine LM, Merchant K, Siderowf A, Sherer T, Tanner C, Marek K, et al. Proposal for a Biologic Staging System of Parkinson's Disease. J Parkinsons Dis. 2023;13(3):297-309.
342. Höglinger GUA, C.H.; Berg, D.; Klein, C.; Outeiro, T.F.; Poewe, W.; Postuma, R.; Stoessl, J.; Lang, A.E. . Towards a Biological Definition of Parkinson’s Disease. Preprints.orgPreprint.
343. Shahnawaz M, Tokuda T, Waragai M, Mendez N, Ishii R, Trenkwalder C, et al. Development of a Biochemical Diagnosis of Parkinson Disease by Detection of alpha-Synuclein Misfolded Aggregates in Cerebrospinal Fluid. JAMA Neurol. 2017;74(2):163-72.
344. Bellomo G, De Luca CMG, Paoletti FP, Gaetani L, Moda F, Parnetti L. alpha-Synuclein Seed Amplification Assays for Diagnosing Synucleinopathies: The Way Forward. Neurology. 2022;99(5):195-205.
345. Shahnawaz M, Mukherjee A, Pritzkow S, Mendez N, Rabadia P, Liu X, et al. Discriminating alpha-synuclein strains in Parkinson's disease and multiple system atrophy. Nature. 2020;578(7794):273-7.

346. Lister-James J, Pontecorvo MJ, Clark C, Joshi AD, Mintun MA, Zhang W, et al. Florbetapir f-18: a histopathologically validated Beta-amyloid positron emission tomography imaging agent. Semin Nucl Med. 2011;41(4):300-4.
347. Fleisher AS, Pontecorvo MJ, Devous MD, Sr., Lu M, Arora AK, Truocchio SP, et al. Positron Emission Tomography Imaging With [18F]flortaucipir and Postmortem Assessment of Alzheimer Disease Neuropathologic Changes. JAMA Neurol. 2020;77(7):829-39.
348. Korat S, Bidesi NSR, Bonanno F, Di Nanni A, Hoang ANN, Herfert K, et al. Alpha-Synuclein PET Tracer Development-An Overview about Current Efforts. Pharmaceuticals (Basel). 2021;14(9).
349. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.
350. Foo JN, Chew EGY, Chung SJ, Peng R, Blauwendraat C, Nalls MA, et al. Identification of Risk Loci for Parkinson Disease in Asians and Comparison of Risk Between Asians and Europeans: A Genome-Wide Association Study. JAMA Neurol. 2020;77(6):746-54.
351. Foo JN, Tan LC, Irwan ID, Au WL, Low HQ, Prakash KM, et al. Genome-wide association study of Parkinson's disease in East Asians. Hum Mol Genet. 2017;26(1):226-32.
352. Pan H, Liu Z, Ma J, Li Y, Zhao Y, Zhou X, et al. Genome-wide association study using whole-genome sequencing identifies risk loci for Parkinson's disease in Chinese population. NPJ Parkinsons Dis. 2023;9(1):22.
353. Sirugo G, Williams SM, Tishkoff SA. The Missing Diversity in Human Genetic Studies. Cell. 2019;177(1):26-31.
354. Global Parkinson's Genetics Program. GP2: The Global Parkinson's Genetics Program. Mov Disord. 2021;36(4):842-51.
355. Chen-Plotkin AS, Albin R, Alcalay R, Babcock D, Bajaj V, Bowman D, et al. Finding useful biomarkers for Parkinson's disease. Sci Transl Med. 2018;10(454).
356. Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. Lancet Neurol. 2021;20(7):559-72.
357. Leonard H, Blauwendraat C, Krohn L, Faghri F, Iwaki H, Ferguson G, et al. Genetic variability and potential effects on clinical trial outcomes: perspectives in Parkinson's disease. J Med Genet. 2020;57(5):331-8.
358. Espay AJ, Kalia LV, Gan-Or Z, Williams-Gray CH, Bedard PL, Rowe SM, et al. Disease modification and biomarker development in Parkinson disease: Revision or reconstruction? Neurology. 2020;94(11):481-94.

I

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 05 February 2021
doi: 10.3389/fneur.2021.631145

APOE and *MAPT* Are Associated With Dementia in Neuropathologically Confirmed Parkinson's Disease

*Jon-Anders Tunold[1,2], Hanneke Geut[3], J. M. Annemieke Rozemuller[4], Sandra Pilar Henriksen[1], Mathias Toft[1,2], Wilma D. J. van de Berg[3] and Lasse Pihlstrøm[1]**

[1] Department of Neurology, Oslo University Hospital, Oslo, Norway, [2] Institute of Clinical Medicine, University of Oslo, Oslo, Norway, [3] Section Clinical Neuroanatomy and Biobanking, Department of Anatomy and Neurosciences, Amsterdam UMC, Location Vrije Universiteit Amsterdam, Amsterdam Neuroscience, Amsterdam, Netherlands, [4] Department of Pathology, Amsterdam UMC, Location Vrije Universiteit Amsterdam, Amsterdam Neuroscience, Amsterdam, Netherlands

Introduction: Cognitive decline and dementia are common and debilitating non-motor phenotypic features of Parkinson's disease with a variable severity and time of onset. Common genetic variation of the Apolipoprotein E (*APOE*) and micro-tubule associated protein tau (*MAPT*) loci have been linked to cognitive decline and dementia in Parkinson's disease, although studies have yielded mixed results. To further elucidate the influence of *APOE* and *MAPT* variability on dementia in Parkinson's disease, we genotyped postmortem brain tissue samples of clinically and pathologically well-characterized Parkinson's donors and performed a survival analysis of time to dementia.

Methods: We included a total of 152 neuropathologically confirmed Parkinson's disease donors with or without clinical dementia during life. We genotyped known risk variants tagging the *APOE* ε4 allele and *MAPT* H1/H2 inversion haplotype. Cox proportional hazards regression analyses adjusted for age at onset, sex and genetic principal components were performed to assess the association between the genetic variants and time from motor onset to onset of dementia.

Results: We found that both the *APOE* ε4 allele (HR 1.82, 95 % CI 1.16–2.83, $p = 0.009$) and *MAPT* H1-haplotype (HR 1.71, 95 % CI 1.06–2.78, $p = 0.03$) were associated with earlier development of dementia in patients with Parkinson's disease.

Conclusion: Our results provide further support for the importance of *APOE* ε4 and *MAPT* H1-haplotype in the etiology of Parkinson's disease dementia, with potential future relevance for risk stratification and patient selection for clinical trials of therapies targeting cognitive decline in Parkinson's disease.

Keywords: parkinson's disease, dementia, neuropathology, genetics, association study, APOE, MAPT

OPEN ACCESS

Edited by:
Ignacio Mata,
Cleveland Clinic, United States

Reviewed by:
Oriol Dols-Icardo,
Sant Pau Institute for Biomedical Research, Spain
Jordi Clarimon,
Sant Pau Institute for Biomedical Research, Spain

***Correspondence:**
Lasse Pihlstrøm
lasse.pihlstrom@medisin.uio.no

Specialty section:
This article was submitted to Neurogenetics, a section of the journal Frontiers in Neurology

Received: *19 November 2020*
Accepted: *11 January 2021*
Published: *05 February 2021*

Citation:
Tunold J-A, Geut H, Rozemuller JMA, Henriksen SP, Toft M, van de Berg WDJ and Pihlstrøm L (2021) APOE and MAPT Are Associated With Dementia in Neuropathologically Confirmed Parkinson's Disease. Front. Neurol. 12:631145. doi: 10.3389/fneur.2021.631145

INTRODUCTION

Parkinson's disease (PD) is a heterogenous disorder in terms of clinical presentation and rate of progression. Dementia is one of the most debilitating non-motor manifestations of the disease, with broad implications for both patients and caregivers (1–3). Longitudinal studies have shown that most patients ultimately develop Parkinson's disease dementia (PDD) if they survive long

enough, although the time of onset is highly variable (4, 5). Cognitive disability is not only a feature of advanced disease, as 36% of patients meet criteria for mild cognitive impairment already at clinical diagnosis (6) and 17% of patients develop dementia within five years from disease onset (7). Identification of biomarkers, including common genetic variants predicting early cognitive decline and dementia, could provide important insights into the biological and molecular underpinnings of PDD, benefit recruitment to clinical trials and identify potential targets for novel therapeutics.

Genome-wide association studies (GWAS) have identified genetic susceptibility loci for sporadic PD, with the latest meta-analysis bringing the number up to 90 risk signals across 78 loci (8). Genetic variability may not only affect the risk of developing PD, but also influence the clinical course of the disease. Several genetic loci have been hypothesized as risk factors for dementia in sporadic PD, among them *APOE* and *MAPT,* showing partly conflicting results in previously published reports (9).

Coding variation in *APOE* on chromosome 19 gives rise to three common alleles: ε2, ε3, and ε4. The *APOE* ε4 allele is a strong and well-established genetic risk factor for Alzheimer's disease (AD) (10), and the top GWAS signal in dementia with Lewy bodies (DLB) (11). While *APOE* does not seem to alter the risk for PD in itself according to GWAS results, the ε4 allele has been studied as a potential risk factor for cognitive decline and development of dementia in PD patients, with several larger studies reporting a significant association (12, 13).

An inversion polymorphism on chromosome 17q21, containing *MAPT* and several other genes, gives rise to the H1 and H2 haplotypes in European populations (14). Single-nucleotide polymorphisms (SNPs) tagging the H1-haplotype have consistently been among the most significant association signals in GWAS of PD-risk (8, 15, 16). The *MAPT* gene encodes the tau protein that is found to aggregate in neurofibrillary tangles (NFT), a core neuropathological feature of AD, but also found in varying degrees in PD and PDD patients upon autopsy (17, 18). Interestingly, the *MAPT* H1-haplotype has also been reported to be associated with an accelerated rate of cognitive decline and earlier development of dementia in PD patients (7, 19, 20), yet larger studies have not been able to replicate this finding (12, 21).

Discrepant results across previous genetic association studies of cognitive outcomes in PD could potentially arise from differences in methodology, in particular with respect to inclusion criteria, duration of follow-up and outcome measures used to assess cognitive decline. A study based on brain bank samples can take advantage of gold standard diagnostics and clinical data that cover the patients' entire lifespan. In this study, we investigated the association of SNPs in the *APOE* and *MAPT* loci with time to dementia by retrospective survival analysis in neuropathologically defined PD brain donors.

METHODS

Subjects

All subjects were neuropathologically confirmed patients with PD or PDD from the Netherlands Brain Bank (NBB, www.brainbank.nl). All brains available from the NBB from 1989 to 2017 (n = 3,853) were considered for study inclusion according to the selection criteria. Written, informed consent for the use of clinical information and tissue samples for research purpose, was collected from the donors or their next of kin.

Standardized brain autopsies and neuropathological examinations were performed by experienced neuropathologists (AR and WB). Neuropathological assessment of Lewy Body (LB)-related α-synuclein pathology was done according to BrainNet Europe guidelines (22) and assessment of AD neuropathologic change was done according to National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines (23).

Clinical information was extracted from the medical records provided by the NBB. The diagnosis of PD was based on the combination of the clinical syndrome of PD [UK Parkinson's Disease Society Brain Bank criteria (24)], and moderate to severe loss of neurons in the substantia nigra in association with Lewy pathology in at least the brainstem with or without limbic and cortical brain regions (25). When dementia had been diagnosed during life, donors fulfilling these criteria were classified as PDD. A diagnosis of dementia was made during life by a neurologist or geriatrician, or retrospectively based on neuropsychological test results showing disturbances in at least two core cognitive domains (26) or Mini-Mental State Examination (MMSE) score <20. Distinction between DLB and PDD was made based on the 1-year rule, where dementia presenting before or within 1 year of parkinsonism onset was diagnosed as DLB, and not included in this study (27). Cases diagnosed as having both PD and AD were also excluded from the study.

Genotyping

DNA was extracted from brain tissue. Genotyping was carried out on the Infinium® NeuroChip Consortium Array (Illumina, San Diego, CA USA) (28). Quality control was carried out in PLINK version 1.9 (29). Samples passing standard quality control, including filtering of variants and individuals based on call rate (< 0.95), Hardy-Weinberg equillibrium ($p < 0.000001$), relatedness (pi-hat > 0.125), excess heterozygosity (> 4SD from mean), sex-check and ancestry assessed by principal component plots, were imputed using the Michigan Imputation Server (30). We selected rs1800547 to discriminate between the *MAPT* H1 and H2 haplotypes, and used rs429358 and rs7412 to define the *APOE* ε2, ε3, and ε4 alleles as previously described (31, 32).

The NeuroChip array was also used to screen for known pathogenic mutations in relevant Mendelian PD genes. Covering the majority of definitely and probably pathogenic variants in the autosomal dominant genes *SNCA*, *LRRK2*, and *VPS35*, we identified no mutation carriers (**Supplementary Table 1**).

Statistical Analysis

All statistical analyses were carried out in R (version 4.0.2; http://www.r-project.org). Differences in baseline demographics and clinical variables between patients with PD and PDD were assessed using *t*-tests for continuous variables and chi-square tests for categorical variables. Ordinal variables (neuropathological scores) were compared using the Wilcoxon Rank Sum Test, while associations between neuropathology

TABLE 1 | Clinical characteristics of cases with Parkinson's disease non-demented (PDnD) and Parkinson's disease dementia (PDD).

	PDnD *N* = 71	PDD *N* = 81	*p*
Sex, male (%)	43 (60.6)	57 (70.4)	0.271
Age at disease onset, mean (SD)	61.3 (13.0)	64.2 (9.5)	0.117
Age at dementia onset, mean (SD)	-	73.7 (7.0)	-
Disease duration, mean (SD)	15.5 (7.7)	13.6 (6.7)	0.102
Motor dementia interval, mean (SD)	-	9.4 (5.8)	-
Dementia duration, mean (SD)	-	4.1 (2.8)	-
Age at death, mean (SD)	77.0 (9.3)	77.8 (6.5)	0.515

SD: standard deviation. P value from t-tests for continuous variables and chi-square tests for categorical variables (sex).

TABLE 2 | Risk variant frequencies and results from Cox proportional hazards regression models with age at onset, sex, and genetic principal components as covariates.

Variant	Frequency	HR	95% CI for HR	*p*
APOE ε4	PDnD: 0.11 PDD: 0.14	1.82	1.16–2.83	0.009*
MAPT H1/H1	PDnD: 0.68 PDD: 0.77	1.71	1.06–2.78	0.03*

APOE, Apoliporotein E; HR, hazard ratio; CI, confidence interval; MAPT, microtubule-associated protein tau.
**P value from the Wald test.*

and genotypes were measured by odds ratios using ordinal logistic regression adjusting for age at death and sex. For the survival analysis we used the R package "survival." Cox proportional hazards regression models were employed to assess the relationship between genotype and dementia onset. The event variable was presence of dementia. As time variable we used disease duration at dementia onset for PDD and disease duration at death for PD. Separate analyses were carried out for each risk locus, with sex, age at motor symptom onset and the first five genetic principal components as covariates. We estimated hazard ratio (HR) and the 95% confidence interval (CI). P values for each covariate were obtained from the Wald test. The results were visualized as Cox regression-adjusted curves using the R package "survminer." A combined plotting and testing approach was employed to check the proportional hazards assumptions. A $p < 0.05$ was used as significance threshold in this study.

RESULTS

One hundred sixty five donors (PD $n = 79$ and PDD $n = 86$) were identified. A total of 13 cases were excluded for missing clinical, neuropathological or genotype data, or failing quality control. A total of 152 cases (PD $n = 71$ and PDD $n = 81$) meeting clinical and neuropathological criteria were included in the final analysis. The demographic and clinical characteristics are displayed in **Table 1**. There were no significant differences in sex distribution, age at disease onset, disease duration or age at death between PD and PDD patients.

Braak α-synuclein stage ($p = 0.01$), Thal amyloid-β (Aβ) phase ($p = 0.001$), Braak NFT stage ($p = 0.003$) and CERAD neuritic plaque score ($p < 0.001$) were all higher in PDD compared to PD patients (**Figure 1** and **Supplementary Table 2**). Applying the NIA-AA criteria, intermediate or high AD co-pathology was present in 7% (5 of 67) of PD patients and 14% (11 of 80) of PDD patients. *APOE* ε4 was significantly associated with Thal Aβ phase (OR 4.85, $p < 0.001$) and CERAD neuritic plaque score (OR 4.97, $p < 0.001$), but not Braak NFT or Braak α-synuclein stage (**Supplementary Table 3**). The *MAPT* H1-haplotype was not significantly associated with any of the neuropathological scores.

In the Cox proportional hazards model the *APOE* ε4 allele was significantly associated with a shorter time between PD onset and diagnosis of PDD (HR per ε4 allele 1.82, 95 % CI 1.16–2.83, $p = 0.009$, **Table 2** and **Figure 2A**). When Thal Aβ phase or CERAD neuritic plaque score were added as covariates, the association with time to dementia was no longer significant ($p = 0.23$ and $p = 0.11$, respectively). The *MAPT* H1-haplotype was also significantly associated with a shorter time to dementia (HR per H1 haplotype 1.71, 95% CI 1.06–2.78, $p = 0.03$, **Table 2** and **Figure 2B**). Later age at onset was significantly associated with shorter time to dementia in both models (HR 1.09, 95% CI 1.06–1.12, $p < 0.001$).

DISCUSSION

In this study we explored the genetic effects of *MAPT* and *APOE* on onset of dementia in PD in a neuropathologically characterized cohort. With the advantages of definite diagnosis and clinical data from the patients' entire lifespan, we found that even in a small sample, both the *APOE* ε4 allele and the *MAPT* H1-haplotype were significantly associated with an accelerated onset of dementia in PD patients.

Several studies have examined the effects of *APOE* ε4 on cognitive decline and dementia in PD. Many of these have had cross-sectional design, and while some have demonstrated an association with *APOE* ε4 and lower cognitive performance (21), others have failed to do so (33). Consistent with our results, a previous study of PD patients demonstrated earlier development of dementia among *APOE* ε4-carriers (HR 1.90, 95% CI 1.05–3.44) (34). In line with our data, two recent meta-analyses reported an increased risk of dementia in PD patients who carried the *APOE* ε4 allele, although regional differences in effect size were noted (35, 36). Longitudinal studies have found associations with *APOE* ε4 and a more rapid cognitive decline measured on both screening instruments for global cognition (37, 38) and battery-style assessment of mental status (12, 39). In a recent GWAS on PD progression using longitudinal data from three large cohorts, the top hit for cognitive progression was rs429358 tagging *APOE* ε4 (40). In contrast, variants in the *APOE*-gene were not associated with cognitive decline or dementia at 3.5, 5, or 10 year follow-up in the CamPaIGN study, a UK incident cohort of PD patients (7, 20), or with shorter time to dementia

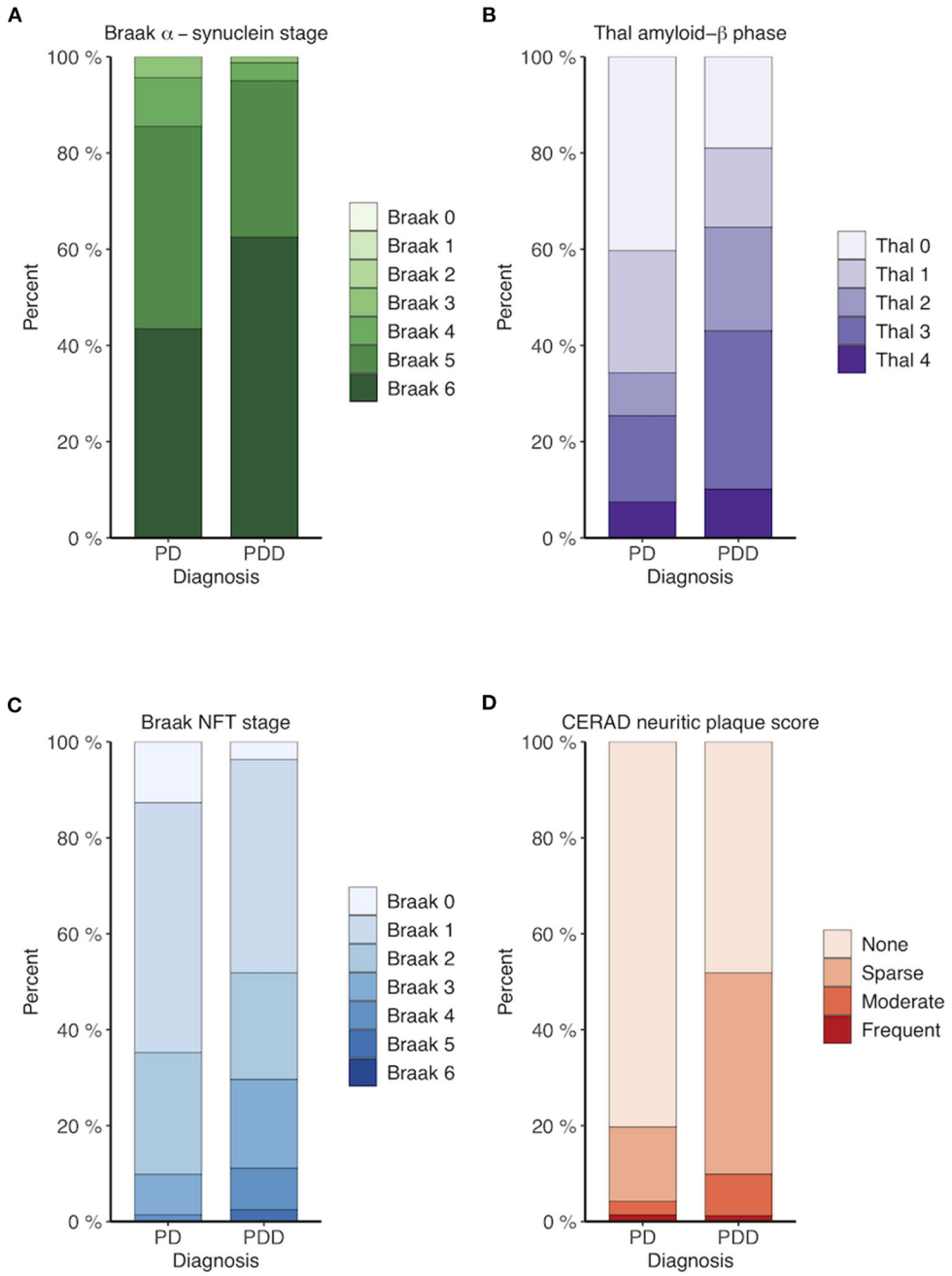

FIGURE 1 | Neuropathological scores for PD and PDD patients. **(A)** Braak α-synuclein stage. **(B)** Thal amyloid-β phase. **(C)** Braak neurofibrillary tangle (NFT) stage. **(D)** CERAD neuritic plaque score. PDD patients display more advanced LB, Aβ, and tau pathology compared to PD patients.

in another longitudinal study (41). While longitudinal designs represent a gold standard for tracking disease progression, they may be hampered by small sample size, short follow-up time and loss to follow-up. Taken together, the weight of evidence favors an effect of *APOE* on cognitive decline and dementia in PD, further supported by our results.

We also found a significant association between *MAPT* H1 and time to dementia in PD. This locus is less established than *APOE* in the previous literature on genetic risk factors of cognitive progression. The CamPaIGN study was the first to report an association between the *MAPT* H1/H1 genotype and cognitive decline in PD (19). The results were confirmed in the subsequent 5- and 10-year follow-up studies, supporting the *MAPT* H1/H1 genotype as predictive of dementia (7, 20). The association between *MAPT* genotype and PDD has later been replicated (42), while other studies have failed to do so

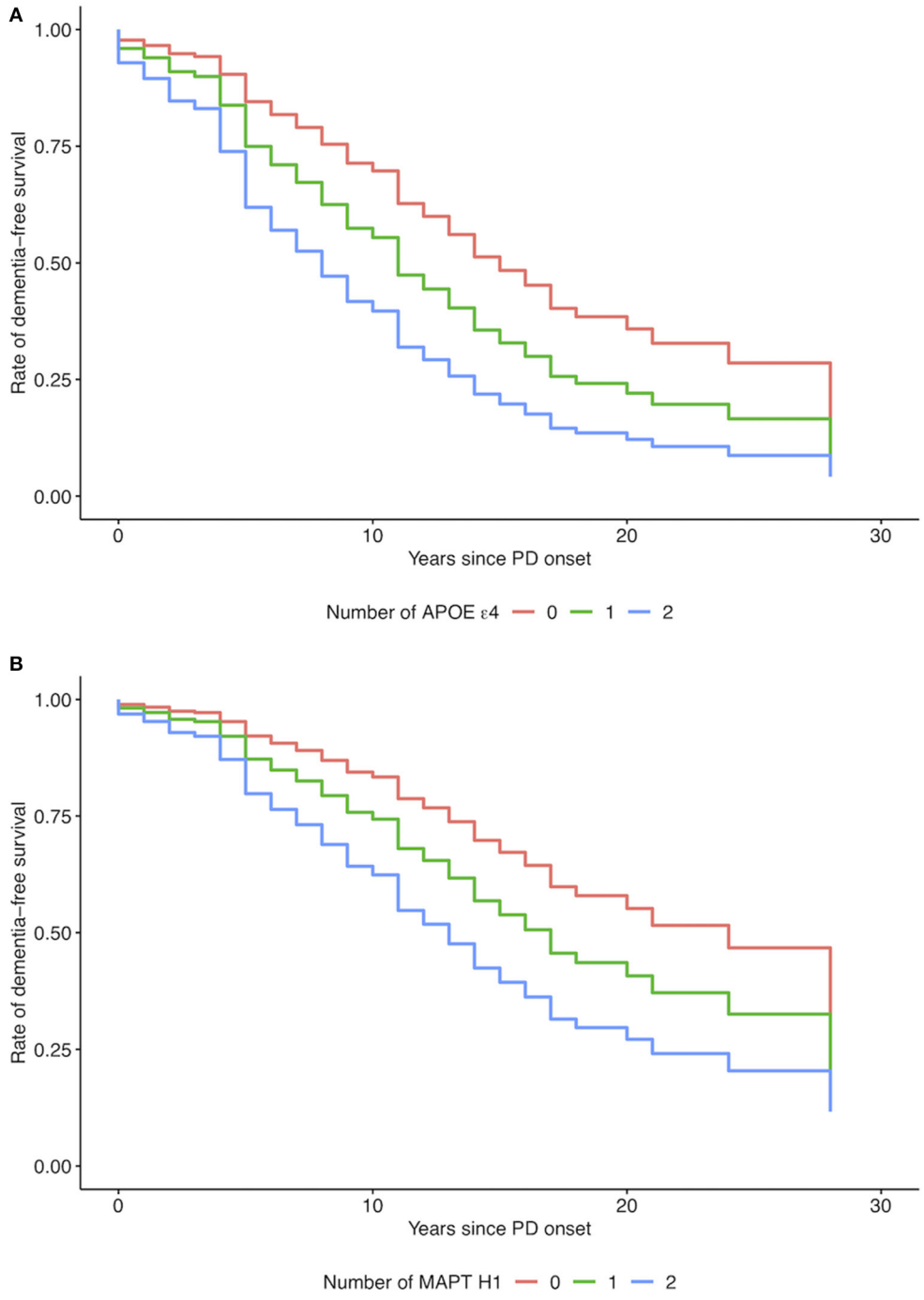

FIGURE 2 | Adjusted survival curves for Cox proportional hazards model. **(A)** Apolipoprotein E (*APOE*) ε4 (0 = negative, 1 = ε4 heterozygous, 2 = ε4 homozygous), and **(B)** Microtubule-associated protein tau (*MAPT*) H1-haplotype (0 = negative, 1 = heterozygous, 2 = homozygous).

(12, 21, 38). Contrary to our results, no association between *MAPT* H1/H1 genotype and dementia onset was found in a previous survival analysis of 298 PD patients where 59 progressed to dementia (34). A prospective investigation of 212 patients noted associations between *MAPT* H1 and specific cognitive outcome measures, but not with the overall rate of cognitive decline (12). The authors of this study hypothesized that the significant signal reported in the CamPaIGN study could represent an effect specific to early dementia development, as the CamPaIGN patients were included at diagnosis and assessed for progression to PDD at 3 years. Our data do not support this explanation of previously discrepant results, as the mean disease

duration at dementia onset in the PDD group was 9–10 years in our study.

The underlying mechanisms linking *APOE* and *MAPT* variants to dementia are unclear, however neuropathological studies suggests that protein aggregation is pivotal in this association. In our study *APOE* ε4 was significantly associated with both Thal Aβ phases and CERAD neuritic plaque scores, supporting that *APOE* ε4 exerts its genetic risk on dementia primarily through Aβ neuropathology. The *MAPT* H1 haplotype was not associated with any neuropathological scores in our study. Concomitant AD pathology (Aβ plaques and NFT) is found in variable amounts upon autopsy in PD and PDD brains, and is more prevalent in PDD compared to PD (17, 43, 44). This is indeed true for our cases, as neuropathological examination revealed significantly more advanced Thal Aβ phases, Braak NFT stages and CERAD neuritic plaque scores in PDD compared to PD samples.

Several lines of evidence support the role of cortical LB pathology as the major pathological driver of dementia in PD (17, 45), and in our study PDD donors had significantly more advanced Braak α-synuclein stages than PD donors. While it seems likely that *APOE* ε4 mediates dementia through an Aβ-dependent pathway, previous studies have also reported an effect of *APOE* ε4 on cognitive outcome and severity of cortical LB pathology in patients with low concomitant AD-pathology (46, 47). Corroborating these findings, two recent experimental studies have shown evidence that *APOE* ε4 may promote LB pathology independent of Aβ pathology (48, 49). In our results, however, the association with dementia was dependent on Aβ, as the signal was no longer significant when adjusting for Thal Aβ phase or CERAD neuritic plaque score.

While the presence of tau pathology has been correlated with reduced time to dementia (50), some evidence also supports that the *MAPT* H1-haplotype may influence the cortical LB burden (51), suggesting *MAPT* also may promote dementia in more than one way. This idea was not supported by our data, but we note that the size of our study provided limited statistical power to disentangle potentially complex correlations between genotype and various neuropathologies. We also acknowledge that although the H1 inversion haplotype on chromosome 17 is commonly named after *MAPT*, it contains a number of other genes, and the mechanism driving the association signal for PD risk has yet to be unequivocally established. Recent evidence suggest that rather than *MAPT*, the disease-relevant gene could be the neighboring *KANSL1*, which is involved in autophagy regulation (52).

The clinical diagnosis of PD can be challenging, with a diagnostic accuracy of 80.6% when pathological examination is used as the gold standard (53). The strength of this study lies in the neuropathological confirmation of diagnosis and the retrospective overview of the clinical disease course from the patients' entire lifespan. Some limitations of our study should be noted. First, clinical information was obtained by retrospective review of medical records posing a risk for information bias, in particular regarding approximation of timing of events. However, the timing of motor symptom onset and dementia onset observed in this study harmonize well with previous reports (17, 54). Second, we acknowledge that lack of extensive neuropsychological evaluation is a limitation. In theory, death and dementia may be competing events and potentially bias the estimated effect of genotypes on dementia development. *APOE* ε4 has been associated with decreased longevity, but we observed similar age at death in PD and PDD, and any theoretical bias from this effect would skew results in the opposite direction of our findings (55). Further corroboration of the genetic associations reported here is warranted, preferably in longitudinal cohorts. Third, given the limited sample size and statistical power of our study, we narrowly selected only two candidate loci among several previously reported as associated with cognition in PD. A broader perspective on the genetic architecture of PDD would have to consider the contribution from loci such as *SNCA*, *GBA*, *COMT* and potentially others (9), and ideally also the possibility of synergistic interactions between these.

In conclusion, our study adds to the growing evidence supporting the role for not only *APOE* ε4 but also the *MAPT* H1 haplotype in development of dementia in PD. Detecting significant associations in a small, but well-characterized neuropathological sample, we anticipate that larger genetic association studies of neuropathological phenotypes will be a fruitful strategy to further disentangle molecular mechanisms in neurodegenerative disorders. Ultimately, a better understanding of genotype-phenotype correlations may facilitate precision medicine in PD, improving risk prediction and patient stratification for novel targeted therapies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committees for Medical and Health Research Ethics, Norway. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-AT performed statistical analyses and drafted the manuscript. HG and JR contributed clinical and neuropathological data. SH contributed to genotyping. MT contributed to study design and organized the study. WB contributed clinical and neuropathological data, contributed to study design and organized the study. LP designed and organized the study and contributed to genotyping, data analyses and drafting of the manuscript. All authors took part in critical revision of the manuscript and approved the submitted version.

FUNDING

This work was funded by grants from the South-Eastern Health Authority, Norway (2019065), the Norwegian Health Association (4999) and the Michael J. Fox Foundation (14227).

ACKNOWLEDGMENTS

The authors thank Michiel Kooreman for technical coordination of NBB brain tissue. In addition, we thank the autopsy team of Dept. Pathology, Amsterdam UMC, location VUmc for assistance with autopsies and Yvonne de Gier and Angela Ingrassia, Amsterdam UMC, location VUmc, for processing the tissue samples for genetic analysis. We also thank Chiara Cappelletti for assistance with DNA sample handling.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.631145/full#supplementary-material

REFERENCES

1. Winter Y, von Campenhausen S, Arend M, Longo K, Boetzel K, Eggert K, et al. Health-related quality of life and its determinants in Parkinson's disease: results of an Italian cohort study. *Parkinsonism Relat Disord*. (2011) 17:265–9. doi: 10.1016/j.parkreldis.2011.01.003
2. Vossius C, Larsen JP, Janvin C, Aarsland D. The economic impact of cognitive impairment in Parkinson's disease. *Mov Disord*. (2011) 26:1541–4. doi: 10.1002/mds.23661
3. Fletcher P, Leake A, Marion MH. Patients with Parkinson's disease dementia stay in the hospital twice as long as those without dementia. *Mov Disord*. (2011) 26:919. doi: 10.1002/mds.23573
4. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov Disord*. (2008) 23:837–44. doi: 10.1002/mds.21956
5. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol*. (2003) 60:387–92. doi: 10.1001/archneur.60.3.387
6. Foltynie T, Brayne CE, Robbins TW, Barker RA. The cognitive ability of an incident cohort of Parkinson's patients in the UK. The CamPaIGN study. *Brain*. (2004) 127:550–60. doi: 10.1093/brain/awh067
7. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain*. (2009) 132:2958–69. doi: 10.1093/brain/awp245
8. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol*. (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
9. Fagan ES, Pihlstrom L. Genetic risk factors for cognitive decline in Parkinson's disease: a review of the literature. *Eur J Neurol*. (2017) 24:561–20. doi: 10.1111/ene.13258
10. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. (1997) 278:1349–56. doi: 10.1001/jama.278.16.1349
11. Guerreiro R, Ross OA, Kun-Rodrigues C, Hernandez DG, Orme T, Eicher JD, et al. Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study. *Lancet Neurol*. (2018) 17:64–74. doi: 10.1016/S1474-4422(17)30400-3
12. Morley JF, Xie SX, Hurtig HI, Stern MB, Colcher A, Horn S, et al. Genetic influences on cognitive decline in Parkinson's disease. *Mov Disord*. (2012) 27:512–8. doi: 10.1002/mds.24946
13. Pankratz N, Byder L, Halter C, Rudolph A, Shults CW, Conneally PM, et al. Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. *Mov Disord*. (2006) 21:45–9. doi: 10.1002/mds.20663
14. Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, et al. A common inversion under selection in Europeans. *Nat Genet*. (2005) 37:129–37. doi: 10.1038/ng1508
15. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. (2014) 46:989–93. doi: 10.1038/ng.3043
16. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. (2009) 41:1308–12. doi: 10.1038/ng.487
17. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol*. (2012) 72:587–98. doi: 10.1002/ana.23659
18. Geut H, Hepp DH, Foncke E, Berendse HW, Rozemuller JM, Huitinga I, et al. Neuropathological correlates of parkinsonian disorders in a large Dutch autopsy series. *Acta Neuropathol Commun*. (2020) 8:39. doi: 10.1186/s40478-020-00914-9
19. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis SJ, Brown J, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann Neurol*. (2007) 62:145–53. doi: 10.1002/ana.21192
20. Williams-Gray CH, Mason SL, Evans JR, Foltynie T, Brayne C, Robbins TW, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J Neurol Neurosurg Psychiatry*. (2013) 84:1258–64. doi: 10.1136/jnnp-2013-305277
21. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Hurtig HI, Van Deerlin VM, et al. APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease. *JAMA Neurol*. (2014) 71:1405–12. doi: 10.1001/jamaneurol.2014.1455
22. Alafuzoff I, Ince PG, Arzberger T, Al-Sarraj S, Bell J, Bodi I, et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. *Acta Neuropathol*. (2009) 117:635–52. doi: 10.1007/s00401-009-0523-2
23. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. (2012) 123:1–11. doi: 10.1007/s00401-011-0910-3
24. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. (1992) 55:181–4. doi: 10.1136/jnnp.55.3.181
25. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol*. (2009) 8:1150–7. doi: 10.1016/S1474-4422(09)70238-8
26. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord*. (2007) 22:1689–707; quiz 837. doi: 10.1002/mds.21507
27. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology*. (2017) 89:88–100. doi: 10.1212/WNL.0000000000004058
28. Blauwendraat C, Faghri F, Pihlstrom L, Geiger JT, Elbaz A, Lesage S, et al. NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. *Neurobiol Aging*. (2017) 57:247 e9–13. doi: 10.1016/j.neurobiolaging.2017.05.009

29. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. (2015) 4:7. doi: 10.1186/s13742-015-0047-8
30. Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. *Nat Genet*. (2016) 48:1284–7. doi: 10.1038/ng.3656
31. Mata IF, Yearout D, Alvarez V, Coto E, de Mena L, Ribacoba R, et al. Replication of MAPT and SNCA, but not PARK16–18, as susceptibility genes for Parkinson's disease. *Mov Disord*. (2011) 26:819–23. doi: 10.1002/mds.23642
32. Williams-Gray CH, Goris A, Saiki M, Foltynie T, Compston DA, Sawcer SJ, et al. Apolipoprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's disease. *J Neurol*. (2009) 256:493–8. doi: 10.1007/s00415-009-0119-8
33. Federoff M, Jimenez-Rolando B, Nalls MA, Singleton AB. A large study reveals no association between APOE and Parkinson's disease. *Neurobiol Dis*. (2012) 46:389–92. doi: 10.1016/j.nbd.2012.02.002
34. Huertas I, Jesus S, Garcia-Gomez FJ, Lojo JA, Bernal-Bernal I, Bonilla-Toribio M, et al. Genetic factors influencing frontostriatal dysfunction and the development of dementia in Parkinson's disease. *PLoS ONE*. (2017) 12:e0175560. doi: 10.1371/journal.pone.0175560
35. Pang S, Li J, Zhang Y, Chen J. Meta-analysis of the relationship between the APOE gene and the onset of parkinson's disease dementia. *Parkinsons Dis*. (2018) 2018:9497147. doi: 10.1155/2018/9497147
36. Sun R, Yang S, Zheng B, Liu J, Ma X. Apolipoprotein E polymorphisms and parkinson disease with or without dementia: a meta-analysis including 6453 participants. *J Geriatr Psychiatry Neurol*. (2019) 32:3–15. doi: 10.1177/0891988718813675
37. Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. *Lancet Neurology*. (2017) 16:66–75. doi: 10.1016/S1474-4422(16)30328-3
38. Paul KC, Rausch R, Creek MM, Sinsheimer JS, Bronstein JM, Bordelon Y, et al. APOE, MAPT, and COMT and parkinson's disease susceptibility and cognitive symptom progression. *J Parkinsons Dis*. (2016) 6:349–59. doi: 10.3233/JPD-150762
39. Tropea TF, Xie SX, Rick J, Chahine LM, Dahodwala N, Doshi J, et al. APOE, thought disorder, and SPARE-AD predict cognitive decline in established Parkinson's disease. *Mov Disord*. (2018) 33:289–97. doi: 10.1002/mds.27204
40. Tan MMX, Lawton MA, Jabbari E, Reynolds RH, Iwaki H, Blauwendraat C, et al. Genome-wide association studies of cognitive and motor progression in parkinson's disease. *Mov Disord*. (2020). doi: 10.1002/mds.28342
41. Phongpreecha T, Cholerton B, Mata IF, Zabetian CP, Poston KL, Aghaeepour N, et al. Multivariate prediction of dementia in Parkinson's disease. *NPJ Parkinsons Dis*. (2020) 6:20. doi: 10.1038/s41531-020-00121-2
42. Seto-Salvia N, Clarimon J, Pagonabarraga J, Pascual-Sedano B, Campolongo A, Combarros O, et al. Dementia risk in Parkinson disease: disentangling the role of MAPT haplotypes. *Arch Neurol*. (2011) 68:359–64. doi: 10.1001/archneurol.2011.17
43. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain*. (2011) 134:1493–505. doi: 10.1093/brain/awr031
44. Sabbagh MN, Adler CH, Lahti TJ, Connor DJ, Vedders L, Peterson LK, et al. Parkinson disease with dementia: comparing patients with and without Alzheimer pathology. *Alzheimer Dis Assoc Disord*. (2009) 23:295–7. doi: 10.1097/WAD.0b013e31819c5ef4
45. Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. *Brain*. (2010) 133:1755–62. doi: 10.1093/brain/awq059
46. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, et al. APOE epsilon4 increases risk for dementia in pure synucleinopathies. *JAMA Neurol*. (2013) 70:223–8. doi: 10.1001/jamaneurol.2013.600
47. Dickson DW, Heckman MG, Murray ME, Soto AI, Walton RL, Diehl NN, et al. APOE epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. *Neurology*. (2018) 91:e1182–95. doi: 10.1212/WNL.0000000000006212
48. Zhao N, Attrebi ON, Ren Y, Qiao W, Sonustun B, Martens YA, et al. APOE4 exacerbates alpha-synuclein pathology and related toxicity independent of amyloid. *Sci Transl Med*. (2020) 12:eaay1809. doi: 10.1126/scitranslmed.aay1809
49. Davis AA, Inman CE, Wargel ZM, Dube U, Freeberg BM, Galluppi A, et al. APOE genotype regulates pathology and disease progression in synucleinopathy. *Sci Transl Med*. (2020) 12:eaay3069. doi: 10.1126/scitranslmed.aay3069
50. Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol*. (2017) 16:55–65. doi: 10.1016/S1474-4422(16)30291-5
51. Robakis D, Cortes E, Clark LN, Vonsattel JP, Virmani T, Alcalay RN, et al. The effect of MAPT haplotype on neocortical Lewy body pathology in Parkinson disease. *J Neural Transm*. (2016) 123:583–8. doi: 10.1007/s00702-016-1552-3
52. Soutar MPM, Melandri D, Annuario E, Welsh NJ, D'Sa K, Guelfi S, et al. *Regulation of Mitophagy by the NSL Complex Underlies Genetic Risk for Parkinson's Disease at Chr16q11.2 and on the MAPT H1 Allele*. BioRxiv (2020). Available online at: https://www.biorxiv.org/content/10.1101/2020.01.06.896241v1.full (accessed December 27, 2020). doi: 10.1101/2020.01.06.896241
53. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology*. (2016) 86:566–76. doi: 10.1212/WNL.0000000000002350
54. Prokopenko I, Miyakawa G, Zheng B, Heikkinen J, Petrova Quayle D, Udeh-Momoh C, et al. Alzheimer's disease pathology explains association between dementia with Lewy bodies and APOE-epsilon4/TOMM40 long poly-T repeat allele variants. *Alzheimers Dement*. (2019) 5:814–24. doi: 10.1016/j.trci.2019.08.005
55. Blauwendraat C, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrom L, et al. Parkinson's disease age at onset genome-wide association study: defining heritability, genetic loci, and alpha-synuclein mechanisms. *Mov Disord*. (2019) 34:866–75. doi: 10.1002/mds.27659

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

II

BRAIN
ORIGINAL ARTICLE

Lysosomal polygenic risk is associated with the severity of neuropathology in Lewy body disease

Jon-Anders Tunold,[1,2] Manuela M. X. Tan,[1] Shunsuke Koga,[3] Hanneke Geut,[4] Annemieke J. M. Rozemuller,[4,5,6] Rebecca Valentino,[3] Hiroaki Sekiya,[3] Nicholas B. Martin,[3] Michael G. Heckman,[7] Jose Bras,[8,9] Rita Guerreiro,[8,9] Dennis W. Dickson,[3] Mathias Toft,[1,2] Wilma D. J. van de Berg,[4,6] Owen A. Ross[3] and Lasse Pihlstrøm[1]

Intraneuronal accumulation of misfolded α-synuclein is the pathological hallmark of Parkinson's disease and dementia with Lewy bodies, often co-occurring with variable degrees of Alzheimer's disease related neuropathology. Genetic association studies have successfully identified common variants associated with disease risk and phenotypic traits in Lewy body disease, yet little is known about the genetic contribution to neuropathological heterogeneity. Using summary statistics from Parkinson's disease and Alzheimer's disease genome-wide association studies, we calculated polygenic risk scores and investigated the relationship with Lewy, amyloid-β and tau pathology. Associations were nominated in neuropathologically defined samples with Lewy body disease from the Netherlands Brain Bank ($n = 217$) and followed up in an independent sample series from the Mayo Clinic Brain Bank ($n = 394$). We also generated stratified polygenic risk scores based on single-nucleotide polymorphisms annotated to eight functional pathways or cell types previously implicated in Parkinson's disease and assessed for association with Lewy pathology in subgroups with and without significant Alzheimer's disease co-pathology.

In an ordinal logistic regression model, the Alzheimer's disease polygenic risk score was associated with concomitant amyloid-β and tau pathology in both cohorts. Moreover, both cohorts showed a significant association between lysosomal pathway polygenic risk and Lewy pathology, which was more consistent than the association with a general Parkinson's disease risk score and specific to the subset of samples without significant concomitant Alzheimer's disease related neuropathology.

Our findings provide proof of principle that the specific risk alleles a patient carries for Parkinson's and Alzheimer's disease also influence key aspects of the underlying neuropathology in Lewy body disease. The interrelations between genetic architecture and neuropathology are complex, as our results implicate lysosomal risk loci specifically in the subset of samples without Alzheimer's disease co-pathology. Our findings hold promise that genetic profiling may help predict the vulnerability to specific neuropathologies in Lewy body disease, with potential relevance for the further development of precision medicine in these disorders.

1 Department of Neurology, Oslo University Hospital, 0424 Oslo, Norway
2 Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, 0372 Oslo, Norway
3 Department of Neuroscience, Mayo Clinic, Jacksonville, FL 32224, USA
4 Department of Anatomy and Neurosciences, Amsterdam UMC, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands
5 Department of Pathology, Amsterdam UMC, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands

Received January 20, 2023. Revised May 08, 2023. Accepted May 10, 2023. Advance access publication May 29, 2023

6 Program Neurodegeneration, Amsterdam Neuroscience, 1081 HV Amsterdam, The Netherlands
7 Division of Clinical Trials and Biostatistics, Mayo Clinic, Jacksonville, FL 32224, USA
8 Department of Neurodegenerative Science, Van Andel Institute, Grand Rapids, MI 49503, USA
9 Division of Psychiatry and Behavioral Medicine, Michigan State University College of Human Medicine, Grand Rapids, MI 49503, USA

Correspondence to: Lasse Pihlstrøm
Department of Neurology, Oslo University Hospital, Sognsvannsveien 20, P.O. Box 4950 Nydalen, 0424 Oslo, Norway
E-mail: lasse.pihlstrom@medisin.uio.no

Keywords: Lewy body disease; Parkinson's disease; genetics; neuropathology; lysosomal pathway

Introduction

Lewy body disease (LBD) represents a continuum of closely related neurodegenerative diseases with overlapping clinical characteristics, genetic risk factors and neuropathological features. The most common manifestations of LBD are Parkinson's disease (PD) and dementia with Lewy bodies (DLB). A defining neuropathological hallmark of LBD is the deposition of α-synuclein (α-syn) rich intraneuronal inclusions called Lewy bodies and Lewy neurites, collectively referred to as Lewy pathology.[1] In addition to Lewy pathology, varying degrees of Alzheimer's disease (AD) co-pathology, including amyloid-β plaques and tau positive neurofibrillary tangles (NFT), are often present.[2] Elucidating biological mechanisms that underlie the heterogenous neuropathological substrates of LBD is crucial for understanding disease aetiology and progression, with the ultimate aim to discover novel therapeutic avenues for disease modification.

Genome-wide association studies (GWAS) have provided insights into the complex polygenic architecture of clinical LBD phenotypes, and successfully identified common genetic risk variants in PD[3] and to a lesser extent in DLB.[4,5] Each GWAS locus explains only a small proportion of disease susceptibility, yet the cumulative effect of many risk variants can be estimated as a polygenic risk score (PRS). A PRS is calculated as the weighted sum of the number of risk alleles an individual carries. In PD, PRSs have been applied successfully to a number of traits, including age at onset, disease status, motor progression and cognitive decline.[6-9] In DLB, the PRS has been linked to disease risk.[10]

The general PRS approach includes all independent single-nucleotide polymorphisms (SNPs) with associated *P*-values below a specified threshold in summary statistics from GWAS. However, stratified PRSs may be generated from subsets of SNPs that are annotated to specific pathways, thus helping to nominate mechanisms that contribute to disease development.[11-13] Pathway-specific PRS studies have provided further support for a number of biological pathways and mechanisms previously implicated in PD, including mitochondrial dysfunction, lysosomal mediated autophagy/lysosomal dysfunction, endocytic membrane trafficking, α-syn misfolding and neuroinflammation.[11,13,14]

A major challenge in understanding how genetic risk influences LBD relates to the clinical and neuropathological heterogeneity. While LBD is defined by the accumulation of Lewy bodies, comorbid AD pathology is common and found more frequently in DLB and PD with dementia than in non-demented PD.[2,15-18] The level of AD co-pathology shows association with the severity of Lewy pathology,[19] which makes it challenging to determine the causal relationships underlying genetic associations. Two recent publications have shown that risk variants in the β-glucocerebrosidase (*GBA*) gene are primarily associated with 'pure' DLB, while the *APOE* ϵ4 allele is a risk factor for DLB with AD co-pathology,[10,20] suggesting the existence of distinct genetic architectures within the LBD continuum.

To assess how common genetic risk variants associated with PD and AD influence the multifaceted neuropathologies of LBD, we generated PD- and AD-susceptibility PRSs as well as stratified PD-PRSs and explored their relationship to key neuropathological markers in two post-mortem cohorts, using the Netherlands Brain Bank ($n = 217$) for discovery and the Mayo Clinic Brain Bank ($n = 394$) for replication. Based on the hypothesis that distinct genetic profiles associate with Lewy pathology depending on the presence or absence of AD co-pathology, we divided the LBD samples into two subgroups based on the level of AD co-pathology.

Materials and methods

Subjects

Cases of LBD with available data from neuropathological assessment of Lewy pathology and AD pathology, as well as genotype data, were considered for inclusion. From the Netherlands Brain Bank (NBB, www.brainbank.nl), donors enrolled from 1989 to 2017 ($n = 3853$) were assessed, and 222 subjects with a neuropathologically confirmed diagnosis of PD or DLB were included. In addition, neurologically healthy controls ($n = 82$) and samples with a neuropathological diagnosis of AD ($n = 64$) were included to evaluate the discriminative ability of AD- and PD-PRSs. Written, informed consent for the use of clinical information and tissue samples for research purposes, was collected from the donors or their next of kin.

Brain dissection was performed according to international guidelines of Brain Net Europe II (BNE) consortium (www.brainnet-europe.org) and the National Institute on Aging-Alzheimer's Association (NIA-AA)[21] by an experienced neuropathologist (A.R.). Formalin-fixed paraffin-embedded 6-μm thick sections were immunostained with antibodies against p-tau (clone AT8, 1:500, Thermo Fisher Scientific), amyloid-β (clone 6F/3D, 1:500, Dako) and α-syn (clone KM51, 1:500, Monosan Xtra), or stained with haematoxylin and eosin (H&E) or Congo red according to current diagnostic guidelines of BrainNet Europe.[22,23]

To assign a Braak NFT stage (Braak NFT 0–VI), NFTs were scored in association cortices (medial frontal gyrus, medial temporal and superior parietal cortex), primary cortices (primary visual cortex and pre/postcentral gyrus), hippocampus (CA1, CA4 and subiculum) and adjacent (trans)entorhinal and fusiform cortex, amygdala, caudate-putamen and cerebellum (if available), as previously described.[22] Thal amyloid-β phases (0–4) were scored according to Thal *et al.*[24] on the medial temporal lobe. For the majority of the cases, a distinction between Thal phase 4 and 5 could not be made, as the cerebellum was not available. Pathological staging

for neuritic plaques in the above-described cortical brain regions was based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score.[25]

Braak Lewy pathology stages, ranging from 3 to 6 in LBD cases, were based on α-syn immunostaining in the neocortices (medial frontal, medial temporal, superior parietal, primary visual and motor cortex), anterior cingulate gyrus, hippocampus (CA1, CA2), (trans)entorhinal cortex, amygdala, basal forebrain, midbrain (substantia nigra), tegmentum (locus coeruleus) and medulla oblongata (dorsal motor nucleus of the vagal nerve), according to the protocol described by Alafuzoff *et al.*[23] Owing to the low number of samples with Braak Lewy pathology stage 3, stages 3 and 4 were collapsed into a single group for the statistical analyses.

Clinical information was extracted from the medical records provided by the NBB. PD was diagnosed based on the combination of UK Parkinson's Disease Society Brain Bank criteria[26] and moderate to severe loss of neurons in the substantia nigra with concurrent Lewy pathology in at least the brainstem.[27] Criteria for DLB were a clinical diagnosis of probable DLB according to the consensus criteria of the DLB Consortium,[28] combined with presence of limbic-transitional or diffuse-neocortical Lewy pathology upon autopsy. Dementia was diagnosed prior to death by a neurologist or geriatrician, or retrospectively based on neuropsychological test results[29] or a Mini-Mental State Examination (MMSE) score <20.

From the Mayo Clinic Jacksonville Brain Bank for Neurodegenerative Disorders, we included a total of 402 autopsy-confirmed LBD cases, characterized by a single neuropathologist (D.W.D.). All subjects were Caucasian, non-Hispanic and unrelated, with written, informed consent for the use of clinical information and tissue samples for research purposes collected from the donors or their next of kin.

Paraffin-embedded 5-μm thick sections mounted on glass slides were stained with thioflavin S. To assign a Braak NFT stage (0–VI) and Thal amyloid-β phase (0–5), NFTs and senile plaques were quantified using thioflavin S fluorescence microscopy in association cortices (frontal, temporal and parietal), primary cortices (visual and motor), hippocampus (CA1, CA4 and subiculum) and adjacent cortex, amygdala, basal ganglia and cerebellum, as previously described.[21,30]

Lewy pathology was assessed in the neocortices (frontal, temporal, parietal, visual and motor), cingulate gyrus, transentorhinal cortex, amygdala, basal forebrain, midbrain, pons and medulla using α-syn immunohistochemistry (NACP, 1:3000 rabbit polyclonal, Mayo Clinic antibody).[31] Lewy pathology was staged as brainstem, transitional or diffuse LBD according to Kosaka *et al.*[32]

Clinical information was extracted from the medical records by three investigators (S.K., H.S. and N.B.M.) to identify the clinical diagnosis and determine the age at onset of either motor symptoms or dementia.[33] Donors with an ante-mortem diagnosis of either PD or DLB were included in the study.[26,28]

We used an adaption of the NIA-AA criteria, where the combination of Thal phase and Braak NFT stage was used to calculate a composite AD-score.[34] Samples with Thal phase 0 or Braak NFT 0 were classified as 'no', Thal phase 1–2 and Braak NFT I-VI or Thal phase 3–5 and Braak NFT I–II as 'low', Thal phase 3 and Braak NFT III–VI or Thal 4–5 and Braak III–IV as 'intermediate' and Thal phase 4–5 and Braak NFT V–VI as 'high'. The LBD samples were divided into two subgroups by severity of AD co-pathology. LBD – AD_{path} was defined as 'no' or 'low' AD-score and LBD + AD_{path} as 'intermediate' or 'high' AD-score.

Genotyping

Genotyping of NBB samples was carried out on the Infinium NeuroChip Consortium Array (Illumina).[35] Mayo Clinic brain bank samples were genotyped on the Infinium OmniExpress-24 (version 1.3) array (Illumina). Standard quality control and filtering were performed and variants imputed using reference data from the Haplotype Reference Consortium as reported previously in detail.[36]

Polygenic risk scores

For each individual, we generated AD-PRS and PD-PRS based on summary statistics from Jansen *et al.*[37] and Nalls *et al.*[3] (including 23andMe, Inc.), respectively, using PRSice2 with standard linkage disequilibrium clumping thresholds (clumping SNPs within a 250 kb window and $r^2 > 0.1$).[38] To improve the linkage disequilibrium estimation for clumping, the 1000 Genomes European samples ($n = 503$) were used as an external reference panel, as is recommended for small datasets in particular.[38] In each of the GWAS summary statistics (the base datasets), duplicated and ambiguous SNPs (C/G or A/T SNPs) were removed as is standard practice.[38] Only variants with a minor allele frequency > 1% were included.

Based on previously published studies of stratified PD-PRS, we selected six pathways (adaptive immune system, α-syn, endocytic membrane trafficking, innate immune system, lysosomal and mitochondrial pathways) and two cell types (microglia and monocytes) of interest for which a significant enrichment of PD risk has been reported.[11-14] One study reported as many as 46 partly overlapping gene sets,[11] and from these we prioritized only a few corresponding to widely studied disease pathways in order to limit multiple testing. We used the same lists of genes or genomic coordinates as these previously published studies to generate pathway-specific PD-PRS, applying the PRSet function in PRSice2. Pathway gene lists were selected by using The Molecular Signatures Database (MsigDB)[39] as well as curated lists of mitochondrial and endocytic membrane trafficking genes applied in previous reports.[13,14] SNPs were mapped to genes using the physical gene boundaries. Cell-type annotations for monocytes and microglia were based on publicly available data on open chromatin regions mapped by assay for transposase-accessible chromatin with sequencing (ATACseq).[40,41]

The PRS algorithm includes SNPs with *P*-values below a user-specified threshold in the original GWAS, which could in theory be less stringent than the threshold for genome-wide significance. To test different thresholds, we evaluated the ability of susceptibility PD-PRS and AD-PRS to discriminate PD and AD samples, respectively, from controls without neurological disease, estimating the area under the receiver operator curve (AUC). For both PD-PRS and AD-PRS, a genome-wide threshold of $P < 5 \times 10^{-8}$ was superior to $P < 1 \times 10^{-5}$ and $P < 0.05$. We therefore chose to use the genome-wide threshold in subsequent analyses, although we acknowledge that assessing susceptibility PRS as predictors for quantitative neuropathologic outcomes in a case-only analysis is principally different from the standard approach differentiating cases from controls. Each PRS was standardized to have a mean of 0 and standard deviation (SD) of 1. The number of SNPs as well as lists of SNPs used to build each PRS are provided in Supplementary Tables 1–25. Genotype imputation ensures that most common SNPs are present in both the NBB and Mayo Clinic datasets, despite not being directly genotyped. Nevertheless, minor differences in the specific SNPs included from the PRSice algorithm were seen for a few of the PRS.

Statistical analyses

All statistical analyses were performed in R version 4.2.1 (www.r-project.org). Demographic data were compared between groups

using Pearson's chi-square test for categorical variables, *t*-tests or the Wilcoxon rank sum test for continuous variables and ordinal variables, as appropriate.

Associations between neuropathology scores (Braak Lewy pathology stage or Kosaka's stage, CERAD neuritic plaque score, Thal amyloid-β phase and Braak NFT stage) and standardized PRS were tested with proportional odds (PO) ordinal logistic regression models to account for the ordered nature of the outcome measure using the vglm() function in the R package 'VGAM'.[42] To assess the PO assumption, we fitted a partial proportional odds (PPO) model where the PO assumption was relaxed for the explanatory variable (i.e. PRS). When comparing PO with PPO models, the likelihood ratio test *P*-values were non-significant, indicating the PO assumption to be reasonable. Due to the small number of LBD + AD_{path} individuals in the NBB cohort with Braak Lewy pathology stage <5, stages 3–5 were collapsed, and associations with Lewy pathology were tested with logistic regression using the R package 'rms'.[43] The models included sex, age at death and first five principal components (PC1–5) as covariates. The odds ratio estimates corresponded to the effect size per 1 SD increase in PRS.

All statistical tests were two-sided. We applied a two-stage design where association signals passing a threshold of $P < 0.05$ in the NBB discovery cohort (NBB) were nominated for independent replication in the Mayo Clinic cohort. We interpreted signals replicating at $P < 0.05$ with a consistent direction of effect across both stages as positive findings.

To further explore the power of PRS to predict neuropathology, we generated an AD co-pathology risk score using coefficients from the ordinal logistic regression in the NBB dataset and assessed the performance of the score in the independent Mayo Clinic dataset. The model included AD-PRS, age at onset and sex. We evaluated the ability of the score to differentiate between LBD – AD_{path} and LBD + AD_{path} samples estimating the AUC from the R package 'pROC'. We also calculated a Lewy pathology risk score in the Mayo Clinic samples using coefficients from the ordinal logistic regression in the NBB cohort, where the model included the lysosomal PD-PRS, age at death, sex and dichotomized AD-score. The AUC was used to assess the power to predict DLBD.

To investigate if the highlighted PRS also influence dementia onset, we conducted survival analysis using the R package 'survival'. Cox proportional hazards regression models were employed to assess the relationship between PRS and time to dementia for the NBB samples. The presence of dementia was used as the event variable. The time variable was the interval between symptom onset and dementia diagnosis for cases who developed dementia prior to death and disease duration at death for non-demented cases. Age at onset, sex and the first five genetic principal components were used as covariates. To assess the proportional hazards assumption, a combined plotting and testing approach was employed.

Data availability

Data on NBB donors that support the findings of this study can be obtained from the Netherlands Neurogenetics Database (https://www.brainbank.nl/nnd-project/). Mayo Clinic data are available from the authors on request. Analysis code used in this manuscript is available on GitHub at https://github.com/lpihlstrom/projects.

Results

After filtering extreme age outliers ($n = 1$) and cases with an atypical distribution of Lewy pathology that prevented the assignment of a Braak Lewy pathology stage ($n = 4$), 217 cases with LBD were included from the NBB in the final analyses. Overall, 161 cases (74%) were classified as LBD – AD_{path} and 56 (26%) as LBD + AD_{path}. The clinical and demographic details split across the LBD – AD_{path} and LBD + AD_{path} groups for NBB cases are summarized in Table 1. Gender distribution and age at death were comparable among the two subgroups of LBD. LBD + AD_{path} subjects were older at disease onset (70.6 versus 64.2 years) and had a shorter disease duration (8.1 versus 13.2 years) but a similar age at death as LBD – AD_{path} cases (77.5 versus 78.8). For 211 NBB cases, dementia status was also available. A larger proportion of LBD + AD_{path} cases had developed dementia prior to death compared to LBD – AD_{path} cases [49/54 (90.7%) versus 95/159 (59.7%)], and LBD + AD_{path} cases had a shorter interval between disease onset and onset of dementia (3.6 years versus 10.1 years). Braak Lewy pathology stage and, as expected, all measures of AD neuropathology were significantly higher in the LBD + AD_{path} subgroup.

In the Mayo Clinic dataset, extreme age outliers ($n = 8$) were excluded and a total of 394 LBD cases included in the final analysis. Of

Table 1 Demographics for NBB samples

	LBD – AD_{path} ($n = 161$)	LBD + AD_{path} ($n = 56$)
Sex, *n* (%)		
Female	56 (34.8)	25 (44.6)
Male	105 (65.2)	31 (55.4)
Age at onset, mean (SD)	64.2 (11.9)	70.6 (8.8)
Age at death, mean (SD)	77.5 (8.0)	78.8 (7.8)
Disease duration, mean (SD)	13.2 (7.5)	8.1 (5.1)
Time to dementia (SD)	10.1 (8.2)	3.6 (5.9)
Braak Lewy pathology stage, *n* (%)		
3–4	14 (8.7)	2 (3.6)
5	60 (37.3)	5 (8.9)
6	87 (54.0)	49 (87.5)
CERAD, median (Q1, Q3)	0 (0, 1)	1 (1, 2)
Thal phase, median (Q1, Q3)	1 (0, 3)	3 (3, 4)
Braak NFT stage, median (Q1, Q3)	I (I, II)	IV (III, IV)

AD_{path} = Alzheimer's disease co-pathology; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; LBD = Lewy body disease; NFT = neurofibrillary tangle; Q1 = 1st quartile; Q3 = 3rd quartile; SD = standard deviation.

Table 2 Demographic table for Mayo Clinic samples

	LBD – AD_{path} ($n = 196$)	LBD + AD_{path} ($n = 198$)
Sex, *n* (%)		
Female	55 (28.1)	67 (33.8)
Male	141 (71.9)	131 (66.2)
Age at onset, mean (SD)	65.9 (10.8)	70.4 (8.3)
Age at death, mean (SD)	76.0 (8.6)	78.3 (6.6)
Disease duration, mean (SD)	10.0 (7.4)	7.9 (5.5)
LBD type/Kosaka, *n* (%)		
BLBD	54 (27.6)	6 (3.0)
TLBD	84 (42.9)	34 (17.2)
DLBD	58 (29.6)	158 (79.8)
Thal phase, median (Q1, Q3)	1 (0, 2)	4 (3, 5)
Braak NFT stage, median (Q1, Q3)	II (II, III)	IV (III, V)

AD_{path} = Alzheimer's disease co-pathology; BLBD = brainstem Lewy body disease; DLBD = diffuse Lewy body disease; NFT = neurofibrillary tangle; Q1 = 1st quartile; Q3 = 3rd quartile; SD = standard deviation; TLBD = transitional Lewy body disease.

these, 196 cases (50%) were categorized as LBD – AD_{path} and 198 cases (50%) as LBD + AD_{path}. A larger male predominance was seen in the LBD – AD_{path} than in the LBD + AD_{path} subgroup [71.9% (141/196) versus 66.2% (131/198)]. LBD + AD_{path} cases had a higher age at onset and age at death (70.4 versus 65.9 and 78.3 versus 76.0 years, respectively) and a shorter disease duration (7.9 versus 10.0 years). The clinical and demographic variables are summarized in Table 2.

AD-PRS is associated with the level of AD co-pathology in LBD

As expected, a higher genetic risk for AD was strongly associated with all measures of AD pathology in the NBB cohort. These included Thal phase, Braak NFT stage and CERAD score as well as the dichotomized AD-score (Table 3). The associations between AD-PRS and measures of AD pathology were replicated in the Mayo Clinic cohort (Table 3). In the Mayo Clinic cohort, these associations were also significant when removing the *APOE* component from the AD-PRS [Mayo Clinic cohort: Thal phase $P = 0.044$, 95% confidence interval (CI) of odds ratio (OR) = 1.0–1.44; Braak NFT stage $P = 0.0095$, 95% CI of OR = 1.06–1.52; AD-score $P = 0.032$, 95% CI of OR = 1.02–1.55].

A risk score predicts AD co-pathology from the AD-PRS, age at onset and sex

To investigate the power to distinguish LBD – AD_{path} from LBD + AD_{path} based on genetics and basic demographic variables, we generated an AD co-pathology risk score for each Mayo Clinic donor based on coefficients from ordinal logistic regression in the NBB data, where the model included AD-PRS, age at onset and sex. The AUC for this score was 0.70 (95% CI 0.65–0.75) (Fig. 1A).

Lysosomal PRS is associated with Lewy pathology in LBD without AD co-pathology

In a sample set including donors both with and without LBD, by definition, diagnosis alone would drive an association between PD-PRS and Lewy pathology. However, there is also an interesting variation 'within' the LBD group, where some have more widespread Lewy pathology than others. This difference is potentially relevant for clinical trials but is not currently well captured by any available *in vivo* biomarker. We hypothesized that the way genetic burden is distributed across specific disease pathways partly determines the extent of Lewy pathology in the individual LBD patient. Several recent reports have indicated that the genetic architecture of 'pure' LBD may be different from cases where Lewy pathology coexists with changes associated with AD. To further investigate this hypothesis in a neuropathological context and identify genetic drivers of Lewy pathology, we split each cohort into two subgroups based on the level of AD co-pathology and assessed ordinal logistic regression models separately for each subgroup. Results from association analyses of Lewy pathology are presented in Table 4. Based on previous reports, we expected genetic risk factors for PD to have the strongest effect on the Lewy pathology stage in the subgroup of cases without AD co-pathology.[20,44,45] In line with this hypothesis, a general PD-PRS was associated with Lewy pathology stage in LBD – AD_{path} samples in the NBB cohort. However, this signal did not replicate in the Mayo Clinic cohort. In contrast, LBD + AD_{path} subgroups showed no clear trend towards association between the Lewy pathology stage and PD-PRS in either cohort.

We then moved on to investigate the association with eight stratified PD-PRS capturing common genetic risk variants annotated to specific pathways and cell types (Fig. 2). One of these risk scores, the lysosomal PD-PRS, was associated with Lewy pathology in the LBD – AD_{path} group in the NBB cohort. This association was replicated in the Mayo clinic cohort (Table 4). We note that the Lewy pathology was classified differently across the two cohorts, although both analyses included three stages. Notwithstanding this methodological difference, the effect size was similar, with 1 SD increase in lysosomal PD-PRS corresponding to an odds ratio of 1.48 (NBB) or 1.46 (Mayo Clinic), respectively, for a higher Lewy pathology stage (Fig. 3). As *GBA* variants are known strong risk factors for both PD and DLB, and have previously been linked to Lewy pathology, we also generated lysosomal PD-PRS excluding the *GBA* region. The association with Lewy pathology stage in LBD – AD_{path} samples remained significant in the Mayo Clinic dataset and showed a similar trend in the NBB dataset, indicating that other lysosomal genes also contribute to Lewy pathology burden in LBD – AD_{path}.

No significant association between stratified PD-PRS were observed in the LBD + AD_{path} subgroup; however, less variation in Lewy pathology was observed with a majority of donors in the highest stages, thus statistical power was limited.

A joint model of AD co-pathology and lysosomal PRS predicts Lewy pathology stage

The neuropathological data indicate that LBD donors with intermediate or high AD co-pathology are likely also to have the most advanced stage of Lewy pathology. In 'pure' LBD, without AD co-pathology, we identified a higher lysosomal genetic burden as a risk factor for higher Lewy pathology stage. From a clinical perspective, the presence of AD co-pathology can be assessed by amyloid PET imaging or CSF biomarkers in living patients. To assess how well we could predict the Lewy pathology stage from genetics combined with data on AD co-pathology in LBD patients, we performed ordinal regression in the full NBB cohort with both dichotomized AD co-pathology status and lysosomal PD-PRS included in the same model. The lysosomal PD-PRS remained independently significant, with only marginally weaker effect size (odds ratio 1.40, 95% CI 1.03–1.89, $P = 0.030$). We used the coefficients from the NBB ordinal logistic regression to generate a joint risk score for neocortical Lewy pathology in the Mayo

Table 3 Association between polygenic risk for AD and measures of AD pathology in samples with LBD, regardless of level of concomitant AD pathology

Outcome	PRS model	OR	95% CI	*P*-value
NBB discovery cohort ($n = 217$)				
CERAD score	AD-PRS	2.14	1.61–2.85	$1.5 \times 10^{-7*}$
Thal phase	AD-PRS	2.08	1.60–2.71	$5.5 \times 10^{-8*}$
Braak NFT stage	AD-PRS	1.39	1.08–1.78	0.010*
AD-pathology score	AD-PRS	1.84	1.33–2.60	$3.2 \times 10^{-4*}$
Mayo Clinic replication cohort ($n = 394$)				
Thal phase	AD-PRS	2.07	1.70–2.52	$3.5 \times 10^{-13*}$
Braak NFT stage	AD-PRS	1.75	1.45–2.11	$5.1 \times 10^{-9*}$
AD-pathology score	AD-PRS	2.04	1.62–2.62	$5.7 \times 10^{-9*}$

Associations were assessed in proportional odds ordinal logistic regression models. AD = Alzheimers' disease; NBB = Netherlands Brain Bank; NFT = neurofibrillary tangle; OR = odds ratio; PRS = polygenic risk score. *$P < 0.05$.

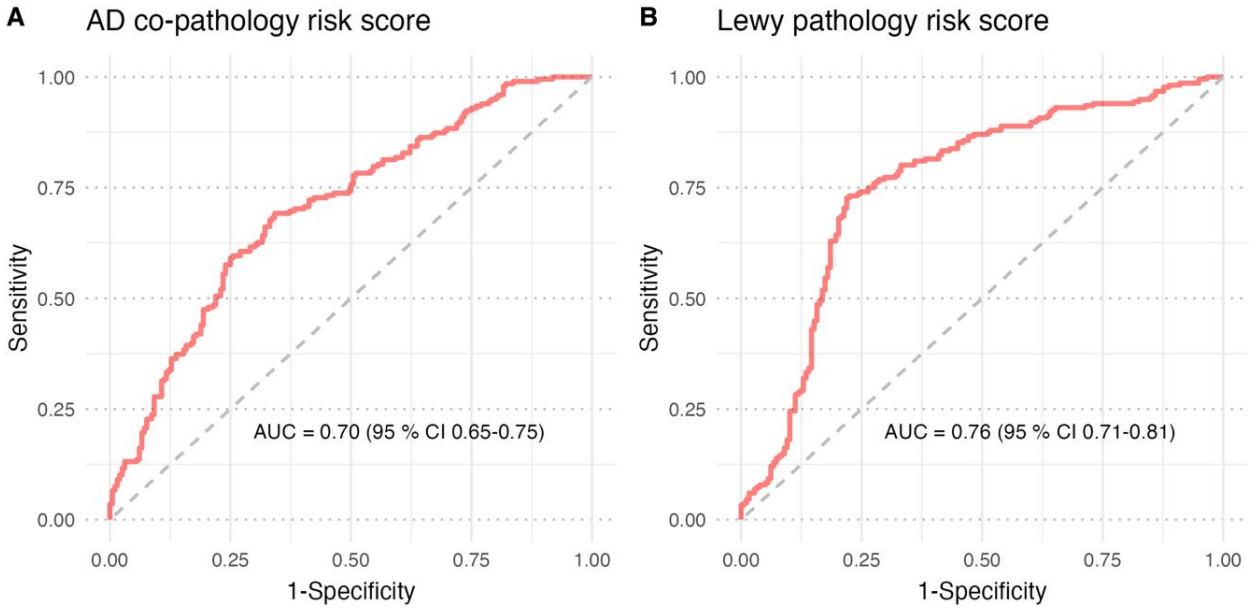

Figure 1 Performance of prediction models for AD co-pathology and Lewy pathology. (**A**) The AD) co-pathology risk score was calculated in Mayo Clinic samples ($n = 394$) based on coefficient weights for AD-PRS, sex and age at onset from ordinal logistic regression in Netherlands Brain Bank (NBB) samples ($n = 213$). (**B**) The Lewy pathology risk score was calculated in Mayo Clinic samples ($n = 394$) based on coefficient weights for lysosomal PD-PRS, AD-pathology, sex and age at onset from proportional odds ordinal logistic regression in NBB samples ($n = 217$). AD-PRS = Alzheimer's disease polygenic risk score; PD-PRS = Parkinson's disease polygenic risk score.

Table 4 Associations between PD polygenic risk scores and Lewy pathology, stratified by level of AD co-pathology

PRS model	LBD − AD_{path}			LBD + AD_{path}		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
NBB discovery cohort (LBD − AD, *n* = 161, LBD + AD, *n* = 56)						
Full PD-PRS	1.62	1.15–2.27	0.0055*	0.70	0.24–2.07	0.52
Adaptive immunity	1.15	0.85–1.57	0.37	0.44	0.16–1.20	0.11
α-Synuclein	1.15	0.84–1.58	0.39	0.62	0.23–1.66	0.34
Endocytic membrane trafficking	0.92	0.67–1.25	0.58	1.16	0.38–3.54	0.80
Innate immunity	1.04	0.76–1.42	0.81	0.52	0.18–1.53	0.24
Lysosomal	1.48	1.04–2.09	0.027*	1.62	0.44–5.94	0.47
Lysosomal excluding *GBA*	1.25	0.90–1.73	0.18	2.32	0.74–7.25	0.15
Microglia	1.21	0.87–1.67	0.25	1.21	0.43–3.39	0.72
Mitochondria	0.93	0.68–1.28	0.66	2.90	0.81–10.4	0.10
Monocytes	1.08	0.78–1.49	0.64	0.77	0.24–2.53	0.67
Mayo Clinic replication cohort (LBD − AD, *n* = 196)						
Full PD-PRS	0.98	0.75–1.28	0.86	–	–	–
Lysosomal	1.46	1.11–1.92	0.0070*	–	–	–
Lysosomal excluding *GBA*	1.42	1.08–1.86	0.011*	–	–	–

Proportional odds (PO) ordinal logistic regression with Braak Lewy pathology stage (NBB) or Kosaka's LBD type (Mayo Clinic) as outcome. Binary rather than ordinal logistic regression was used in the NBB LBD + AD_{path} analysis (see 'Materials and methods' section). AD_{path} = Alzheimer's disease co-pathology; CI = confidence interval; LBD = Lewy body disease; NBB = Netherlands Brain Bank; OR = odds ratio; PD-PRS = Parkinson's disease polygenic risk score. *$P < 0.05$.

Clinic dataset. The score had an AUC of 0.76 (95% CI 0.71–0.81) for predicting DLBD (Fig. 1B).

AD-PRS and lysosomal PD-PRS are associated with dementia onset

To further investigate if the highlighted PRS also associate with disease progression we performed survival analysis for AD-PRS and lysosomal PD-PRS and time to dementia in NBB donors. In a Cox proportional hazards model, AD-PRS was associated with a shorter time between disease onset and dementia diagnosis (hazard ratio 1.36 per SD increase in PRS, 95% CI 1.15–1.61, $P = 0.00040$) when all NBB samples were analysed together. When the samples were split based on the level of AD co-pathology the lysosomal PD-PRS was also associated with a shorter time to dementia in the LBD − AD_{path} samples (hazard ratio 1.31 per SD increase in PRS, 95% CI 1.07–1.62, $P = 0.010$), but not in LBD + AD_{path} samples (hazard ratio 0.81 per SD increase in PRS, 95% CI 0.54–1.22, $P = 0.32$).

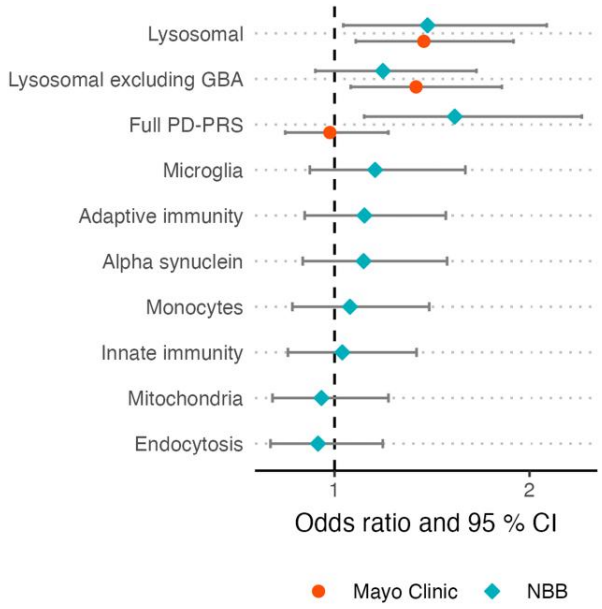

Figure 2 Associations between Lewy pathology and PRS in the subgroup without AD co-pathology. The figure shows the effect size and confidence intervals of the association between different polygenic risk scores (PD-PRS) and Lewy pathology stage in the subgroup without Alzheimer's disease co-pathology (LBD – AD_{path}). Hypothesis testing was performed using proportional odds ordinal logistic regression models and associations passing $P < 0.05$ in the Netherlands Brain Bank (NBB) cohort ($n = 161$) were followed-up in the Mayo Clinic cohort ($n = 196$). AD_{path} = Alzheimer's disease co-pathology; LBD = Lewy body disease; PD-PRS = Parkinson's disease polygenic risk score.

Discussion

An increasing number of genetic variants are recognized as risk factors for LBD, but the specific genetic architecture of the underlying neuropathological substrate of disease remains undetermined. To address this knowledge gap, we explored the association between AD-PRS, PD-PRS, and pathway-stratified PD-PRSs with key neuropathological measures in thoroughly characterized LBD samples from two independent brain bank cohorts. The relationship between neuropathological outcomes and PRS were assessed using ordinal logistic regression models that take into account the ordered fashion of the neuropathological stages. Firstly, we showed that AD-PRS was associated with co-morbid amyloid-β and tau pathology in samples with LBD. Secondly, we provide evidence that a stratified PD-PRS reflecting the genetic burden on the lysosomal pathway is associated with Lewy pathology in LBD, specifically in the subgroup without AD co-pathology. Interestingly, this pathway-stratified PRS showed a stronger and more consistent association with Lewy pathology than the overall PD-PRS, which includes a larger number of risk variants reflecting several biological pathways. Thirdly, our data suggests that both the AD-PRS and lysosomal PD-PRS are associated with an accelerated onset of dementia, the latter specifically in donors without AD co-pathology. Our findings provide novel insights into the complex relationships between neuropathology and genetics and indicate a future potential for the use of multiple, specific PRSs in clinical patient stratification.

As expected, we observed a strong association between AD-PRS and AD co-pathology, including measures of both amyloid-β and tau pathology in both datasets. AD-PRS also remained strongly associated with AD pathology in the larger Mayo Clinic dataset after removing the *APOE* component, indicating that genetic risk variants beyond *APOE* influence amyloid-β and tau pathology. Previously, PRS based on AD GWAS have also been shown to be associated with AD pathology in brain bank cohorts of AD patients.[46-48] In samples from LBD patients, the *APOE* ϵ4 allele has in several reports been associated with higher likelihood of both amyloid-β pathology and tau NFT co-pathology,[2,36,49-51] including studies from the NBB[36] and Mayo Clinic[51] based on sample sets overlapping those of our present study. Moreover, a 'clinical-genetic risk score' based on *APOE* ϵ4 alleles, two additional risk SNPs (*BIN1* and *SORL1*) and age at onset, was reported to predict the presence of intermediate or high AD co-pathology in samples with LBD.[52] Our study adds to prior findings by showing association between a full AD-PRS and the two key measures of AD-pathology in post-mortem LBD samples, also demonstrating significance of polygenic burden when the strong *APOE* effect is excluded.

Genetic association studies of Lewy pathology have thus far focused primarily on targeted variants in the *SNCA*, *MAPT*, *GBA*, and *APOE* loci.[20,44,49,51,53] In a previous Mayo Clinic study, Heckman and colleagues did not find an association between a PD genetic risk score and Lewy body count or LBD subtype, yet the analysis did not stratify by the presence of AD co-pathology.[54] In the present study, the full PD-PRS was associated with Lewy pathology only in the LBD – AD_{path} subgroup of the NBB cohort, but did not replicate in the Mayo Clinic cohort. The absence of a consistent strong signal for the general PD-PRS indicates that not all PD risk factors act through mechanisms that increase Lewy pathology, underlining the rationale for a pathway-stratified PRS approach. In principle, genetic variants not associated with Lewy pathology could increase PD risk through mechanisms that primarily cause neuronal loss.

To our knowledge, there are no previous reports examining associations between pathway-stratified PRS and neuropathological outcomes in samples with LBD. Testing eight selected PRS stratified by specific pathways or cell-types we found that the lysosomal PD-PRS was associated with Lewy pathology in both the discovery and replication cohort. This result is highly plausible considering previous research. Genetic studies have provided a link between lysosomal function and LBD risk. Heterozygous mutations in the *GBA* gene, which in the biallelic state are known to cause the lysosomal storage disorder Gaucher's disease, are major genetic risk factors for both PD[55,56] and DLB,[57] conferring a more than 5-fold increase of PD risk,[55] 6-fold increase in risk of PD with dementia[57] and more than 8-fold increase in DLB risk.[57] Moreover, low-frequency variants in the *GBA* locus have consistently shown significant association with both PD and DLB in GWAS.[3-5,58,59] Neuropathological studies of patients carrying *GBA* variants have demonstrated that these patients tend to have severe Lewy pathology,[44,45] although a small neuropathological study found no significant association comparing to sporadic PD.[60]

In the current study, we suspected variation in the *GBA* locus to be a strong driver of the lysosomal PD-PRS signal. However, even after removing the *GBA* component, the lysosomal PD-PRS remained significantly associated with Lewy pathology in the LBD – AD_{path} samples from the Mayo Clinic, indicating that lysosomal variants beyond *GBA* are involved. Substantial evidence suggests a wider contribution of lysosomal mechanisms in LBD liability and pathogenesis. In addition to *GBA*, other genes involved in lysosomal functioning have been nominated by both PD and DLB GWAS, including *SCARB2*, *TMEM175*, *CTSB*, ATP6V0A1, *GALC*, *GUSB*, *GRN* and *NEU1*.[3,4,58,59,61] Moreover, an excessive burden of

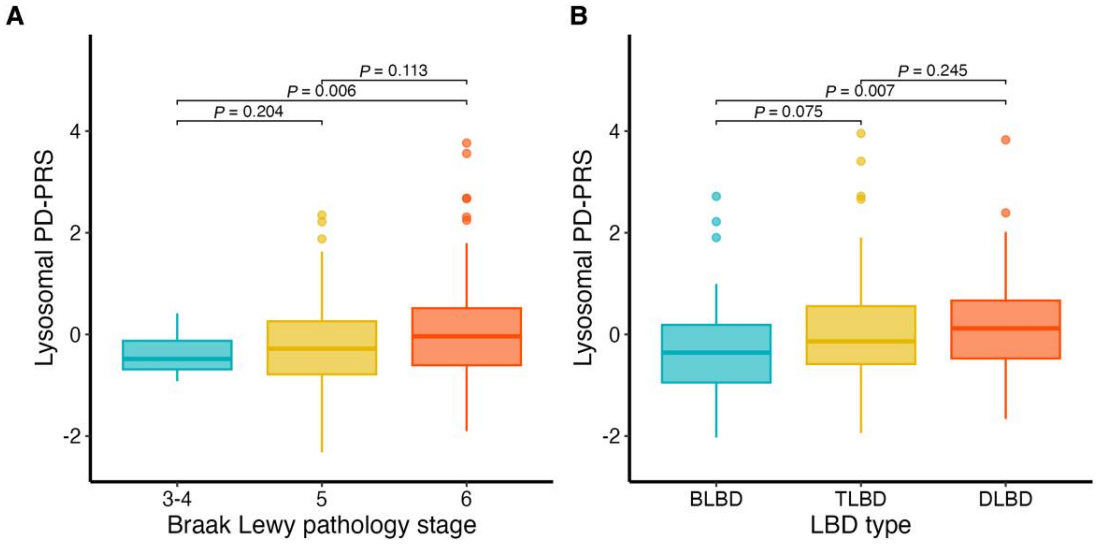

Figure 3 Box plots illustrating increasing lysosomal PD-PRS with higher Lewy pathology stages in LBD – AD_{path} samples. The figure shows box plots of lysosomal PD-PRS for different stages of Lewy pathology in the Netherlands Brain Bank (NBB) (**A**) and Mayo Clinic (**B**) data for the subgroup without AD co-pathology ($n = 161$ and 196, respectively). Plots are created with the default R parameters where the box ranges from the first (Q1) to the third (Q3) quartile and whiskers extend to the most extreme observation that is less than 1.5 times the Q1–Q3 distance from the box. Mean lysosomal PD-PRS between the samples with different levels of Lewy pathology were compared with *t*-tests. AD = Alzheimer's disease; BLBD = brainstem Lewy body disease; DLBD = diffuse Lewy body disease; PD-PRS = Parkinson's disease polygenic risk score; TLBD = transitional Lewy body disease.

lysosomal storage disorder gene variants has been found in PD.[56] Our findings add to these insights by showing that the cumulative effect of multiple gene loci converging on the lysosomal pathway increases the Lewy pathology burden in LBD.

Interestingly, the association between lysosomal PD-PRS and Lewy pathology was specific to the subgroup of donors with no or low AD co-pathology. In agreement with this result, *GBA* has been proposed to be associated with 'pure' LBD with extensive Lewy pathology and less severe AD co-pathology, supported by several autopsy studies.[20,44,45] Data from neuropathological post-mortem studies are corroborated by a recent study where CSF biomarkers were used as an in vivo proxy of AD co-pathology. Here, van der Lee and colleagues found the *GBA* p.E365K variant to be more strongly associated with 'pure' DLB than DLB with AD co-pathology.[10] In a large neuropathological study of different forms of dementia, a DLB-PRS was associated with Lewy pathology stage only if the *APOE* component was excluded.[48] The reason why genetic association signals differ depending on the degree of AD co-pathology is currently unclear. One possible explanation could be that AD-related changes make the brain more susceptible to additional neuropathologies, creating a vulnerable environment where genetic risk factors specific to LBD are relatively less important.

Genetics and neuropathology probably also contribute to shaping the clinical progression of disease. LBD + AD_{path} donors had a later disease onset and more rapid progression to dementia than LBD – AD_{path} donors. Further, we showed that the AD-PRS was associated with a more rapid progression to dementia, in line with longitudinal and cross-sectional studies that have shown that the *APOE* E4 allele is associated with an increased risk of cognitive decline and dementia in PD.[36,62-64] Notably, the lysosomal PD-PRS was also associated with a faster development of dementia, exclusively in the LBD – AD_{path} samples. Several studies have shown that GBA variants (both pathogenic and non-pathogenic) adversely affects the prognosis of PD, including increasing the risk of dementia in PD patients.[65-67]

A strength of our study is the relatively large sample size, including a total of more than 600 neuropathologically characterized LBD cases. However, the NBB and Mayo Clinic cohorts differ in several important ways, the latter being larger and having a much larger proportion of donors positive for AD co-pathology. Furthermore, there are differences in neuropathological assessment between the two brain banks. In particular, the protocols for defining the Lewy pathology stage and Thal phases were not identical. However, both staging schemes for Lewy pathology are based on the same assumption of a generally caudal to rostral progression Lewy pathology within the CNS,[23] and a high correlation between the two protocols for determining Thal phases used in this study has previously been reported.[68] Differences in neuropathological methodology are likely to make signals less reproducible across the cohorts. This represents a clear limitation to our study design, yet with respect to the findings that we do highlight as consistent across both datasets, similar results across heterogeneous independent cohorts are arguably also an indication of methodologically robust signals.

We acknowledge that post-mortem datasets are skewed towards advanced disease stages and will not be representative of living patients the same age, limiting the relevance for e.g. patient stratification in clinical trials. Furthermore, we chose to emphasize the neuropathology of LBD as a common group, with the caveat that there might be relevant differences between PD and DLB that are not captured by our design. Limited statistical power led us to select eight pathways and cell types for stratified PD-PRS based on previous literature, although a hypothesis-free approach would have been preferable. The analysis of time to dementia was performed only in 211 donors with available data in the NBB dataset and should therefore be regarded as exploratory and interpreted with particular caution. Finally, our analysis was restricted to donors of European ancestry, a reminder that efforts to extend PD research to underrepresented populations should also involve brain bank donor programs.

Our study holds promise that PRS may be useful as an enrichment marker of Lewy pathology and AD co-pathology in future clinical trials assessing the effect of therapeutics targeting α-syn, amyloid-β or tau. Future work should also aim to further characterize the relationship between the correlations studied here and clinical outcomes in LBD. A number of large clinico-genetic studies have recently started to shed light on the genetics basis of clinical variability in PD, yet the positive associations with established risk variants from GWAS have been few and inconsistent.[63,67,69] Our findings suggest that endophenotypes such as neuropathology could capture relevant pathological processes with higher precision than clinical symptoms, thereby providing a promising path for further genetic association studies.

Conclusion

In this study on neuropathologically defined samples from two independent cohorts we show that genetic variants known to be associated with the risk of AD and PD also influence key neuropathological measures in LBD donors. We extend the current knowledge about the influence of AD risk variants on AD co-pathology. Furthermore, we provide novel evidence that genetic variants linked to the lysosomal pathway are associated with higher Lewy pathology stage in 'pure' LBD. Our findings hold promise that larger genetic association studies of neuropathology and other endophenotypes will provide further insights into the pathogenic mechanisms of LBD. With further refinements, it is also our hope that a more fine-grained understanding of polygenic risk will make stratified PRS a useful tool for patient stratification in a precision medicine context.

Acknowledgements

The PD GWAS summary statistics used in this study were generated in a meta-analysis including data from 23andMe, Inc. We would like to thank the research participants and employees of 23andMe for making this work possible. The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/dataset-access/ for more information and to apply to access the data. The authors are grateful to the Netherlands Brain Bank and its funders for providing the samples that made the study possible.

Funding

J.-A.T. and L.P. were funded by grants from the South-Eastern Norway Regional Health Authority (Helse Sør-Øst RHF, Norway). W.B. received funding from the Dutch Parkinson association, Health Holland, and Rotary Club Aalsmeer-Uithoorn. O.A.R. is supported by NIH (RF1 NS085070; U54-NS100693; U01 NS100620; R01 AG056366; U19 AG071754), DOD (W81XWH-17-1-0249), The Michael J. Fox Foundation, The Little Family Foundation, the Mayo Clinic Dorothy and Harry T. Mangurian Jr. Lewy Body Dementia Program at Mayo Clinic, Ted Turner and family with the Functional Genomics of LBD Program and the Mayo Clinic LBD Center without Walls (U54-NS110435). Mayo Clinic (O.A.R.) was a recipient of the inaugural Cure One, Cure Many Award from the American Brain Foundation for the study of Lewy body dementia. Mayo Clinic is an American Parkinson Disease Association (APDA) Mayo Clinic Information and Referral Center, an APDA Center for Advanced Research, and a Lewy Body Dementia Association (LBDA) Research Center of Excellence.

Competing interests

The authors declare no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997;388:839-840.
2. Robinson JL, Lee EB, Xie SX, *et al*. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. *Brain*. 2018;141:2181-2193.
3. Nalls MA, Blauwendraat C, Vallerga CL, *et al*. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2019;18:1091-1102.
4. Chia R, Sabir MS, Bandres-Ciga S, *et al*. Genome sequencing analysis identifies new loci associated with Lewy body dementia and provides insights into its genetic architecture. *Nat Genet*. 2021;53:294-303.
5. Guerreiro R, Ross OA, Kun-Rodrigues C, *et al*. Investigating the genetic architecture of dementia with Lewy bodies: A two-stage genome-wide association study. *Lancet Neurol*. 2018;17:64-74.
6. Escott-Price V, Nalls MA, *et al*. Polygenic risk of Parkinson disease is correlated with disease age at onset. *Ann Neurol*. 2015;77:582-591.
7. Ibanez L, Dube U, Saef B, *et al*. Parkinson Disease polygenic risk score is associated with Parkinson disease status and age at onset but not with alpha-synuclein cerebrospinal fluid levels. *BMC Neurol*. 2017;17:198.
8. Pihlstrom L, Morset KR, Grimstad E, Vitelli V, Toft M. A cumulative genetic risk score predicts progression in Parkinson's disease. *Mov Disord*. 2016;31:487-490.
9. Paul KC, Schulz J, Bronstein JM, Lill CM, Ritz BR. Association of polygenic risk score with cognitive decline and motor progression in Parkinson disease. *JAMA Neurol*. 2018;75:360-366.
10. van der Lee SJ, van Steenoven I, van de Beek M, *et al*. Genetics contributes to concomitant pathology and clinical presentation in dementia with Lewy bodies. *J Alzheimers Dis*. 2021;83:269-279.
11. Bandres-Ciga S, Saez-Atienzar S, Kim JJ, *et al*. Large-scale pathway specific polygenic risk and transcriptomic community network analysis identifies novel functional pathways in Parkinson disease. *Acta Neuropathol*. 2020;140:341-358.
12. Andersen MS, Bandres-Ciga S, Reynolds RH, *et al*. Heritability enrichment implicates microglia in Parkinson's disease pathogenesis. *Ann Neurol*. 2021;89:942-951.
13. Billingsley KJ, Barbosa IA, Bandres-Ciga S, *et al*. Mitochondria function associated genes contribute to Parkinson's disease risk and later age at onset. *NPJ Parkinsons Dis*. 2019;5:8.
14. Bandres-Ciga S, Saez-Atienzar S, Bonet-Ponce L, *et al*. The endocytic membrane trafficking pathway plays a major role in the risk of Parkinson's disease. *Mov Disord*. 2019;34:460-468.

15. Hamilton RL. Lewy Bodies in Alzheimer's disease: A neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. *Brain Pathol*. 2000;10:378-384.
16. Irwin DJ, White MT, Toledo JB, *et al*. Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol*. 2012;72:587-598.
17. Dugger BN, Adler CH, Shill HA, *et al*. Concomitant pathologies among a spectrum of parkinsonian disorders. *Parkinsonism Relat Disord*. 2014;20:525-529.
18. Walker L, Stefanis L, Attems J. Clinical and neuropathological differences between Parkinson's disease, Parkinson's disease dementia and dementia with Lewy bodies—current issues and future directions. *J Neurochem*. 2019;150:467-474.
19. Irwin DJ, Grossman M, Weintraub D, *et al*. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol*. 2017;16:55-65.
20. Kaivola K, Shah Z, Chia R, International LBDGC, Scholz SW. Genetic evaluation of dementia with Lewy bodies implicates distinct disease subgroups. *Brain*. 2022;145:1757-1762.
21. Montine TJ, Phelps CH, Beach TG, *et al*. National institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. 2012;123:1-11.
22. Alafuzoff I, Arzberger T, Al-Sarraj S, *et al*. Staging of neurofibrillary pathology in Alzheimer's disease: A study of the BrainNet Europe consortium. *Brain Pathol*. 2008;18:484-496.
23. Alafuzoff I, Ince PG, Arzberger T, *et al*. Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe consortium. *Acta Neuropathol*. 2009;117:635-652.
24. Thal DR, Rub U, Schultz C, *et al*. Sequence of Abeta-protein deposition in the human medial temporal lobe. *J Neuropathol Exp Neurol*. 2000;59:733-748.
25. Mirra SS, Heyman A, McKeel D, *et al*. The consortium to establish a registry for Alzheimer's disease (CERAD). part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991;41:479-486.
26. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1988;51:745-752.
27. Dickson DW, Braak H, Duda JE, *et al*. Neuropathological assessment of Parkinson's disease: Refining the diagnostic criteria. *Lancet Neurol*. 2009;8:1150-1157.
28. McKeith IG, Boeve BF, Dickson DW, *et al*. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB consortium. *Neurology*. 2017;89:88-100.
29. Emre M, Aarsland D, Brown R, *et al*. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord*. 2007;22:1689-1707.
30. Koga S, Zhou X, Dickson DW. Machine learning-based decision tree classifier for the diagnosis of progressive supranuclear palsy and corticobasal degeneration. *Neuropathol Appl Neurobiol*. 2021;47:931-941.
31. Dickson DW, Liu W, Hardy J, *et al*. Widespread alterations of alpha-synuclein in multiple system atrophy. *Am J Pathol*. 1999;155:1241-1251.
32. Kosaka K, Yoshimura M, Ikeda K, Budka H. Diffuse type of Lewy body disease: Progressive dementia with abundant cortical Lewy bodies and senile changes of varying degree–a new disease? *Clin Neuropathol*. 1984;3:185-192.
33. Koga S, Sekiya H, Kondru N, Ross OA, Dickson DW. Neuropathology and molecular diagnosis of synucleinopathies. *Mol Neurodegener*. 2021;16:83.
34. Hyman BT, Phelps CH, Beach TG, *et al*. National Institute on Aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*. 2012;8:1-13.
35. Blauwendraat C, Faghri F, Pihlstrom L, *et al*. Neurochip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. *Neurobiol Aging*. 2017;57:247 e249-247 e213.
36. Tunold JA, Geut H, Rozemuller JMA, *et al*. APOE And MAPT are associated with dementia in neuropathologically confirmed Parkinson's disease. *Front Neurol*. 2021;12:631145.
37. Jansen IE, Savage JE, Watanabe K, *et al*. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51:404-413.
38. Choi SW, O'Reilly PF. PRSice-2: Polygenic Risk Score software for biobank-scale data. *Gigascience*. 2019;8:giz082.
39. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1:417-425.
40. Corces MR, Buenrostro JD, Wu B, *et al*. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. *Nat Genet*. 2016;48:1193-1203.
41. Nott A, Holtman IR, Coufal NG, *et al*. Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. *Science*. 2019;366:1134-1139.
42. VGAM: Vector Generalized Linear and Additive Models. Version R package version 1.1–7. 2022. https://CRAN.R-project.org/package=rms
43. *rms: Regression Modeling Strategies*. Version R package version 6.3–0. 2022. https://CRAN.R-project.org/package=rms
44. Clark LN, Kartsaklis LA, Wolf Gilbert R, *et al*. Association of glucocerebrosidase mutations with dementia with Lewy bodies. *Arch Neurol*. 2009;66:578-583.
45. Tsuang D, Leverenz JB, Lopez OL, *et al*. GBA Mutations increase risk for Lewy body disease with and without Alzheimer disease pathology. *Neurology*. 2012;79:1944-1950.
46. Tan CH, Bonham LW, Fan CC, *et al*. Polygenic hazard score, amyloid deposition and Alzheimer's neurodegeneration. *Brain*. 2019;142:460-470.
47. Hannon E, Shireby GL, Brookes K, *et al*. Genetic risk for Alzheimer's disease influences neuropathology via multiple biological pathways. *Brain Commun*. 2020;2:fcaa167.
48. Spencer BE, Jennings RG, Fan CC, Brewer JB. Assessment of genetic risk for improved clinical-neuropathological correlations. *Acta Neuropathol Commun*. 2020;8:160.
49. Compta Y, Parkkinen L, O'Sullivan SS, *et al*. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: Which is more important? *Brain*. 2011;134(Pt 5):1493-1505.
50. Lashley T, Holton JL, Gray E, *et al*. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. *Acta Neuropathol*. 2008;115:417-425.
51. Dickson DW, Heckman MG, Murray ME, *et al*. APOE Epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. *Neurology*. 2018;91:1182-1195.
52. Dai DL, Tropea TF, Robinson JL, *et al*. ADNC-RS, a clinical-genetic risk score, predicts Alzheimer's pathology in autopsy-confirmed Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol*. 2020;140:449-461.
53. Colom-Cadena M, Gelpi E, Marti MJ, *et al*. MAPT H1 haplotype is associated with enhanced alpha-synuclein deposition in dementia with Lewy bodies. *Neurobiol Aging*. 2013;34:936-942.
54. Heckman MG, Kasanuki K, Diehl NN, *et al*. Parkinson's disease susceptibility variants and severity of Lewy body pathology. *Parkinsonism Relat Disord*. 2017;44:79-84.

55. Sidransky E, Nalls MA, Aasly JO, *et al*. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med*. 2009;361:1651-1661.
56. Robak LA, Jansen IE, van Rooij J, *et al*. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. *Brain*. 2017;140:3191-3203.
57. Nalls MA, Duran R, Lopez G, *et al*. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol*. 2013;70:727-735.
58. Nalls MA, Pankratz N, Lill CM, *et al*. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. 2014;46:989-993.
59. Chang D, Nalls MA, Hallgrimsdottir IB, *et al*. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet*. 2017;49:1511-1516.
60. Parkkinen L, Neumann J, O'Sullivan SS, *et al*. Glucocerebrosidase mutations do not cause increased Lewy body pathology in Parkinson's disease. *Mol Genet Metabol*. 2011;103:410-412.
61. Do CB, Tung JY, Dorfman E, *et al*. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet*. 2011;7:e1002141.
62. Huertas I, Jesus S, Garcia-Gomez FJ, *et al*. Genetic factors influencing frontostriatal dysfunction and the development of dementia in Parkinson's disease. *PLoS One*. 2017;12:e0175560.
63. Tan MMX, Lawton MA, Jabbari E, *et al*. Genome-Wide association studies of cognitive and motor progression in Parkinson's disease. *Mov Disord*. 2021;36:424-433.
64. Liu G, Peng J, Liao Z, *et al*. Genome-wide survival study identifies a novel synaptic locus and polygenic score for cognitive progression in Parkinson's disease. *Nat Genet*. 2021;53:787-793.
65. Stoker TB, Camacho M, Winder-Rhodes S, *et al*. Impact of GBA1 variants on long-term clinical progression and mortality in incident Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 2020; 91: 695-702.
66. Winder-Rhodes SE, Evans JR, Ban M, *et al*. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. *Brain*. 2013;136(Pt 2): 392-399.
67. Iwaki H, Blauwendraat C, Leonard HL, *et al*. Genetic risk of Parkinson disease and progression: An analysis of 13 longitudinal cohorts. *Neurol Genet*. 2019;5:e348.
68. Thal DR, Ronisz A, Tousseyn T, *et al*. Different aspects of Alzheimer's disease-related amyloid β-peptide pathology and their relationship to amyloid positron emission tomography imaging and dementia. *Acta Neuropathol Commun*. 2019;7:178.
69. Iwaki H, Blauwendraat C, Leonard HL, *et al*. Genomewide association study of Parkinson's disease clinical biomarkers in 12 longitudinal patients' cohorts. *Mov Disord*. 2019;34:1839-1850.

III

Lysosomal polygenic burden is associated with cognitive progression in Parkinson's disease patients with low risk of Alzheimer co-pathology

Jon-Anders Tunold[1,2,*], Manuela Tan[1,*], Mathias Toft[1,2], Owen Ross[3], Wilma D. J. van de Berg[4,5] and Lasse Pihlstrøm[1]

[1]Oslo University Hospital, Department of Neurology, Oslo, Norway
[2]University of Oslo, Institute of Clinical Medicine, Oslo, Norway
[3]Mayo Clinic, Department of Neuroscience, Jacksonville, FL, USA
[4]Amsterdam UMC, Vrije Universiteit, Department of Anatomy and Neurosciences, Amsterdam, Netherlands
[5] Amsterdam Neuroscience, Program Neurodegeneration, Amsterdam, Netherlands

*Contributed equally

Abstract

Background: Genetics influence cognitive progression in Parkinson's disease, possibly through mechanisms related to Lewy and Alzheimer's disease pathology. Lysosomal polygenic burden has recently been linked to more severe Lewy pathology post mortem.

Objectives: To assess the influence of lysosomal polygenic burden on cognitive progression in Parkinson's disease patients with low Alzheimer's disease risk.

Methods: Using Cox regression we assessed association between lysosomal polygenic scores and time to Montreal Cognitive Assessment score ≤ 21 in the Parkinson's Progression Markers Initiative cohort (n=374), with replication in data from the Parkinson's Disease Biomarker Program (n=777). Patients were stratified by Alzheimer's disease polygenic risk.

Results: The lysosomal polygenic score was associated with a higher risk of cognitive impairment in patients with low Alzheimer's disease risk in both datasets (p=0.0032 and p=0.0054, respectively).

Conclusion: Our study supports complex interplay between genetics and neuropathology in Parkinson's disease-related cognitive impairment, emphasizing the role of lysosomal polygenic burden.

Introduction

Dementia is a highly disabling non-motor manifestation of Parkinson's disease, impacting patients, caregivers and health care systems[1, 2]. While the majority of patients may eventually develop Parkinson's disease dementia (PDD), the timing of dementia onset is highly heterogenous.[3, 4] Understanding the risk factors and molecular mechanisms contributing to cognitive decline in PD is essential in order to improve prognostics and develop targeted treatment.

Advanced limbic and neocortical Lewy pathology is the most consistent neuropathological feature of PDD. However, Alzheimer's disease (AD)-related amyloid-β and tau co-pathologies are also common and have shown independent association with cognitive impairment in PD.[5] In line with this duality of neuropathology, established genetic risk loci for cognitive progression in PD include both *GBA1*,[6-8] which is implicated in PD risk and Lewy pathology, and *APOE*,[8, 9] the major common AD susceptibility locus.

In dementia with Lewy bodies (DLB), two recent studies have found evidence of distinct genetic architectures depending on the extent of concomitant AD-pathology.[10, 11] Using either neuropathology[10] or CSF biomarkers[11] to stratify DLB patients into subgroups with or without significant AD co-pathology, *APOE* was specifically associated with the former, AD-positive group, and *GBA1* with the latter, "pure" DLB group. Similarly, we recently showed that the severity of Lewy pathology is associated with lysosomal polygenic burden specifically in the subset of PD and DLB donors without AD co-pathology.[12]

In the present study, we aimed to investigate if these findings can be extended to cognitive progression in the early phase of PD. Based on our recent observations in brain bank donors, we hypothesized that stratification of PD patients based on the vulnerability to AD pathology may be important for genetic studies of cognitive progression. Using data from two longitudinal cohorts, we show that a lysosomal polygenic risk score is associated with progression to dementia in patients with low risk of AD co-pathology. Our study supports that cognitive decline in PD is both neuropathologically and genetically heterogenous, highlighting the importance of patient stratification for future translational research.

Methods

Sample description

We used data from two longitudinal PD cohorts; the Parkinson's Progression Markers Initiative (PPMI) and the National Institute of Neurological Disorders and Stroke (NINDS) Parkinson's Disease Biomarker Program (PDBP). While PPMI included PD patients within 2

years of diagnosis and without symptomatic therapy at baseline (n = 423), PDBP recruited patients at various stages of disease (n = 884). Detailed descriptions of the cohorts have been published elsewhere.[13, 14] All patients included in the present study had a clinical diagnosis of PD. In addition, PPMI subjects had a positive dopamine transporter (DAT) SPECT. The PPMI data were obtained from the PPMI database (www.ppmi-info.org/access-data-specimens/download-data) on September 14, 2020 and included CSF measures (supplementary methods) and clinical data for baseline and annual follow up visits at year 1-5. The PDBP data and whole genome sequencing data (supplementary methods) for both cohorts were obtained from the Accelerating Medicines Partnership®-Parkinson's Disease v2.5 (AMP-PD; www.amp-pd.org) initiative, on January 4, 2023. All procedures were approved by ethical committees and written informed consent obtained from participants in both studies.

Cognition was assessed using the Montreal Cognitive Assessment (MoCA)[15] adjusted for education, with a cut-point of 21/30 for classification of cognitive impairment.[16]

To determine the optimal threshold for AD polygenic risk score (PRS) to discriminate between samples with and without significant AD co-pathology, we analyzed data from 217 neuropathologically characterized Lewy body disease (LBD) samples from the Netherland's Brain Bank (NBB) as previously described.[12]

Calculation of Polygenic Risk Scores

Individual Parkinson's disease PRS (PD-PRS), lysosomal PD-PRS with and without *GBA1* and AD-PRS were calculated in PRSice2 using summary statistics from recent PD[17] and AD[18] GWAS meta-analyses respectively, and PRSs were standardized to have a mean of 0 and SD of 1 (supplementary methods and supplementary table 1-4).

Statistical analyses

All statistical analyses were performed in R version 4.3.1 (www.r-project.org).

AD-PRS was used to stratify patients for low or high vulnerability to AD co-pathology. To identify the optimal cut-point we took advantage of NBB data where Lewy body disease donor brains were classified as with or without intermediate to high AD co-pathology based on a composite score of Thal amyloid-β phase and Braak neurofibrillary tangle (NFT) stage.[12] 70 % of the samples were used for model training, while the remaining 30 % were held-out to serve as an independent validation. Samples were stratified for approximate balance of the presence of AD co-pathology. To reduce the risk of overfitting

we performed k-fold (k = 10) repeated (r = 3) cross-validation on the training data using the r package "caret". The resulting model was validated on the held-out data, and the optimal cut-point determined based on the Youden index was 0.29 SD, and achieved an AUC of 0.71. Patients with an AD-PRS below the threshold were considered having a low vulnerability to AD co-pathology.

We applied CSF cut-offs previously determined in AD (supplementary methods).[19, 20] CSF measures were log-transformed to normalize the distribution and compared between groups using t-tests.

Time to cognitive impairment was assessed by survival analysis using the r package "survival". Cox proportional hazard models, adjusted for age at diagnosis, sex, education and first five genetic principal components were used to determine the association between the PRS and time from diagnosis to the follow-up visit at which cognitive impairment was first measured. The proportional hazards assumption was assessed using a combined testing and plotting approach with the function *ggcoxzph* from the r package "survminer".

All statistical tests were two-sided. We applied a two-stage design with discovery in the PPMI cohort and replication in the PDBP cohort. PRS signals replicating at $p < 0.05$ with a consistent direction of effects across both stages were interpreted as positive findings.

Results:

A total of 374 individuals from PPMI and 777 from PDBP passing QC and with available demographic variables were included in the study. Demographic variables are displayed in Table 1.

Table1 Demographic table for the PPMI and PDBP cohorts

	PPMI (n = 374)	**PDBP** (n = 777)
Sex, N (%)		
Male	244 (65.2)	498 (64.1)
Female	130 (34.8)	279 (35.9)
Age at diagnosis (years), mean (SD)	61.4 (9.5)	58.8 (10.2)
Age at inclusion (years), mean (SD)	61.9 (9.5)	64.5 (9.0)
Disease duration (years) at baseline, mean (SD)	0.5 (0.5)	5.8 (5.6)
Years of education, mean (SD)	15.5 (3.0)	
<12 years		21 (2.7)
12-16		502 (64.6)
>16		252 (32.4)
UPDRS 1 score, mean (SD)	5.6 (4.2)	9.5 (6.0)
UPDRS 2 score, mean (SD)	5.8 (4.2)	10.8 (7.8)
UPDRS 3 score, mean (SD)	20.7 (8.8)	25.6 (13.5)
UPDRS 4 score, mean (SD)	NA	2.1 (3.5)
UPDRS total score, mean (SD)	32.1 (13.1)	47.8 (23.8)
MoCA, mean (SD)	26.5 (3.4)	25.4 (3.5)
Follow-up time (months), median (SD)	52.7 (16.4)	16.5 (19.2)
APOE E4 alleles, N (%)		
0	253 (67.6)	581 (74.8)
1	81 (21.7)	181 (23.3)
2	8 (2.1)	15 (1.9)
AD-PRS above cut-off, N (%)	126 (33.7)	259 (33.3)

UPDRS = Unified Parkinson's Disease Rating Scale, MoCA = Montreal Cognitive Assessment; AD-PRS = Alzheimer's disease polygenic risk score.

When considering all PPMI subjects, the lysosomal PD-PRS was not significantly associated with time to cognitive impairment (Table 2). Next, in light of our previous results showing association with lysosomal PD-PRS limited to brain donors without AD co-pathology, we selectively analyzed the subset of PPMI subjects with negative AD CSF biomarkers (Table 2). CSF $A\beta_{1-42}$-based stratification classified ~70 % of samples as low AD risk, and in these cases, the lysosomal PD-PRS was associated with a faster cognitive decline ($p = 0.039$).

Stratifying by CSF t-tau or p-tau yielded nearly identical groups with ~50 % of samples below the cut-point, where the lysosomal PD-PRS was not associated with cognitive progression. Among AD CSF biomarkers, the strongest association with cognitive decline in PD is found for low CSF $A\beta_{1-42}$ levels,[21-23] and this stratification also provided the best statistical power for analyses in the low AD risk group in our data. We therefore interpret the CSF $A\beta_{1-42}$ -stratified result as suggestive evidence of an association, although we note that the tau and $A\beta_{1-42}$ results are semi-independent and we did not correct the significance threshold for multiple testing of both these CSF biomarkers.

As the availability of CSF biomarker data is limited in larger sample series, we next investigated whether stratification based on polygenic AD risk could also capture the relevant subgroup with sufficient accuracy. Splitting the PPMI samples based on the previously determined AD-PRS cut-point, patients with a high vulnerability to AD co-pathology (n = 126 (34 %)) exhibited a significantly lower mean baseline CSF $A\beta_{1-42}$ compared to those with a low-vulnerability to AD co-pathology ($p = 0.0025$). In the samples with a low vulnerability to AD co-pathology (n = 248 (66 %)) the lysosomal PD-PRS was significantly associated with a shorter time to cognitive impairment (Table 2).

In order to replicate the PPMI results in an independent dataset we analyzed samples from PDBP using an identical approach with AD-PRS stratification and Cox regression. We replicated the association between the lysosomal PD-PRS and time to dementia in samples with a low AD risk (Table 2). As we expected *GBA1* to be a strong driver of the lysosomal PD-PRS signal, we repeated the analysis with the lysosomal PD-PRS excluding *GBA1*. The lysosomal PD-PRS remained significantly associated with a shorter time to dementia in PPMI subjects with a normal baseline CSF $A\beta_{1-42}$ (HR 1.45, 95 % CI 1.0-2.1, $p = 0.0475$) and in the PDBP subjects with a low vulnerability to AD co-pathology (HR 1.33, 95 % CI 1.05-1.67, $p = 0.0162$). There were no associations between the full PD-PRS and time to cognitive impairment using CSF or PRS measures to determine vulnerability to AD co-pathology.

Table 2 Results from Cox proportional hazards regression

Cohort	Determination of AD risk	HR	95 % CI	P value	Total (n)	Total (n) with low AD risk	Events (n)
PPMI	-	1.15	0.87-1.53	0.316	374	374	59
PPMI	$A\beta_{1-42}$	1.42	1.02-1.99	0.0386*	363	246	31
PPMI	pTau	0.99	0.61-1.59	0.960	338	153	20
PPMI	tTau	0.97	0.6-1.56	0.889	358	168	24
PPMI	AD-PRS	1.89	1.24-2.88	0.0032*	374	248	33
PDBP	AD-PRS	1.31	1.08-1.58	0.0054*	777	517	93

Associations between the lysosomal PD polygenic risk score and progression to cognitive impairment with age at diagnosis, sex, education and first five genetic principal components as covariates. HR = Hazard ratio; CI = confidence interval. *P < 0.05.

Discussion:

Using data from two longitudinal PD cohorts we have shown that the cumulative burden of PD susceptibility variants converging on the lysosomal pathway is associated with an earlier onset of cognitive impairment in subjects with a low vulnerability to AD co-pathology, extending on our recently published results in neuropathologically confirmed LBD samples.[12]

Genetic studies have highlighted a broad contribution of genes linked to lysosomal functions in PD,[24, 25] and a functional association between lysosomal impairment and alpha-synuclein aggregation has been demonstrated for several of these.[26-28] *GBA1* is a major lysosomal risk locus for both PD[29] and DLB.[30] Variants in *GBA1* have been linked to more rapid cognitive decline and increased risk of dementia in PD.[6-8, 31, 32] Additionally, our results suggest lysosomal variants beyond *GBA1* contribute to cognitive decline, as the association between the lysosomal PD-PRS excluding *GBA1* and time to cognitive impairment remained significant in PPMI and PDBP subjects with a low CSF and genetic vulnerability to AD co-pathology respectively.

Neuropathological changes in limbic and cortical brain regions are believed to be the substrate of cognitive symptoms in PD.[5] Consequently, it might be expected that genetic variants associated with more widespread neuropathology should also increase the susceptibility to cognitive impairment. In line with this, an abundance of evidence supports a role of both *APOE* E4 and *GBA1* in cognitive progression in PD.[7, 9] *APOE* E4 is strongly

linked to more severe AD co-pathology,[33-35] whereas *GBA1* is associated with cortical Lewy pathology, with some reports suggesting *GBA1* carriers have a "purer" Lewy body disease with less advanced AD co-pathology.[10, 36, 37] In the present study, we hypothesized that the cumulative lysosomal genetic burden might be part of an overlapping genetic architecture of vulnerability to both more widespread Lewy pathology and earlier cognitive progression in PD. The results confirm this hypothesis for a subset of patients, also highlighting the heterogeneous genetic and neuropathological underpinnings of cognitive decline in PD.

We acknowledge that our study has some limitations. The sample size is limited, yet our results were replicated across both cohorts. We note that both PPMI and PDBP subjects have contributed to the PD meta-GWAS[17] from which the PRS allele weights were derived. Independent datasets are typically preferred for testing the predictive performance of PRSs, in order to avoid overfitting. We acknowledge that this would have been ideal, yet in our study a completely different outcome was assessed, using weights from a case-control risk analysis in a case-only analysis of cognitive progression. Consequently, a partial sample overlap is not of major concern for the interpretation of our results.

We are mindful that our attempts to stratify patients based on the susceptibility to AD co-pathology are no gold standard. CSF $A\beta_{1-42}$ may distinguish between individuals with and without AD co-pathology, although the optimal cut-point in PD remains to be determined and may differ from established AD cut-points.[38, 39] Additionally, our data suggests that the AD-PRS can serve as a proxy for AD co-pathology on a group level. While clearly not as accurate as stratifying by AD pathology measures as we recently did in our genetic study of post-mortem neuropathology,[12] the AD-PRS was able to serve a similar purpose for meaningful stratification of clinical samples into subgroups in the present association study. Supporting the validity of this proxy, PPMI patients with AD-PRS above the cut-point had lower $A\beta_{1-42}$, likely reflecting a higher level of AD co-pathology in these samples. Nevertheless, the suggested cut-point was determined in a small sample and should be interpreted with caution.

The temporal sequence of protein pathology in PD is not known. Several investigations have documented that reduced CSF $A\beta_{1-42}$ at baseline is associated with cognitive decline,[21, 23] yet the optimal threshold remains to be established. $A\beta_{1-42}$ continues to decrease over the course of disease,[40] possibly mirroring increase in AD co-pathology.[38, 41] Thus, early prediction of patients who will develop AD co-pathology using CSF measures alone remains elusive. PRSs offer an advantage over other biomarkers by providing risk

assessment at an earlier disease stage, preceding the typical rise in risk over the disease course observed with other biomarkers.

In conclusion we highlight the burden of lysosomal variants in progression to cognitive impairment in PD patients with a low vulnerability to AD co-pathology. Further, our results provide novel evidence for stratification by the polygenic burden of AD risk alleles, which may enable a more precise understanding of the genetic influence of cognitive impairment in PD. Additional research with larger cohorts and more comprehensive assessment of cognition is needed to validate and expand upon these findings. With further improvement, we hope that the PRSs may inform individual prognosis and facilitate detection of therapeutic targets within a precision medicine framework.

Acknowledgements

J.A.T., M.M.X.T. and L.P were funded by grants from the South-Eastern Regional Health Authority, Norway. The PD GWAS summary statistics used in this study were generated in a meta-analysis including data from 23andMe, Inc. We would like to thank the research participants and employees of 23andMe for making this work possible. The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/dataset-access/ for more information and to apply to access the data. The authors are grateful to the Netherlands Brain Bank and its funders for providing the samples that made the study possible.

Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/access-data-specimens/download-data), RRID:SCR 006431. For up-to-date information on the study, visit www.ppmi-info.org.

PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including AbbVie, Curex Therapeutics, Allergan, Amathus Therapeutics, Aligning Science Across Parkinson's (ASAP), Avid Radiopharmaceuticals, Bial, Biogen, BioLegend, Biohaven pharmaceuticals, BlueRock Therapeutics, Bristol-Meyers Squibb, Calico, Celgene, Cerevel, Coave Therapeutics, DaCapo, Denali Therapeutics, Edmond J. Safra Philanthropic Foundation, 4D Pharma plc, GE Healthcare, Genentech, GlaxoSmithKline, Golub Capital, Gain Therapeutics, Handl Therapeutics, Insitro, Janssen Neuroscience, Lilly, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Pfizer, Piramal Healthcare, Prevail

Therapeutics, Roche, Sanofi-Genzyme, Servier, Sparc, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, Weston Family Foundation and Yumanity Therapeutics. The PPMI investigators have not participated in reviewing the data analysis or content of the manuscript

Data used in the preparation of this article were obtained from the Accelerating Medicine Partnership® (AMP®) Parkinson's Disease (AMP PD) Knowledge Platform. For up-to-date information on the study, visit https://www.amp-pd.org.

The AMP® PD program is a public-private partnership managed by the Foundation for the National Institutes of Health and funded by the National Institute of Neurological Disorders and Stroke (NINDS) in partnership with the Aligning Science Across Parkinson's (ASAP) initiative; Celgene Corporation, a subsidiary of Bristol-Myers Squibb Company; GlaxoSmithKline plc (GSK); The Michael J. Fox Foundation for Parkinson's Research ; Pfizer Inc.; AbbVie Inc.; Sanofi US Services Inc.; and Verily Life Sciences.

ACCELERATING MEDICINES PARTNERSHIP and AMP are registered service marks of the U.S. Department of Health and Human Services.

Clinical data and biosamples used in preparation of this article were obtained from the (i) NINDS Parkinson's Disease Biomarkers Program (PDBP) and (ii) MJFF Parkinson's Progression Markers Initiative (PPMI).

The Parkinson's Disease Biomarker Program (PDBP) consortium is supported by the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health. A full list of PDBP investigators can be found at https://pdbp.ninds.nih.gov/policy. The PDBP investigators have not participated in reviewing the data analysis or content of the manuscript.

References

1. Aarsland D, Batzu L, Halliday GM, et al. Parkinson disease-associated cognitive impairment. Nat Rev Dis Primers 2021;7(1):47.
2. Backstrom D, Granasen G, Domellof ME, et al. Early predictors of mortality in parkinsonism and Parkinson disease: A population-based study. Neurology 2018;91(22):e2045-e2056.
3. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord 2008;23(6):837-844.
4. Williams-Gray CH, Evans JR, Goris A, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. Brain 2009;132(Pt 11):2958-2969.
5. Smith C, Malek N, Grosset K, Cullen B, Gentleman S, Grosset DG. Neuropathology of dementia in patients with Parkinson's disease: a systematic review of autopsy studies. J Neurol Neurosurg Psychiatry 2019;90(11):1234-1243.
6. Cilia R, Tunesi S, Marotta G, et al. Survival and dementia in GBA-associated Parkinson's disease: The mutation matters. Ann Neurol 2016;80(5):662-673.
7. Iwaki H, Blauwendraat C, Leonard HL, et al. Genetic risk of Parkinson disease and progression:: An analysis of 13 longitudinal cohorts. Neurol Genet 2019;5(4):e348.
8. Szwedo AA, Dalen I, Pedersen KF, et al. GBA and APOE Impact Cognitive Decline in Parkinson's Disease: A 10-Year Population-Based Study. Mov Disord 2022;37(5):1016-1027.
9. Tan MMX, Lawton MA, Jabbari E, et al. Genome-Wide Association Studies of Cognitive and Motor Progression in Parkinson's Disease. Mov Disord 2021;36(2):424-433.
10. Kaivola K, Shah Z, Chia R, International LBDGC, Scholz SW. Genetic evaluation of dementia with Lewy bodies implicates distinct disease subgroups. Brain 2022;145(5):1757-1762.
11. van der Lee SJ, van Steenoven I, van de Beek M, et al. Genetics Contributes to Concomitant Pathology and Clinical Presentation in Dementia with Lewy Bodies. J Alzheimers Dis 2021;83(1):269-279.
12. Tunold JA, Tan MMX, Koga S, et al. Lysosomal polygenic risk is associated with the severity of neuropathology in Lewy body disease. Brain 2023.

13. Marek K, Chowdhury S, Siderowf A, et al. The Parkinson's progression markers initiative (PPMI) - establishing a PD biomarker cohort. Ann Clin Transl Neurol 2018;5(12):1460-1477.
14. Rosenthal LS, Drake D, Alcalay RN, et al. The NINDS Parkinson's disease biomarkers program. Mov Disord 2016;31(6):915-923.
15. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53(4):695-699.
16. Dalrymple-Alford JC, MacAskill MR, Nakas CT, et al. The MoCA: well-suited screen for cognitive impairment in Parkinson disease. Neurology 2010;75(19):1717-1725.
17. Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol 2019;18(12):1091-1102.
18. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nat Genet 2019;51(3):404-413.
19. Blennow K, Shaw LM, Stomrud E, et al. Predicting clinical decline and conversion to Alzheimer's disease or dementia using novel Elecsys Abeta(1-42), pTau and tTau CSF immunoassays. Sci Rep 2019;9(1):19024.
20. Shaw LM, Waligorska T, Fields L, et al. Derivation of cutoffs for the Elecsys((R)) amyloid beta (1-42) assay in Alzheimer's disease. Alzheimers Dement (Amst) 2018;10:698-705.
21. Siderowf A, Xie SX, Hurtig H, et al. CSF amyloid beta 1-42 predicts cognitive decline in Parkinson disease. Neurology 2010;75(12):1055-1061.
22. Parnetti L, Farotti L, Eusebi P, et al. Differential role of CSF alpha-synuclein species, tau, and Abeta42 in Parkinson's Disease. Front Aging Neurosci 2014;6:53.
23. Alves G, Lange J, Blennow K, et al. CSF Abeta42 predicts early-onset dementia in Parkinson disease. Neurology 2014;82(20):1784-1790.
24. Robak LA, Jansen IE, van Rooij J, et al. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain 2017;140(12):3191-3203.
25. Hopfner F, Mueller SH, Szymczak S, et al. Rare Variants in Specific Lysosomal Genes Are Associated With Parkinson's Disease. Mov Disord 2020;35(7):1245-1248.
26. Mazzulli JR, Xu YH, Sun Y, et al. Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell 2011;146(1):37-52.

27. Jinn S, Drolet RE, Cramer PE, et al. TMEM175 deficiency impairs lysosomal and mitochondrial function and increases alpha-synuclein aggregation. Proc Natl Acad Sci U S A 2017;114(9):2389-2394.
28. Park JS, Blair NF, Sue CM. The role of ATP13A2 in Parkinson's disease: Clinical phenotypes and molecular mechanisms. Mov Disord 2015;30(6):770-779.
29. Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 2009;361(17):1651-1661.
30. Nalls MA, Duran R, Lopez G, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. JAMA Neurol 2013;70(6):727-735.
31. Brockmann K, Srulijes K, Pflederer S, et al. GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study. Mov Disord 2015;30(3):407-411.
32. Koros C, Brockmann K, Simitsi AM, et al. Impact of APOE Genotype on Cognition in Idiopathic and Genetic Forms of Parkinson's Disease. Mov Disord 2023.
33. Lashley T, Holton JL, Gray E, et al. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol 2008;115(4):417-425.
34. Compta Y, Parkkinen L, O'Sullivan SS, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain 2011;134(Pt 5):1493-1505.
35. Robinson JL, Lee EB, Xie SX, et al. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. Brain 2018;141(7):2181-2193.
36. Clark LN, Kartsaklis LA, Wolf Gilbert R, et al. Association of glucocerebrosidase mutations with dementia with lewy bodies. Arch Neurol 2009;66(5):578-583.
37. Irwin DJ, Grossman M, Weintraub D, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. Lancet Neurol 2017;16(1):55-65.
38. Irwin DJ, Xie SX, Coughlin D, et al. CSF tau and beta-amyloid predict cerebral synucleinopathy in autopsied Lewy body disorders. Neurology 2018;90(12):e1038-e1046.
39. Weinshel S, Irwin DJ, Zhang P, et al. Appropriateness of Applying Cerebrospinal Fluid Biomarker Cutoffs from Alzheimer's Disease to Parkinson's Disease. J Parkinsons Dis 2022;12(4):1155-1167.
40. Baek MS, Lee MJ, Kim HK, Lyoo CH. Temporal trajectory of biofluid markers in Parkinson's disease. Sci Rep 2021;11(1):14820.

41. Buongiorno M, Antonelli F, Compta Y, et al. Cross-Sectional and Longitudinal Cognitive Correlates of FDDNP PET and CSF Amyloid-beta and Tau in Parkinson's Disease1. J Alzheimers Dis 2017;55(3):1261-1272.